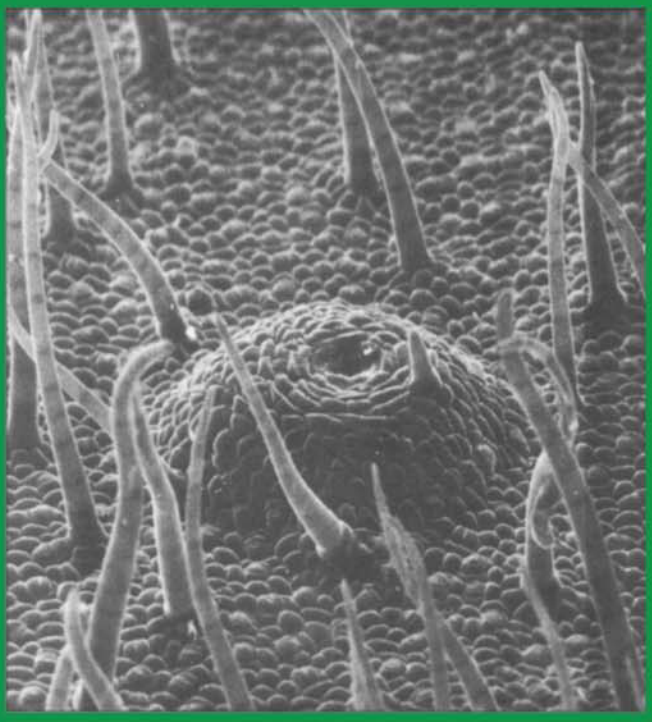
Advances in BOTANICAL RESEARCH



Volume 17

edited by J. A. CALLOW

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Edited by

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PREFACE

In this volume of *Advances in Botanical Research* we start with two reviews on aspects of primitive floras and angiosperm evolution. Collinson's article is concerned with the Early Tertiary or Palaeogene era, a time of major geological and evolutionary change producing new microhabitats and competitive pressures. The resultant radiations of flora and fauna (including the second radiation of angiosperms) represent the early stages in the evolution of the communities and biological diversity that characterize the modern world. Collinson's review is concerned with the study of the floras of this period as they aid in the deduction of ancient climate and ecology. A more complete understanding of the latter is not irrelevant to current predictions of global climatic change! The review examines the geological and biological contexts of the period before embarking on a reconstruction of climate as inferred from physiological indicators such as carbon isotope ratios, from comparative anatomy and the study of fossil communities and plant–animal associations.

Moving to a slightly earlier geological period, during the last few years there has been a number of spectacular discoveries of well-preserved Early and mid-Cretaceous flowers. Study of floral development and biology of living archaic angiosperms has also intensified. In their chapter, Friis and Endress argue that the time is ripe for the two areas of study to come together, to encourage a synthesis of these sources of information into a new theory of how flowers evolved. This extensive review provides a detailed morphological description of both fossil and extant 'primitive' flowers and related fossil gymnosperms. The various standard evolutionary theories are discussed and then evaluated in relation to the new sources of evidence. The conclusion is reached that none is entirely compatible with the new sources of evidence and in the final sections the authors outline their views on the most likely major evolutionary steps.

The study of the cyclic bacterial-leaf nodule symbioses of plants such as *Psychotria*, and *Ardisia*, is probably the least researched and most overlooked of all higher plant–microbe interactions. This obscurity may be partly due to the fact that with one exception (*Dioscorea*) the host plants are not of any great commercial importance, but a major factor must be the fact that the systems are proved to be relatively intractable. Even the identity of

the microorganisms involved is still confused and Koch's postulates have not been satisfied for any of them. Yet these relationships are of fundamental importance to the student of symbiosis since they must rank as the most intimate of relationships, they are totally obligate, neither host plants nor microbes can be effectively grown in the absence of each other and it seems likely that the external infection events may have occurred several million years ago, the intervening period permitting such extensive co-evolution that neither partner can exist in free-living form. Miller's critical and timely review examines aspects of the systematics of host plants involved and provides the first comprehensive analysis of the infection cycles and host–microbe interfaces using modern microscopical techniques. The problems in identifying the microorganisms involved are considered, and the review speculates on the potential biotechnological value of these symbioses if they could be manipulated.

The structural integrity of plant tissues can be expressed largely in terms of fracture properties. Clearly these properties are fundamental to the sedentary life style of most plants where environmental vicissitudes often have to be accommodated rather than avoided. An analysis of such properties can be expected to shed light on the evolutionary pressures that plants have been subjected to. Controlled fracture in dehiscence or abscission for example, is also vital to many plant developmental processes. In addition, analysis of plant durability is an important consideration in relation to the agricultural value of crop plants. The article by Vincent injects more rigour into the analysis of plant fracture than is common hitherto because it approaches it from the viewpoint of the engineer and materials scientist rather than the botanist. In this chapter a number of general misconceptions and inadequate methodologies are exposed, better techniques are discussed, and properties of individual plants and plant tissues are reviewed. The chapter should enable materials scientists and botanists to communicate more effectively with each other in the future.

Finally I would like to pay my thanks to each of the contributors in the present volume for their patience with the editor and their efforts to make his task easier.

J. A. CALLOW

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Plant Evolution and Ecology During the Early Cainozoic Diversification

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I. INTRODUCTION AND SCOPE

The Early Tertiary or Palaeogene spans a period from 66.5 to 25.2 million years ago (Haq *et al.*, 1987). It was the time of major modernization of world vegetation and floras, the second radiation of angiosperms and extensive changes in terrestrial vertebrate faunas. The Cretaceous/Tertiary boundary is marked by major extinctions in the marine realm, final extinction of the dinosaurs and ecological disruption of land habitats (Officer *et al.*, 1987; Wolfe and Upchurch, 1987b). Placental mammals underwent a major diversification during the Early Tertiary and the large herbivorous dinosaurs were not replaced by a similar diversity of large herbivorous mammals until the middle Eocene (Friis *et al.*, 1987; Wing and Tiffney 1987a,b). Thus, a diversity of new microhabitats and new competitive pressures typified this time. The resultant radiations of flora and fauna represent the early stages of evolution of the communities and diversity which characterize the modern world. In this chapter I aim to review our current understanding of Early Tertiary floras concentrating on the evidence these provide concerning the evolution and diversification of land vegetation, origination of modern plant groups and co-evolution of floras and faunas. I also consider early Tertiary floras as a basis for interpreting ancient climates.

This chapter is not a comprehensive review article. My intention is rather to emphasize those areas in which I consider major innovations are occurring both in study methods and in resultant knowledge. Even for this I can only give selected examples and apologize in advance to colleagues who might feel that their contribution has been covered inadequately.

I have also tried to provide sufficient guidance to the literature for most plant groups recorded in the Early Tertiary from most areas of the world. This article is not concerned with palynology and readers wishing to pursue this aspect should initially consult Muller (1981, 1985), Frederiksen (1985) and Traverse (1988).

Selected aspects of Tertiary floras which I consider of particular interest and promise in the context of this article are as follows:

1. Studies on the biology of whole plants (including their reconstruction from detached parts) in contrast to the more usual studies of isolated organs.
2. Innovative approaches in systematic and evolutionary studies, often involving 1 above, including thorough investigations of fossil and modern representatives of individual plant groups and utilizing computing technology.
3. Investigations of fossils in their sedimentological and taphonomic context leading to more reliable reconstruction of ancient communities.
4. Application of ecological anatomy and palaeophysiology to elucidate further details of these communities.
5. Evidence concerning interaction between animals and plants in these ancient communities.

6. Reconstructions of large scale vegetational pattern and of physical parameters such as climate and the palaeoatmosphere.

7. Studies on geographic areas or geological sections where Tertiary floras were previously poorly known.

In the latter part of this chapter each of these topics will be covered in detail (Section IV). Initially however, I will introduce important geological and physical factors which may have influenced plant life in the Early Tertiary and provide a general overview of the floras themselves and the methods by which they are investigated.

II. THE GEOLOGICAL CONTEXT AND BIOLOGICAL NATURE OF EARLY TERTIARY FLORAS

A. GEOLOGICAL SETTING

The Cainozoic era includes the Tertiary and Quaternary periods. The Early Tertiary or Palaeogene spans just over 40 million years of earth history from the Cretaceous/Tertiary boundary at 66.5 million years before present (mybp). The Early Tertiary is subdivided into three epochs: the Palaeocene, 66.5–54 mybp; the Eocene, 54–36 mybp; and the Oligocene, 36–25.2 mybp (Haq *et al.*, 1987). There is still some discussion concerning this absolute dating, for example the Oligocene/Miocene boundary is placed at 23.7 mybp by Berrgren *et al.* (1985) and at 24.6 mybp by Harland *et al.* (1982). I follow the time scale of Haq *et al.* (1987) as this is the most recent study and presents a consensus of the most reliable dates obtained by a variety of methods.

Biostratigraphy of the Early Tertiary relies heavily on marine plankton (foraminifera, dinoflagellates, calcareous nanofossils and radiolaria; Berrgren *et al.*, 1985; Haq *et al.*, 1987) and this inevitably poses problems in continental sequences where much of the evidence for land floras is preserved. In Europe, some of the Early Tertiary sequence includes marine incursions thus minimizing this problem (see comments of Collinson and Hooker, 1987). In North American continental sequences a separate system of land mammal ages is used. The mammals are distinct from those in Europe after the early Eocene. Correlation can be improved through magnetostratigraphy (Berrgren *et al.*, 1985; Haq *et al.*, 1987) but this has not yet been fully refined. It is therefore not yet possible to make precise comparisons between synchronous Tertiary floras from different areas of the world, although the broad correlation of the epochs (and their subdivisions) is generally established.

B. MAJOR GEOLOGICAL AND BIOLOGICAL EVENTS

Several important geological events occurred during the Early Tertiary

(Harland *et al.*, 1982; Shackleton, 1986a; Parrish, 1987). The Laramide phase of orogeny (mountain building) occurred early in the Palaeocene, with another phase, the Pyrenean or Alpine, near the end of the Eocene.

Two of the boundaries, the Cretaceous/Tertiary (i.e. Maastrichtian/Palaeocene) and the Eocene/Oligocene are the subject of much controversy (Pomerol and Premoli-Silva, 1986; Officer *et al.*, 1987). At both, meteoritic impacts are said to have caused profound biological disturbance including ecological disruption with ensuing extinctions. Evidence in favour of extra-terrestrial impact has been drawn from the occurrence of anomalously high concentrations of elements like iridium, otherwise rare on earth; from the presence of impact-formed glasses or tektites (as spherules); from the presence of shocked quartz; and from the sudden nature of the extinctions (Alvarez *et al.*, 1982).

1. Cretaceous/Tertiary (K/T) Boundary

Intense global vulcanism is an alternative explanation for events at the K/T boundary (Officer *et al.*, 1987), especially in view of the emplacement of extensive flood basalts in the Deccan Traps (western India) at this time (Courtillot *et al.*, 1988). In the volcanic scenario, emissions from vulcanism led to acid rain, reduced alkalinity and pH of the surface ocean, global atmospheric temperature changes and ozone-layer depletion. In the alternative extraterrestrial-impact scenario, a huge dust cloud was ejected into the atmosphere or stratosphere causing a decrease in solar radiation resulting in darkness at the earth's surface and global climatic cooling (Alvarez *et al.*, 1982). Acid rain, due to build up of NO_2, has also been included in this scenario (Crutzen, 1987). Wolbach *et al.* (1988) invoke global fire, resulting from meteoritic impact, producing a soot cloud which caused darkness and cooling. Their evidence comes from enrichment of elemental carbon (mainly soot) in the boundary layers.

It is now recognized that several of the geological signatures previously thought to imply extraterrestrial origin, including iridium anomalies and shocked quartz may be the result of volcanic activity, the iridium being derived from the mantle. Furthermore, the microspherules may be the result of diagenetic replacement (Officer *et al.*, 1987). Note, however, that Owen and Anders (1988) claim that new cathodoluminescence evidence shows that some shocked quartz did not originate from volcanic sources.

The extinctions of the K/T boundary are by no means instantaneous nor do they affect all groups equally. In particular there is controversy concerning whether the dinosaur extinctions are any more unusual in rate or magnitude at the K/T boundary than elsewhere (see Officer *et al.*, 1987). The catastrophist reply is to invoke comet showers rather than a single impact in explanation of the periodic nature of the extinctions (Hut *et al.*, 1987).

Differential extinctions at the K/T boundary have long been known. In the marine realm, the deep sea benthos was little affected but planktonic organ-

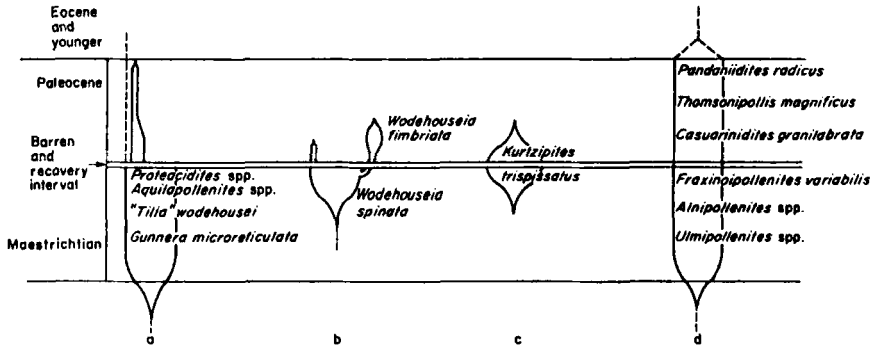

Fig. 1. Patterns of extinction and survival in pollen across the K/T boundary, Western Interior, North America. (a) Abrupt regional disappearance but the same genera found in younger rocks elsewhere; (b) Extinction or attenuation followed by appearance of derived species; (c) extinction in the Palaeocene; (d) little affected by K/T event. From Tschudy and Tschudy (1986) with permission of the Geological Society of America and the surviving author.

isms with calcareous skeletons and tropical reef invertebrates were severely hit. In the terrestrial realm, of greatest interest here, a number of groups were little affected including freshwater vertebrates, snakes, mammals and many plants. In contrast land vertebrates of large size were hit most severely (Officer *et al.*, 1987).

In view of the significance of events at the K/T boundary in opening up new habitats and altering competitive interactions on land, it is pertinent here to summarize our knowledge of the influence of these events on land plants.

Influence on land plants: palynological evidence. The first clear indications of changes in land vegetation at the K/T boundary came from the Raton Basin in Western North America. Here Tschudy *et al.* (1984) noted, in a palynological study, that the boundary was marked by an abrupt increase in the proportion of fern spores compared with angiosperm pollen. This peak in a graph of spore frequencies became known as the "fern spike" and has subsequently been recognized in spore counts throughout the western interior of North America, Canada (Tschudy and Tschudy, 1986) and Japan (Saito *et al.*, 1986). The latter work is particularly significant as the "fern spike" is there recognized for the first time in a marine sequence thus confirming the correlated timing of events in the marine and terrestrial realms. Tschudy and Tschudy (1986), studying several sections in the Western Interior of North America, documented four major patterns of extinction and survival of angiosperm pollen across the boundary (Fig. 1). These range from abrupt regional disappearance to survival almost without change.

Late Cretaceous vegetation of the Northern hemisphere has been subdivided into two floral provinces based on pollen data: the *Aquilapollenites*

province and the Normapolles province (Herngreen and Chlonova, 1981; Batten, 1984). The former included western North America and eastern Siberia, the latter eastern North America and Europe. Details of the K/T boundary events mentioned above refer only to the *Aquilapollenites* province. Muller (1985) mentions some turnover in the Normapolles province and Hickey (1981, fig. 2) shows that turnover there was much reduced compared with that in the *Aquilapollenites* province. Detailed studies like that of Tschudy and Tschudy (1986) have generally not been made elsewhere. However, Ashraf and Erben (1986) have studied boundary sections in Spain and France through marine strata. They found changes in pollen and spore floras implying a post-boundary increase in conifer abundance. However, as they also record dinosaur egg shell fragments within their Tertiary strata, the dating of their events is equivocal (for recent discussion for and against the existence of Palaeocene dinosaurs, see Van Valen, 1988).

Few detailed palynological investigations of boundary sections in the Southern hemisphere floristic provinces have been published and evidence from this region is essential in order to obtain a global understanding of K/T events. This is especially true if volcanism in the Deccan Traps is to be invoked as a possible causal factor (e.g. Courtillot *et al.*, 1988). Askin (1988a,b) has recorded the pollen and spore flora across the K/T boundary on Seymour, Antarctica, and adjacent islands. There is no abrupt change in the sequence and no "fern spike". Major vegetational disruption apparently did not occur in this area. However, there is a long-term floral turnover, with certain Late Cretaceous angiosperm pollen absent from Palaeocene strata and some forms first appearing in the Palaeocene.

Influence on land plants: megafossil evidence. Comparable information, based on plant megafossils (i.e. leaves, fruit, wood, etc.), has been rather sparse. General texts, acknowledging the very limited data base and the problems associated with analyses of global diversity patterns, have not recorded an especially significant turnover at the K/T boundary (Niklas *et al.*, 1985; Tiffney, 1981a). Hickey (1981), working only with North American data, has also recorded only gradual change in land plants.

A series of elegant studies by Wolfe and Upchurch (1986, 1987b) and Wolfe (1987) has provided for the first time clear evidence for plant megafossil changes across the K/T boundary. These authors have examined leaves and dispersed leaf cuticles by detailed sampling at numerous localities in western North America, from New Mexico in the south to Alberta in the north. The extinction patterns contrast sharply with those of the pollen data from the same sequence. Most of the leaves (75%) from the Late Cretaceous are absent from the Early Tertiary of the southernmost sites (Raton Basin). This contrasts with extinction of only a few pollen species (Tschudy and Tschudy, 1986). However, pollen species are generally less diagnostic than leaves and cuticles, and thus the "species" recognition may differ. Also many

of the leaf extinctions relate to archaic forms, especially of Laurales, a group whose pollen is rarely preserved owing to a thin exine. In the northernmost area sampled, extinction rate among leaf forms was only 24%, and intermediate sites showed intermediate levels of extinction.

The extinctions were also differential in the kinds of plants they affected. In particular, evergreen types were replaced by deciduous forms. This is considered to have had a major influence on future northern hemisphere vegetation and is discussed in more detail elsewhere (Section IV.F.1).

These studies also revealed a short interval, at and near the boundary, dominated by fern foliage and containing very few dicotyledonous cuticles. Leaf assemblages of dicotyledons above and below the "fern spike" differed profoundly in their form or physiognomy. The differences are consistent with destruction of climax vegetation followed by recolonization, initially by ferns. The nearest living relative of the fossil fern encountered by Wolfe and Upchurch is *Stenochlaena* a primary colonizer today. Ferns have often been noted as important primary colonizers in many situations where modern climax vegetation is destroyed, e.g. following the volcanic eruptions of Krakatoa and El Chichon (Richards, 1952; Spicer *et al.*, 1985). Subsequent recolonization by dicotyledons, in the Palaeocene, is marked by early successional forms but over a much longer duration than in comparable modern circumstances. Many archaic forms were replaced by types referable to modern families or genera. Many evergreen forms were replaced by deciduous types, e.g. certain Taxodiaceae amongst conifers. Vegetation never returned to its pre-boundary condition and the events thus left a permanent mark on Tertiary floras. The authors consider that these changes are best explained by a short cold period, particularly the selection for deciduous taxa, i.e. those able to enter a period of dormancy. They thus favour the extraterrestrial explanation, with the short cold period produced by an "impact winter". It does seem that the volcanic hypothesis (involving darkness and cold) might be equally plausible, bearing in mind dormancy of modern high latitude conifers and potential dormancy of seeds in the seed bank. The carbon isotope record (Shackleton, 1986b), for coccoliths and planktonic foraminifera (but not benthos) documents a sharp excursion at the boundary. Shackleton (1986b) considers that this can best be explained by a drop in global photosynthesis at the ocean surface. This might be caused by cold or darkness or both.

Whatever the cause, it is clear that ecological disruption marked the K/T boundary at least in the *Aquillapollenites* province of the Northern palaeohemisphere. This resulted in destruction of previous climax vegetation and the temporary dominance of ferns. Recolonization followed a pattern comparable to that for some recent disturbed habitats, culminating in new climax vegetation of a more modern aspect. The influence of this event seems to have been strongest at the more equatorial palaeolatitudes, i.e. in more tropical palaeoclimates.

2. Eocene/Oligocene Boundary

Extraterrestrial impacts have also been invoked to explain extinctions at the Eocene/Oligocene boundary, which are mainly in the marine realm especially amongst foraminifera. Vegetational changes are usually explained in terms of climatic cooling inferred from leaf physiognomy (see Section IV.F), and by independent physical means (see Section II.C). Detailed studies of the kind undertaken across the K/T boundary are lacking but in general terms one may view the change as being from broad-leaved evergreen forest under subtopical conditions to temperate broad-leaved deciduous forest (Wolfe, 1978; Pomerol and Premoli-Silva, 1986). These changes are documented in mid-high latitudes of the Northern hemisphere and are gradual rather than abrupt, perhaps beginning early in the Late Eocene (Pomerol and Premoli-Silva, 1986). Floristic changes in southern England give further support to the gradual nature of this change (Collinson *et al.*, 1981; Collinson and Hooker, 1987) as do the patterns observed in other biotic and physical events (Pomerol and Premoli-Silva, 1986). Changes in land mammal faunas are also documented in Europe and North America but the extinctions seem to be related to the incoming of new forms rather than to external causes. Asian mammal faunas show little change at this boundary (Pomerol and Premoli-Silva, 1986). The climatic cooling seems more likely to be due to global tectonic and orogenic events (the alpine orogeny) rather than an extraterrestrial impact. Such an impact may have occurred, as indicated by an extensive microtektite field stretching from North America westwards to the Indian Ocean. However, the gradual nature of biotic and other physical changes weigh against this being the major cause (Pomerol and Premoli-Silva, 1986). Shackleton (1986b) has confirmed "beyond reasonable doubt" that the final sharp cooling event at the Eocene-Oligocene boundary followed the foraminiferal extinctions and the microtektite events. Whatever the cause, the Eocene-Oligocene boundary events also influenced future floras. The major changes are those resulting from climatic cooling and southward displacement of climatic and hence vegetational belts (see Section IV.F).

C. PALAEOCLIMATIC SETTING

One major line of evidence for Early Tertiary climatic changes is leaf physiognomic studies. These, and other evidence from the plants themselves, are discussed later (Sections IV.F and IV.I). Here I give the climatic pattern derived primarily from oxygen isotope data (Buchardt, 1978; Shackleton, 1984, 1986a,b; Parrish, 1987). These techniques have proved most reliable on open ocean carbonates (e.g. benthic and planktonic foraminifera). They therefore give ocean temperatures which are difficult to extrapolate to terrestrial environments. They do, however, provide a general pattern of global temperature change.

The latest Cretaceous, Palaeocene and Eocene were much warmer at corresponding latitudes than the Oligocene and later Tertiary or the present day. The pattern implies a warming during the Palaeocene with temperature maxima in the late Early or early Middle Eocene. Subsequent cooling accelerated near the Eocene/Oligocene boundary, giving temperature minima at that time. Global temperature gradients were much lower than today, in particular the temperature difference between mid- and high-latitudes was less pronounced. The fall in temperature at the Eocene/Oligocene boundary indicated by isotopic evidence from bethnic foraminifera is much more pronounced than that from planktonic forms. This cooling was global in extent in deep ocean waters. Mid- and high-latitudes show similar cooling in surface waters (evidence from planktonic foraminifera). Shackleton (1986b) considers that temporary glaciation on Antarctica was responsible for the cooling event. It may also reflect the temperature of bottom waters generated at high latitudes, but circum-polar bottom-currents probably had not originated at this time (see Section II.D). Prentice and Matthews (1988) used evidence from oxygen isotope studies of benthic and planktonic foraminifera to infer the Cainozoic history of global ice volume. They concluded that the period from 65 to 50 mybp was intermittently ice-free and by 40 mybp (Late Eocene) a significant ice budget existed comparable to the present day. They also considered that the polar regions did not export significant amounts of cold bottom waters until about 20 mybp (Early Miocene).

Parrish *et al.* (1982) reconstructed global rainfall patterns for the Mesozoic and Cainozoic using reconstructed palaeogeographic maps to infer the influence of continental position on atmospheric circulation. They tested their results using the distribution of coals and evaporites. This showed that past climatic zones were largely parallel to latitude as they are today. However, the influence of palaeogeography on monsoonal circulation was considered important during the Tertiary. Early Tertiary rainfall patterns differed from those of the present, in that much of Europe, Africa, Asia and North America were more humid. Conditions equivalent to modern deserts were largely absent. Their maps, although preliminary (see also Parrish, 1987) have important implications for palaeoclimatic data inferred from leaf floras. It is important to consider the palaeogeographic context of a study site before inferring global climatic change. If tectonic evidence indicates that the site is shifting into an area believed to be drier, then clearly climatic and associated floral changes might only be local in nature (Parrish *et al.*, 1982).

D. PALAEOGEOGRAPHY, TECTONIC EVENTS AND SEA LEVEL CHANGES

Sea level changes (Haq *et al.*, 1987) may have influenced continental connections, especially those across shallow seaways. They may have also influenced continental margins, erosion patterns and available land for

colonization. The Early Tertiary was characterized by high sea levels which had still fallen little from the mid-Cretaceous maximum. Short term peaks occurred during the Early Eocene and Early Oligocene. Short term troughs also occurred in the early Late Palaeocene, at the Early/Middle and Middle/Late Eocene boundaries and the declining levels of the later Tertiary began with a major fall in the mid-Oligocene.

Figure 2, is the palaeogeographic map of the Lutetian (Middle Eocene, approximately 47 mybp) by Ziegler *et al.* (1983). Changes before and after this time, pertinent to this article, are reviewed below following Ziegler *et al.* (1983), Tiffney (1985a, b), Parrish (1987), Shackleton (1986a) and Pomerol and Premoli-Silva (1986).

1. Late Cretaceous–Middle Eocene

Connection between North America and Europe existed across two possible land bridges during the advanced stages of North Atlantic rifting; one between Greenland and Svalbard in Scandinavia (the DeGeer route), and the other along the Iceland/Faroe ridge from Scotland to southern Greenland (the Thulean route). The latter, being more southerly, would have been more favourable for passage of warm-loving biota but this existed only during the Palaeocene and early in the Early Eocene (Tiffney, 1985a,b; Parrish, 1987). The former, at higher latitudes, might have persisted longer (Tiffney, 1985a,b) but not according to Parrish (1987).

Connections between South America and Africa were probably severed by the early Late Cretaceous (Parrish, 1987). Those between South and North America and between Europe and Africa are complex and may have included temporary connections during the Early Tertiary. A recent assessment of Early Tertiary floras (Taylor, 1988b) suggests that interchange was possible between North and South America during the Late Cretaceous and Early Tertiary. Separations of South America and Australasia from Antarctica are complicated by limited data from Antarctica. The final separation of South America probably occurred between the Late Cretaceous and mid-Tertiary and that of Australasia during the Eocene.

2. Middle Eocene–End Oligocene

The northward movements of Australasia, Africa and India continue. India collided with Asia in the late Eocene (beginning the Alpine orogenic phase). Connections between Africa and Europe may have been established in the Oligocene or Miocene. Temporary connections between South America and North America might have existed. The shallow seaway, the Turgai Straits, between Europe and Asia (Fig. 2, longitudinal belt of light stipple) was breached by the Early Oligocene, but the main European landmass (south-west) was at that time separated from the Russian platform (northeast) by the Polish seaway. As Australasia and South America moved northwards a circum-polar Antarctic current became established, reducing the influence of

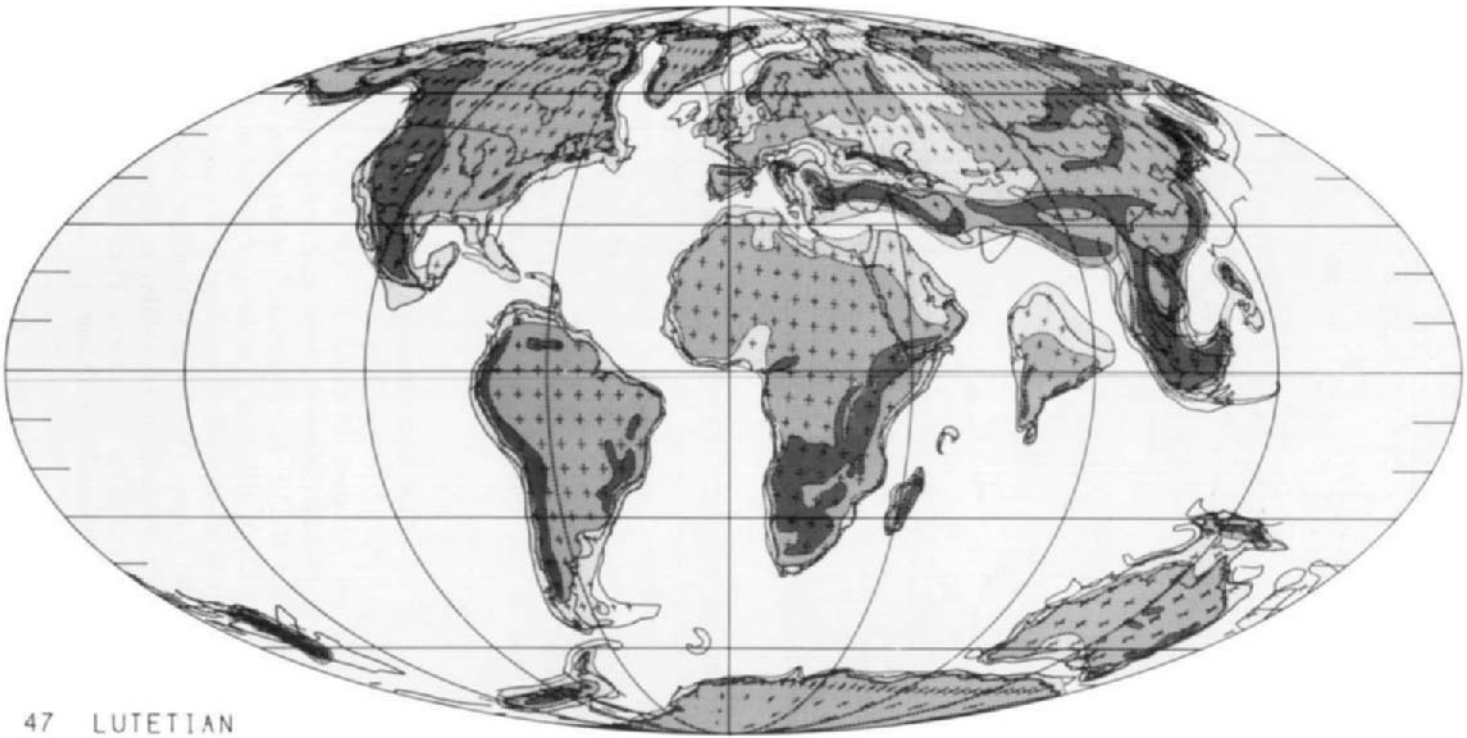

Fig. 2. Palaeogeographic map for the Middle Eocene, Lutetian 47 mybp. Unshaded deep oceans; light shading shallow seas; intermediate shading low land; dense shading high land; + modern latitudes at 5 intervals. Illustration courtesy of A. M. Ziegler. From Ziegler *et al.* (1983) with permission of the publisher.

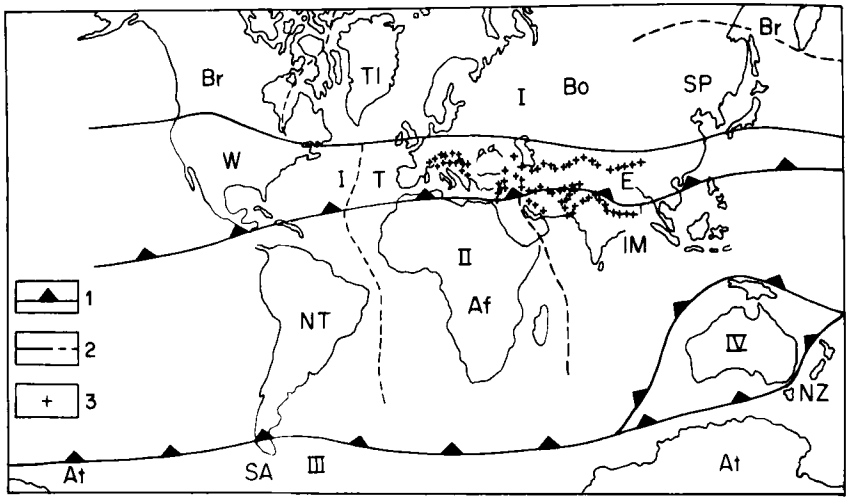

Fig. 3. Palaeofloristic zonation of the Early–Middle Eocene. Boundaries between kingdoms (1) and provinces (2), shore line of Tethys ocean (3). Four kingdoms: I, Holarctic; II, Tropical; III, Notal; IV, Australian. Areas: Af, African; At, Antarctic; Bo, Boreal; IM, Indomalesian; NT, Neotropical; NZ, New Zealand; SA, South American; T, Tethyan. Provinces: in the Boreal area—Br, Beringian; SP, Sakhalin-Primorskian; TI, Tulean; in the Tethyan area—E, Eastern; W, Western. From M. A. Akhmetyev (1987) with permission.

tropical waters at high latitudes. Cold bottom-water circulation resulted as water depths increased with continued rifting. The overall cooling on Antarctica led to ice accumulation. Early stages of these events might have been partly responsible for the global cooling at the end of the Eocene. Prentice and Matthews (1988) concluded that an essentially modern ice budget existed by 40 million years ago (latest Eocene) but that significant export of cold bottom waters from the poles did not occur until about 20 million years ago (Early Miocene).

E. PALAEOFLORISTICS

Cainozoic floristics are discussed in detail by Akhmetyev (1987). His zonation for the Early–Middle Eocene is reproduced in Fig. 3. Four major kingdoms are recognized (I–IV). The Holarctic kingdom has two major areas, one (boreal) in the high, the other (Tethyan) in the mid northern latitudes; each is subdivided into longitudinal provinces which largely reflect the opening of the Atlantic Ocean. The Tropical kingdom has three areas; present day South America, Africa and Indo-Malaysia. Floras from here are poorly known (see Section II.G for references). The Australian and Notal (Southern hemisphere high latitudes) kingdoms are largely extrapolations from continental positions and Cretaceous pollen zonations. They have little basis in known megafossil floras (but see Romero, 1986a,b; Tanai, 1986;

Sections IV.B.1 and IV.F.2, for some details). For this reason the new studies on Australian floras (Section IV.G) are very important.

In the Holarctic kingdom, where more data are available, there is room for discussion concerning the validity of this model. Latitudinal zonation into a northern (boreal) more temperate and a southern (Tethyan) more tropical zone is well established, as is the positioning of these belts in much higher latitudes than similar conditions today (Wolfe, 1985, 1986; Parrish, 1987) (see also Section IV.F). These two zones formed the basis for the Geoflora concept (for discussion and references see Crane, 1987; Tiffney, 1985a; Manchester and Meyer, 1987). According to this concept, fossil floras were assigned comparable status to the "Clementsian" modern communities as "super organisms" composed of strongly interdependent plant groupings which were largely inviolable. The two geofloras, the Arcto-Tertiary (boreal above) and Palaeotropical or Neotropical (Tethyan above) were said to have maintained themselves with little change over long periods of the Early Tertiary, merely shifting northwards or southwards with fluctuating climate. This Geoflora concept has been discredited by Wolfe (1977) after a detailed review (see also Manchester and Meyer, 1987), as it is a very misleading oversimplification. Recent studies concerning vegetational patterns of the Early Tertiary are discussed in Section IV.F.

F. FLORAL COMPOSITION

1. Fungi, Algae and Bryophytes

Stubblefield and Taylor (1988) provide a review of palaeomycology. They give recent references to Tertiary fungal work and discuss significant aspects of fungal interactions with the environment.

I am not concerned here with marine algae. Charophyta are the best represented freshwater algae but usually only gyrogonites are found and they have been used primarily in age correlation of rocks. They do show a major diversification in the Early Tertiary (Grambast, 1974; Tappan, 1980).

The Palaeogene record of Bryophyta is extremely poor. The scattered evidence, reviewed by Miller (1984), includes several epiphytic leafy liverworts, an epiphyllous moss and various other mosses including species of *Sphagnum* from the Canadian Arctic. This record is interesting as peat-forming *Sphagnum* communities are only known from the Quarternary (Collinson and Scott, 1987b) but ancient analogues are sometimes invoked as past coal-forming vegetation (Clymo, 1987).

2. Pteridophytes

Although the pteridophytes (here taken to include lycopsids, sphenopsids and ferns) were ousted from dominance by the diversification of angiosperms (Crane, 1987; Lidgard and Crane, 1988; Fig. 17) they remained very important elements in some Early Tertiary floras. At the K/T boundary in western

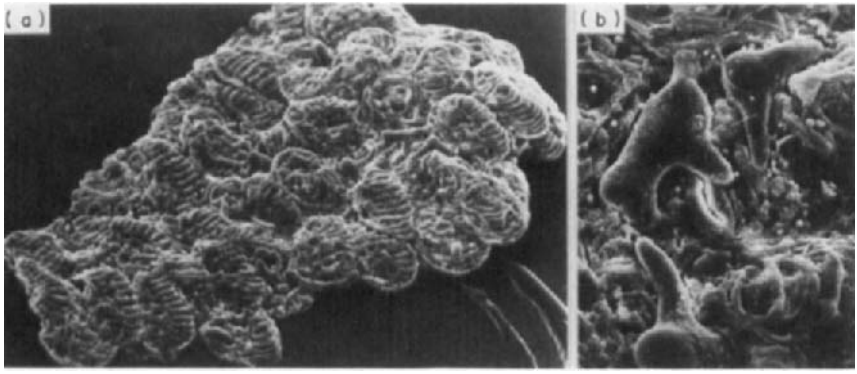

Fig. 4. Fertile fern (Polypodiaceae *s.l.*). *Acrostichum anglicum* Collinson (1978), latest Eocene, southern England. (a) Cluster of sporangia, ×49.5; (b) detail showing paraphyses, ×198. SEM.

North America a fern-dominated vegetation was established during recolonization following ecological disruption (Wolfe and Upchurch, 1986, 1987b). Ferns are important in high latitude Early Tertiary floras (Crane, 1987) and in some communities at lower latitudes (Collinson, 1983a; Collinson and Hooker, 1987). However very few Tertiary ferns, featuring fertile material, have been studied in detail and this is especially the case for the family Polypodiaceae *s.l.* which must have diversified at this time (Thomas, 1985). The only exceptions are species of *Acrostichum* (Collinson, 1978) (Fig. 4). Fern rachides and rhizomes similar to those of modern Dennstaedtiaceae are known from the London Clay flora of England (Fig. 5b,c); from the Palaeocene of England and from the Clarno chert of Oregon (Arnold and Daugherty, 1964; Ribbins and Collinson, 1978; Collinson, 1986a).

Some other fern families, e.g. Schizaeaceae and Osmundaceae, were well established prior to the Tertiary (Stewart, 1983), but nevertheless are known from few well-documented Tertiary examples. One exception is the *Lygodium* described by Manchester and Zavada (1987). Fertile and sterile foliage were found in organic connection (Fig. 6a). One species was widespread in Europe and North America and a very similar form occurred in South America and Australasia. This excellent fossil record of *Lygodium* demonstrates that forms with strongly dimorphic foliage typified the Early Tertiary contrasting with most modern forms which are only weakly dimorphic. The fossils probably represented climbing ferns like the modern species (Manchester and Zavada, 1987; A. C. Rozefelds, personal communication, 1988, on Australasian material).

Other Schizaeaceae (Chandler, 1955; Barthel, 1976; Collinson, 1986a) are also abundant in the European Early Tertiary (Fig. 6b,c). Osmundaceae have an extensive Tertiary record which includes foliage and fertile material (Miller, 1971; Barthel, 1976) and a recently described trunk (Tidwell and Parker, 1987).

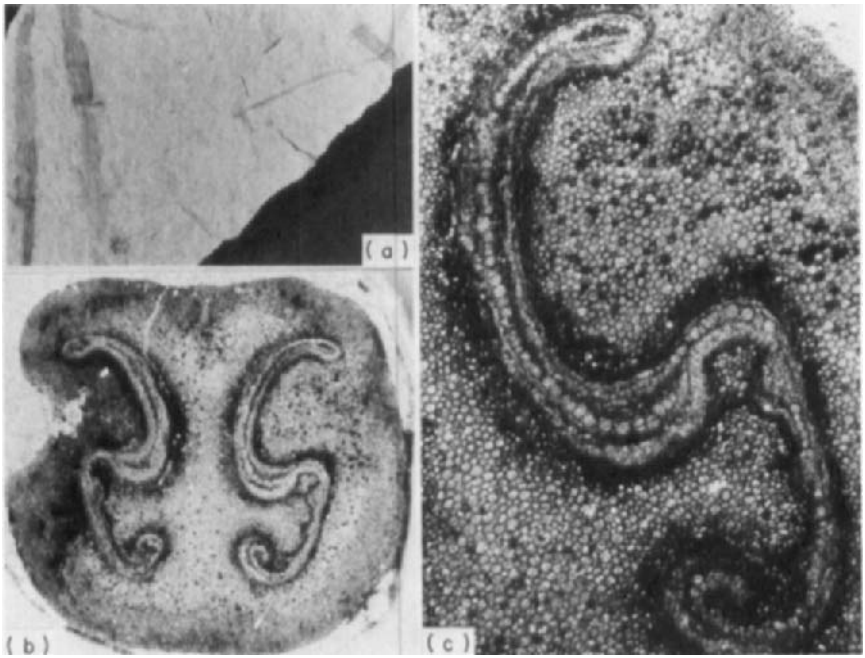

Fig. 5. (a) *Equisetum* fragments including axes with leaf sheaths, Eocene, USA, ×0.97; (b) polished transverse surface of pyrite permineralized fern rachis from the early Eocene London Clay flora, southern England (Ribbins and Collinson, 1978), ×9.7; (c) detail of (b) ×24.25.

Heterosporous water ferns have an excellent Tertiary record (Collinson, 1980a, 1988a; Mai, 1985) including megaspores, microspore massulae and whole fertile and sterile plants. Megaspores of *Azolla* (Fig. 7a,b) are particularly well documented (Fig. 8) and show clearly a pattern of decreasing "float" number through time. They also show that mechanisms for attaching microspore massulae to megaspores with grapnel spines have existed at least since the Palaeocene (Fig. 7b). The numbers of species represented by whole plants, including one currently being studied (Fig. 9) provide an excellent opportunity to study the evolution of this plant group (Collinson, in progress).

Lycopsids are well represented in European and North American Palaeocene floras by megaspores (Collinson *et al.*, 1985; Collinson and Hooker, 1987; Crane, 1987). Some forms similar to those of modern *Selaginella* have a distinctive wall ultrastructure (Fig. 10) which occurs in some modern *Selaginella* species (Minaki, 1984) and may have conferred resistance to desiccation (M. E. Collinson, in preparation). Megaspores similar to those of modern Isoetales have attached monolete microspores (Fig. 11a). They occur *in situ* in plants named *Isoetites* (Fig. 11b; Hickey, 1977; Melchior, 1977). These plants seem to represent an extinct group related to modern Isoetales which

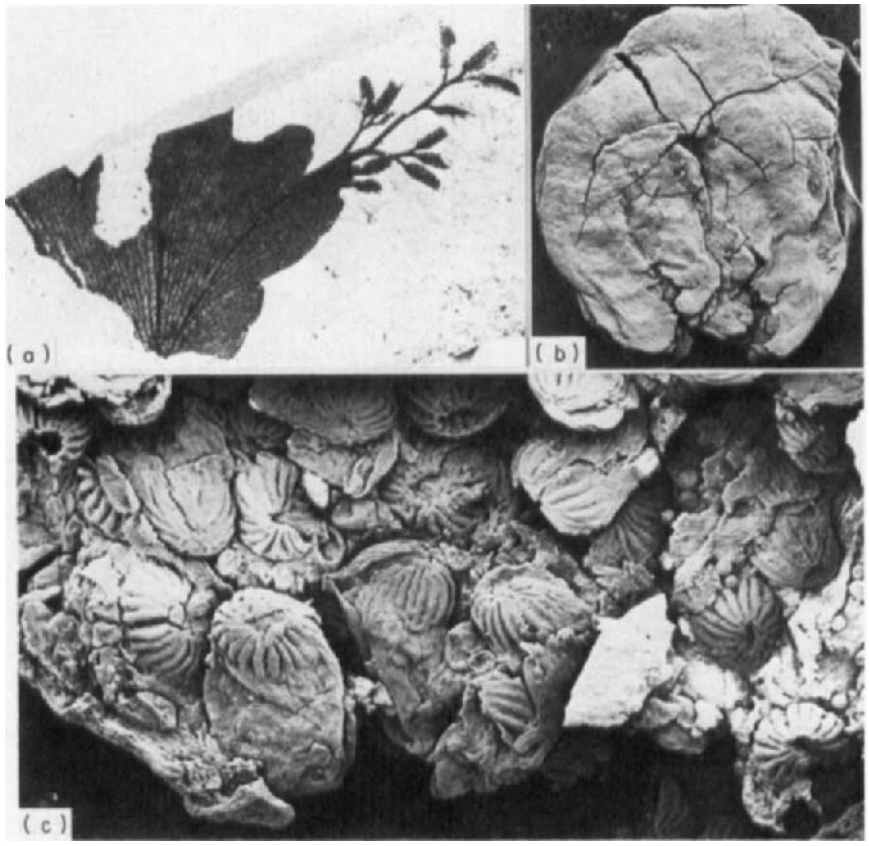

Fig. 6. Fertile ferns, Schizaeaceae. (a) *Lygodium* showing fertile and sterile foliage, late Eocene, USA, × 1.5. Illustration courtesy of S. R. Manchester. From Manchester and Zavada (1987) with permission of the publisher. © 1987 by the University of Chicago. All rights reserved. (b),(c) *Anemia* from the Palaeocene of southern England (Collinson, 1986a); (b) single fertile pinnule with recurled margins, sporangia visible at torn base, SEM, × 15; (c) detail of sporangia from another specimen, SEM, × 100.

was widespread in the wetland floras of the Northern hemisphere Palaeocene (Collinson, in progress).

Herbaceous sphenopsids indistinguishable from modern *Equisetum* in vegetative form have existed since early in the Mesozoic or Late Palaeozoic (Collinson, 1988a). They occur in the Tertiary (Fig. 5a) but there are few detailed studies, e.g. Brown (1975), and I know of no comprehensive treatment of Tertiary fossils of this group.

3. Conifers

Conifers have an extensive fossil record from the Carboniferous onwards with many modern families being recognized in the Mesozoic (Florin, 1963; Stewart, 1983). The Early Tertiary marked the extinction of several Creta-

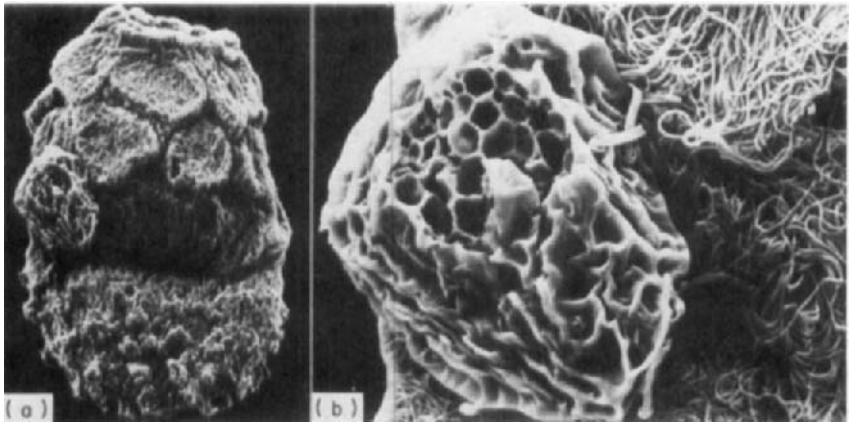

Fig. 7. Water fern, *Azolla teschiana* Florschutz from the Dutch Palaeocene (D. J. Batten collection). (a) Megaspore with attached microspore massula at left, × 114; (b) detail of massula showing attachment by grapnel spines most of which arise from the surface adjacent to the megaspore, × 475. SEM. (See also 1 on Fig. 8.)

ceous types and the appearance of modern forms, especially of Taxodiaceae which includes the swamp cypresses (Wolfe and Upchurch, 1986, 1987b; Spicer *et al.*, 1987). Taxodiaceae were clearly dominant trees especially at high latitudes and in wetland sites in the Late Cretaceous and Early Tertiary of the northern hemisphere where they often contributed to coal formation (Fowler *et al.*, 1973; Basinger, 1981, 1984, 1987a,b,c; Collinson and Scott, 1987a,b; DiMichele *et al.*, 1987; Francis and McMillan, 1987; Collinson, 1988a). *Metasequoia milleri* (Taxodiaceae) is one of the most completely reconstructed Tertiary conifers (Basinger, 1981, 1984; Rothwell and Basinger, 1979). It is known from permineralized material of leaves, stems, wood, pollen cones and seed cones (Fig. 12) and is largely indistinguishable from modern *M. glyptostroboides*. The single modern species is restricted to a small area of China but the genus was widespread in the Northern hemisphere in the Early Tertiary. Other modern Taxodiaceae have restricted distributions in either western or eastern North America or eastern Asia, e.g. *Sequoia*, *Sequoiadendron*, *Glyptostrobus*, *Taxodium*. All were widespread in the Northern hemisphere during the Early Tertiary not suffering restriction to their modern relict distributions until the Late Tertiary or Quaternary (Sporne 1965; Stewart, 1983; Axelrod, 1986).

The modern Southern hemisphere conifer families Araucariaceae and Podocarpaceae, especially the former, also have extensive fossil records in the Mesozoic. The Araucariaceae were formerly widespread in the Northern hemisphere (Florin, 1963; Stewart, 1983) but by the Early Tertiary had become restricted to the Southern hemisphere in Australasia and Argentina (Bigwood and Hill, 1985; Hill and Bigwood, 1987). Like the Northern hemisphere Taxodiaceae, Araucariaceae also now have a relict distribution, much

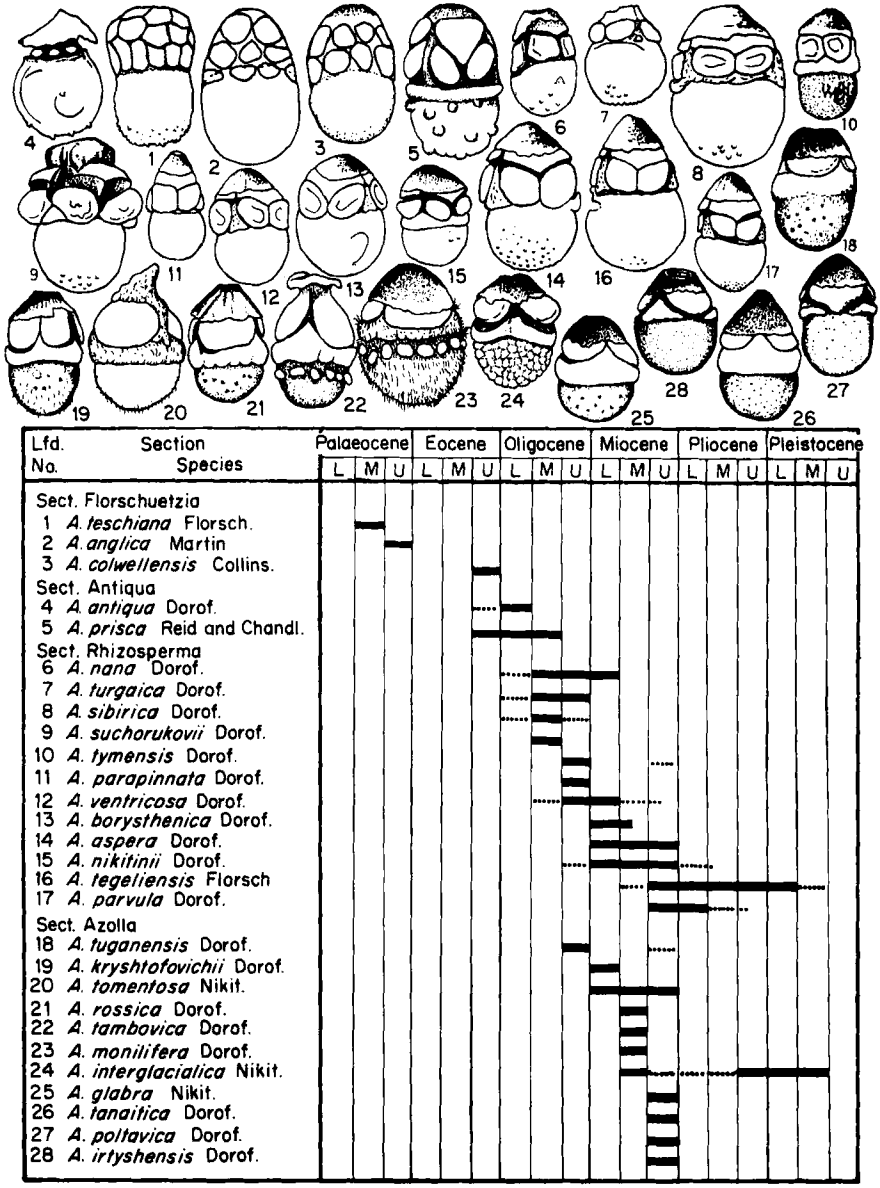

Fig. 8. Stratigraphic distribution of the water fern *Azolla* from Europe, western Asia and Siberia showing megaspore morphology (see also Fig. 7). From Mai (1985) with permission of the publisher, VEB Gustav Fischer Verlag, Jena.

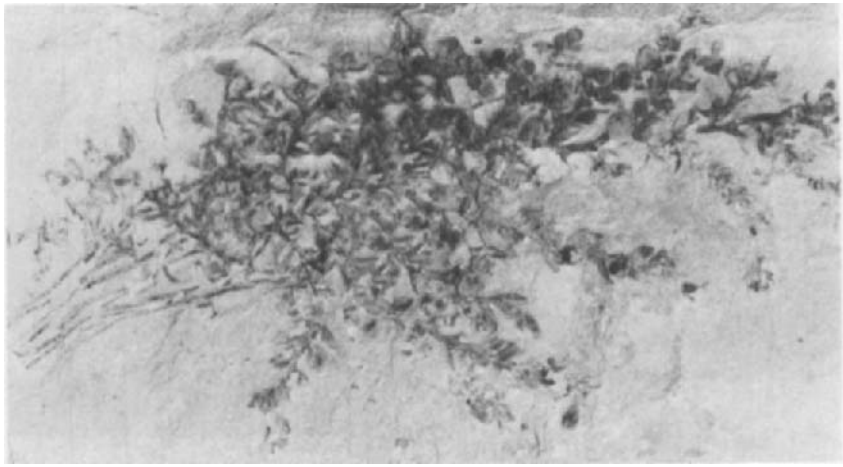

Fig. 9. Whole plant of an extinct species of *Azolla* (water fern), Eocene, USA, Smithsonian Institution, Washington, Paleobiology Department specimen number 372415, × 3.

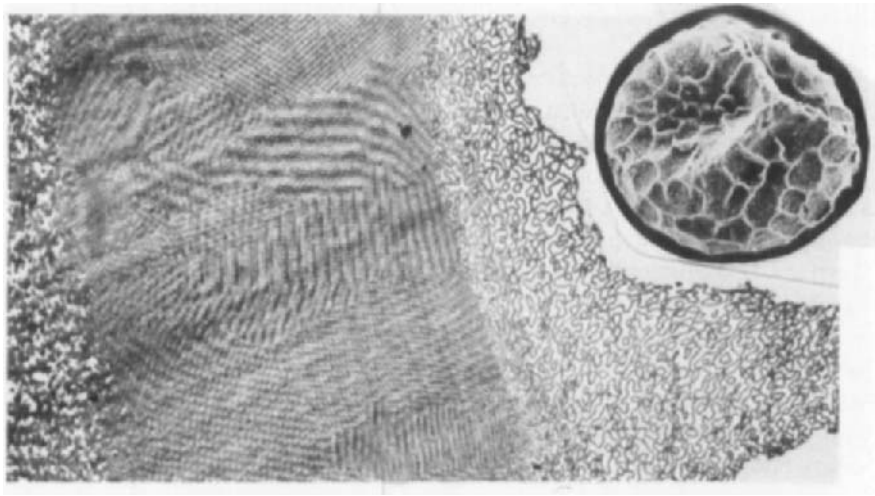

Fig. 10. Palaeocene lycopsids. *Erlansonisporites* megaspore, southern England (Collinson *et al.*, 1985). TEM showing complex wall ultrastructure similar to that of some modern *Selaginella* species, external surface at right, × 2137.5; inset shows megaspore SEM, × 57. TEM preparation by Tony Brain.

reduced from their Early Tertiary range when, for example, both *Araucaria* and *Agathis* were present in Tasmania. Podocarpaceae have a mainly Southern hemisphere fossil record. Hill (1989) describes several species of *Phyllocladus* from the Early Tertiary of Tasmania. Greenwood (1987) recorded species from a further five modern genera in the Eocene of Anglesea, Austra-

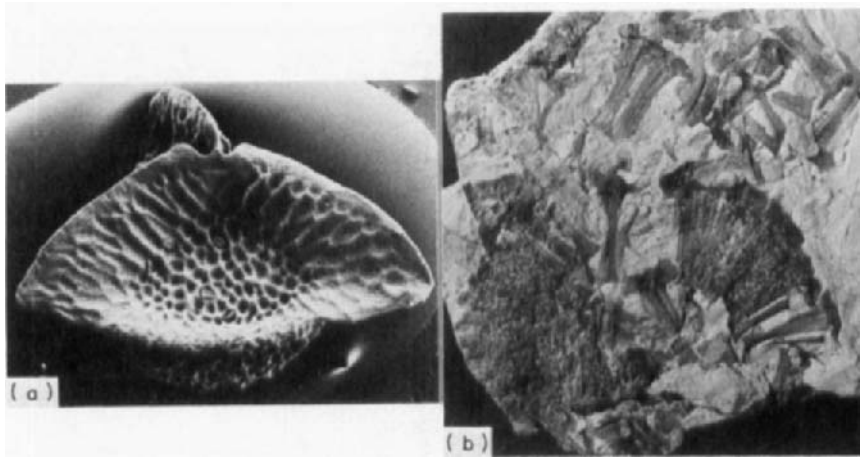

Fig. 11. Palaeocene lycopsids. (a) *Minerisporites* megaspore with adherent monolete microspores on the surface (Collinson *et al.*, 1985), from southern England, SEM, × 66.5; (b) *Isoetites* plant showing sporophyll bases (see Hickey, 1977) and megasporangia containing fragmentary megaspores as in (a). M. E. Collinson and P. R. Crane collection, USA Field Museum of Natural History, Chicago, Geology department specimen number PP33936, × 1.425.

lia; a diversity which is far greater for any one site than exists in modern vegetation.

Some Pinaceae have a very extensive and diverse Tertiary fossil record which has permitted detailed evolutionary analyses. Such is the case for *Pinus* (Mai, 1986b; Axelrod, 1986). *Pinus* fossils are known from foliage and cones some of which are permineralized (Fig. 13) preserving exquisite cellular detail (Stockey, 1984). Figure 14 shows the inferred stages in *Pinus* evolution indicating that modern groupings of the genus were established in the Early Tertiary. Subsequent diversifications may be related to various environmental changes (Axelrod, 1986; see Section II.A–D). Mai (1986b) emphasized an endemic development in Europe of Early Tertiary *Pinus* species whose relationships lay with modern forms from Europe and Asia rather than North America. "Mummified" material of many conifers, including Taxodiaceae and Pinaceae (Fig. 15), from the Arctic fossil forests (Basinger, 1987a,b,c) offers enormous potential for future study of Early Tertiary conifers and coniferous forests.

4. *Cycads and* Ginkgo

Although cycads have an extensive fossil record in the Mesozoic their Tertiary records are rather poor. Again, like some conifers, they show very restricted modern distributions but details of the first occurrences of modern genera of cycads and their distribution in the Tertiary are generally lacking. Exciting new material from Australia (Hill, 1978, 1980) provides a fossil record and evidence of a more extensive past distribution for two modern genera, *Bowenia* and *Lepidozamia*. One extinct genus has also been described.

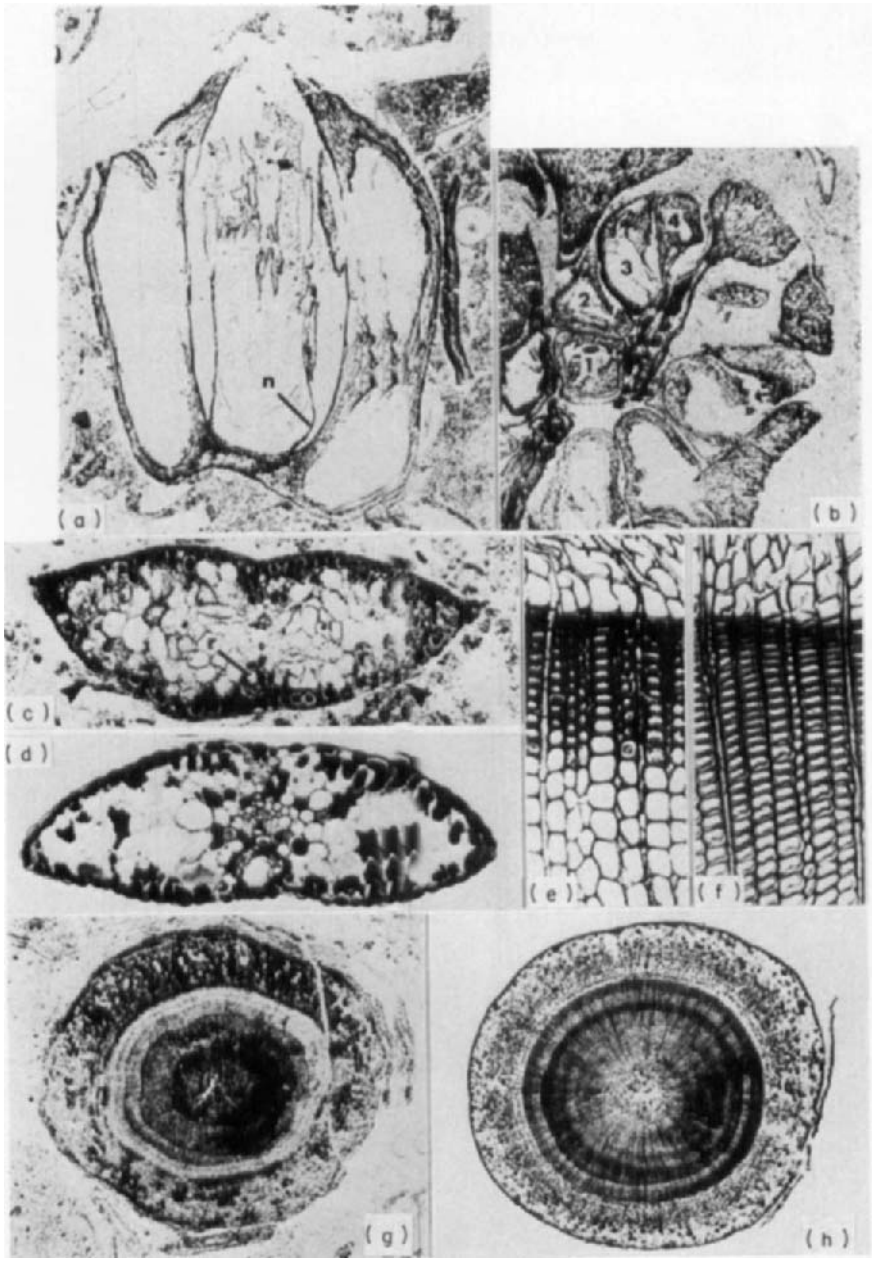

Fig. 12. Elements from the taxodiaceous conifer *Metasequoia milleri* from the Middle Eocene of Canada. (a),(b),(c),(e),(g) Permineralized fossil material; (d),(f),(h) Modern Taxodiaceae for comparison. (a) Longitudinal section of seed showing central body with nucellus (n) and lateral wings, × 13.58; (b) oblique section of seed cone showing four seeds fused to upper scale and two to lower scale, × 5.335; (c),(d) transverse sections of leaves, (d) *Taxodium distichum*, (c) × 55.29, (d) × 67.9; (e–h) transverse sections of woody twigs, (f),(h) *Metasequoia glyptostroboides*. (e),(f) showing details at a growth ring boundary, (e) × 61.11, (f) × 84.39, (g) × 10.67, (h) × 10.185. Illustrations courtesy of J. F. Basinger. From Basinger (1981, 1984) with permission.

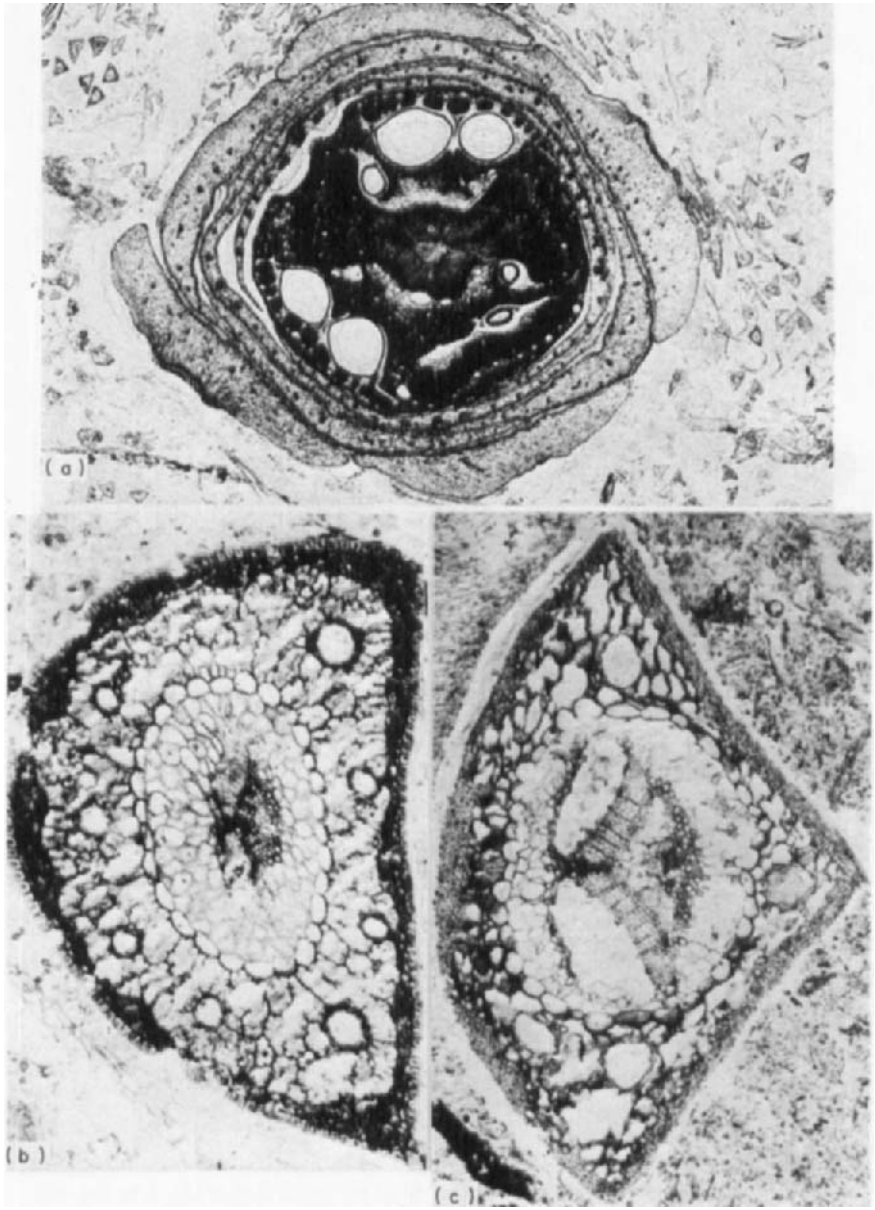

Fig. 13. Permineralized *Pinus* from the middle Eocene of Canada. These fossils have been assigned to three distinct species. (a) Transverse section of seed cone showing two seeds per cone scale, note the numerous leaves in the matrix around the cone, × 4.05; (b),(c) transverse sections of leaves from a two-needled (b) and three-needled (c) pine, × 60.3. Illustrations courtesy of R. A. Stockey. From Stockey (1984) with permission of the publisher.

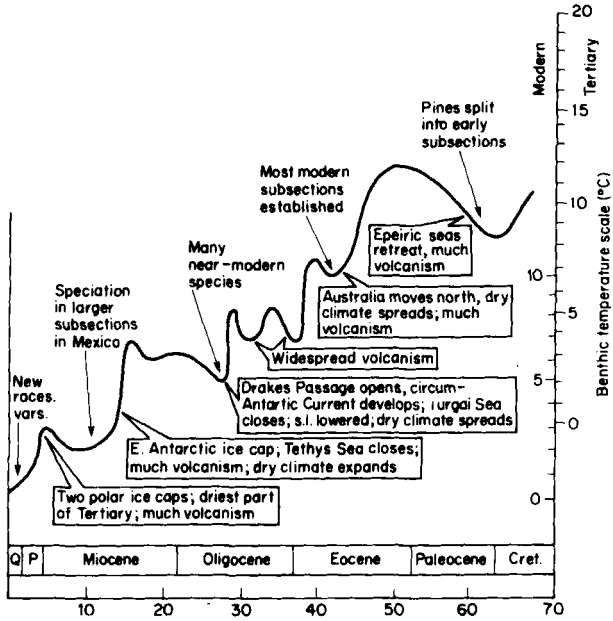

Fig. 14. Inferred correlation of environmental changes with evolution of western American pines. From Axelrod (1986) with permission.

Barthel (1976) named cycad foliage from the European Middle Eocene *Eostangeria* as it shared features with modern African *Stangeria*. All these records are based on cuticle characteristics and we should look forward to the recognition of reproductive structures which seems very possible in the Australian deposits.

Plants similar to the living *Ginkgo biloba* may have existed in the Mesozoic and were apparently widespread in Northern hemisphere Tertiary especially at high latitudes (Stewart, 1983; Samylina and Chelebayeva, 1986). However, much of this record consists only of leaves. New Palaeocene material (Fig. 16), from North America (Crane *et al.*, 1990) is exciting because of the association of leaves and seeds (Fig. 16a). Moreover some seeds have their fleshy layer preserved (Fig. 16b), thus confirming this aspect of similarity with modern *Ginkgo*.

5. *Angiosperms*

Early Tertiary floras are dominated by angiosperms (Muller, 1981, 1985; Dilcher and Crepet, 1985; Friis *et al.*, 1987). During the late Early and early Late Cretaceous the angiosperms came to dominate land vegetation over much of the world (Crane, 1987; Lidgard and Crane, 1988). Although this replacement occurred later at higher latitudes (Spicer *et al.*, 1987) it was complete by the Early Tertiary. Figure 17 illustrates this replacement as documented by statistical analysis of leaf floras (Crane, 1987, 1989a; Lidgard and

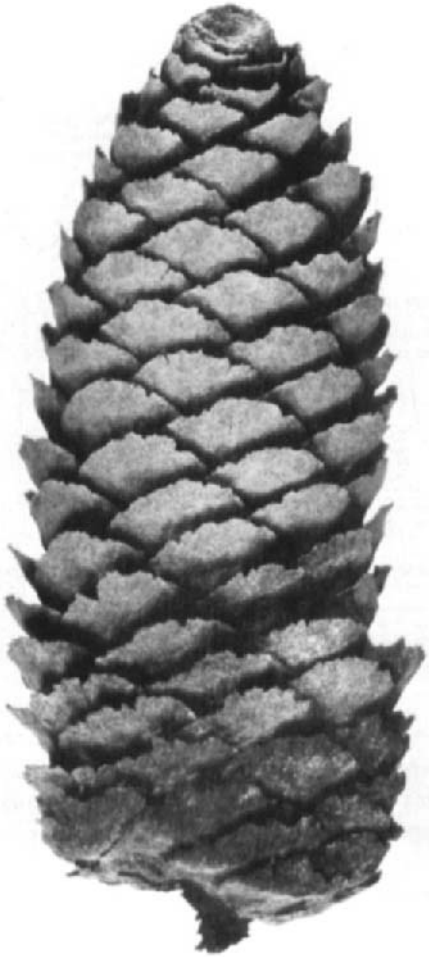

Fig. 15. Exceptional preservation in a "mummified" *Picea* cone from a Late Eocene fossil forest in the Canadian high arctic (see Basinger, 1987a,b,c), × 1.2. Illustration courtesy of J. F. Basinger.

Crane, 1988). Diversity within plant communities (measured by species richness) began to increase following the first major diversification of angiosperms and continued to do so throughout the Early Tertiary (Knoll, 1986; Knoll and Niklas, 1987).

The Early Tertiary marked a second major radiation and diversification of angiosperms (Friis *et al.*, 1987; Dilcher and Crepet, 1985; Muller, 1981, 1985). Many fossils are indistinguishable from equivalent organs of modern plants. Most can be accommodated within modern families and many in modern genera. Collinson (1986b) discussed the nomenclatural problems created by this procedure; see also other papers in Spicer and Thomas (1986).

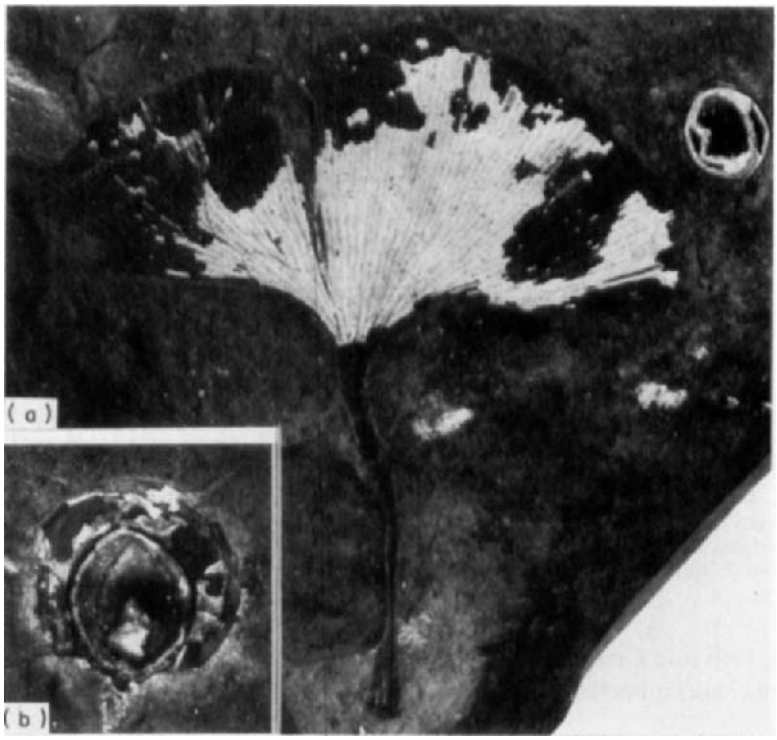

Fig. 16. Palaeocene *Ginkgo*, USA (Crane *et al.*, 1990). (a) Leaf, showing bilobation, with a seed, lacking fleshy tissues, on the same block (top right), × 1; (b) seed with evidence of fleshy outer layer, × 1.5. Illustrations courtesy of P. R. Crane.

The origins of many modern families can be traced to the Early Tertiary (for examples see Section IV.A).

The literature on Early Tertiary angiosperms is too extensive to permit a general review. The references cited in Section IV include many of the more important recent papers. Several papers on specific topics are worth mentioning here. Wetland plants and their roles in ancient lakes are reviewed by Collinson (1988a) and Mai (1985). Floristic similarity between Europe, Asia and North America is reviewed by Tiffney (1985a,b). Monocotyledons are reviewed by Daghlian (1981) and seagrasses are described by Lumbert *et al.* (1984). The literature is dominated by works dealing largely with fruits and seeds (e.g. Collinson, 1983b; Mai, 1987a,b; Kirchheimer, 1957, Tiffney, 1981b) or leaves (e.g. Wolfe, 1977; Hill, 1982; Tanai, 1986; Axelrod, 1987) and some studies integrate both (e.g. Takhtajan, 1982, 1984; Mai and Walther, 1985; Manchester and Meyer, 1987). Many trunk woods (e.g. Bande and Prakash, 1984; Suss, 1986; references in Wheeler *et al.*, 1987; Duperon, 1988) and some twig woods (e.g. Wilkinson, 1988) have also been described. More recently an extensive literature on flowers has accumulated

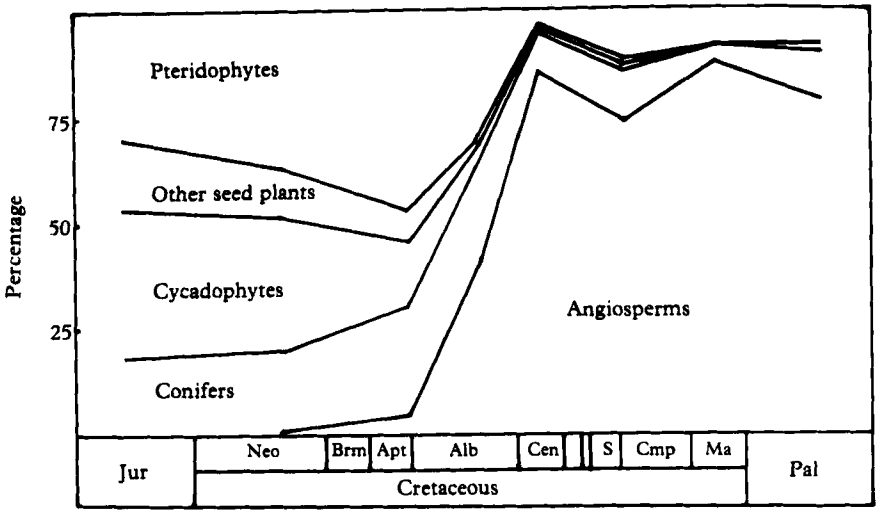

Fig. 17. Changes in the proportions of plant groups in leaf floras from the Jurassic (Jur) to Palaeocene (Pal), Neo, Neocomian; Brm, Barremian; Apt, Aptian; Alb, Albian; Cen, Cenomanian; S, Santonian; Cmp, Campanian; Ma, Maastrichtian. From Crane (1987) with permission.

(e.g. Friis and Crepet, 1987; Crepet, 1985) and integrated studies of "whole plants" are enumerated in Section IV.A.

Palaeocene. Amongst the angiosperms of the northern hemisphere middle palaeolatitudes in the Early Palaeocene are palms and evergreen dicots including protolauraceans, other Laurales, Euphorbiaceae, Tiliaceae and deciduous dicots of the Platanaceae, Betulaceae and Juglandaceae. At higher palaeolatitudes the deciduous taxa were more dominant and included Hamamelidaceae and Trochodendrales (Crane, 1987, 1989a; Wolfe and Upchurch, 1986, 1987b; Upchurch and Wolfe, 1987; Spicer *et al.*, 1987). Late Palaeocene floras were a little more diverse (Collinson 1986a; Collinson and Hooker, 1987; Mai, 1987b; Crane *et al.*, 1990), though in individual fossil floras species counts are generally less than 50. Crane *et al.* (1990) listed over 20 references to northern hemisphere Palaeocene floras, to which could be added Collinson (1986a), Mai (1987b), Crane (1988) and Crane *et al.* (1988). A revision of the Palaeocene flora of Mull, Scotland, has recently been completed (Kvaček and Boulter, 1989). Mai (1987b) usefully reviewed the fruits and seeds from the European Palaeocene.

In the Southern hemisphere *Nothofagus* is an important element of Palaeocene floras (Tanai, 1986; Romero, 1986a,b; Case, 1988).

Eocene and Oligocene. Eocene floras are, in contrast to those of the Palaeocene, very numerous and diverse. Over 300 species are described from the famous London Clay flora of southern England (summarized by Collinson, 1983b; Collinson and Hooker, 1987). Over 50 Eocene plant bearing

levels are recorded in southern England and their floras are summarized in Collinson and Hooker (1987). An extensive data base on fruits and seeds is accumulating through researches on European Eocene and Oligocene material (see Section II.G) which will be of considerable value in understanding patterns of evolution when applied in studies like those described in Section IV.B.2 for *Stratiotes*. In North America, in contrast, most of the fossil floras are represented by assemblages of leaves. These provide valuable data for leaf physiognomic analyses and hence for palaeoclimatic interpretations (see Section IV.F). Both data sets are of value in interpreting plant community evolution and global vegetational patterns provided that fossils are studied in their taphonomic and sedimentological context and that time correlation is relatively accurate (see Section IV.C–F). Information from rare "whole plants" (see Section IV.A) may be integrated with these abundant data from isolated organs to provide good evidence of the evolution of modern plant groups, e.g. Manchester (1987) for Juglandaceae.

G. GLOBAL COVERAGE

A great deal of our data on Early Tertiary floras comes from the Northern hemisphere, from North America, Europe, and northern Asia. In comparison, other regions, especially the Southern hemisphere, have provided little data. In this section I aim to cite review articles and examples of recent papers to guide the reader into the relevant literature for each area. I comment briefly where appropriate.

Southern England: Collinson and Hooker (1987), Chandler (1964).
Scotland: Crane *et al.* (1988), Crane (1988), Kvaček and Boulter (1989).
France: Vaudois-Mieja (1986), Grambast (1962).
Germany (East and West): Ruffle (1976), Kirchheimer (1957), Collinson (1988b), Schaarschmidt and Wilde (1986), Schaarschmidt (1986, 1988), Mai and Walther (1985), Mai (1987a,b).
U.S.S.R.: Takhtajan (1982, 1984; the first two in a series of volumes revising the entire Tertiary floras of the area); Mai (1986a; bibliography of the works of Dorofeev); Makulbekov (1983), Iljinskaja (1988).
Bulgaria: Palamarev (1973).
North polar regions: inclusing Alaska, Greenland, Canada, Kamchatka: Axelrod (1984), Spicer *et al.* (1987), Chelbayeva and Akhmeteva (1983), Basinger (1987a,b,c), Francis and McMillan (1987).
USA: Lamotte (1952), Wolfe (1977, 1978, 1985, 1987), Crane *et al.* (1990), Manchester (1981), Manchester and Meyer (1987), Wolfe and Tanai (1987), Wolfe and Wehr (1988), Tiffney (1981b), Mazer and Tiffney (1982), Wing (1987), Taylor (1988b), McClammer and Crabtree (1989).

Canada: (see also USA), Basinger (1984, 1987a,b,c), Stockey (1984), Cevallos-Ferriz and Stockey (1988a,b).

South polar regions: Axelrod (1984), Romero (1986a,b), Tanai (1986), Francis (1986), Askin (1988a,b), Case (1988).

Australasia: Axelrod (1984), Basinger and Christophel (1985), Christophel *et al.* (1987), Tanai (1986), Hill and Read (1987), Hill (1987, 1988a,b), Hill and Christophel (1988); (see Section IV.G for detailed discussion of exciting new work on this area).

Africa: Boureau *et al.* (1983), Bown *et al.* (1982), Rao and Kumaran (1988).

India: Prakash (1973; general), Awasthi (1982; Himalaya region), Bhattacharya (1983; Meghalaya), Lakhanpal *et al.* (1984; Cutch).

Deccan Trap permineralized flora: Basinger and Rothwell (1977, pp. 1985–1986, reference list), Friis and Crepet (1987, p. 161 and note their comment that some of these floras may be latest Cretaceous), Bande and Prakash (1982, 1984, 1986), Paradkar and Patki (1987, reference list).

Iran: Makulbekov (1984).

Central America: Graham (1987).

South America: Romero (1986a,b), Tanai (1986).

South East Asia: Bande and Prakash (1986).

China: Hsu (1983), Guo (1985), Academia Sinica (1978).

Japan: Huzioka and Takahasi (1970), Tanai (1972; much of the Japanese Tertiary is Neogene).

III. METHODS OF STUDY

Initially field collecting should be undertaken with care to avoid bias in the sample, e.g. it should not favour spectacular material or just a single horizon to the detriment of the total flora (see Scott and Collinson, 1983; Ferguson, 1985, pp. 172–184; Shute and Cleal, 1987). A lithological log (e.g. Figs 39 and 41) with sedimentological and biotic information should be produced. If collecting megafossils a sample should be retained for palynological investigation. Ensure sufficient material is collected for the purposes intended, e.g. large sample size for leaf physiognomic study (Wolfe, 1971). In indurated strata collecting will be by splitting bedding planes (or occasionally surface picking). In soft rock, bulk sediment samples may also be collected for disaggregation using sieving techniques allowing total recovery of fossil material.

In order to undertake any serious investigation of Tertiary fossils a modern comparative collection is necessary. This may be a herbarium, a fruit and seed collection, a slide collection of prepared sections of woods or a slide collection of cleared leaves with cuticle preparations. Most Tertiary workers prepare much of their own comparative material (e.g. Figs 12d,h, 18a,d, 34a, and 50a,d,g) but often rely on the collections in botanic gardens and

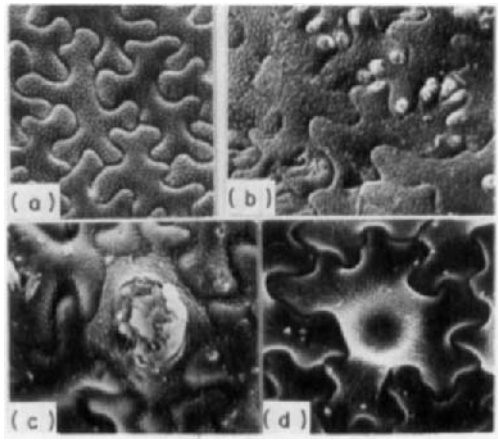

Fig. 18. Diagnostic seed surface cell patterns in modern and fossil Nymphaeaceae (waterlilies) (Collinson, 1980b). (a) Modern *Brasenia*, × 125; (b) Fossil *Sabrenia*, × 500; (c) fossil *Brasenia*, × 300; (d) modern *Cabomba*, × 175. All SEM (see Fig. 33).

museums as their source of supply and for reference collections, e.g. of wood slides, prepared by plant anatomy laboratories.

Fossil plants in the Early Tertiary may be preserved as impressions, compressions, permineralizations or petrifactions. For a detailed account of plant fossil preservation consult Schopf (1975). Each requires a special means of investigation as does each of the different organ types.

Petrified material must be studied by means of ground thin sections but permineralized material may be investigated using the peel technique (Joy *et al.*, 1956; Stewart and Taylor, 1965; Matten, 1973, pp. 169–170; Basinger, 1981; Chitaley, 1985). Wheeler *et al.* (1987) describe techniques recently applied in wood anatomical studies including the use of computer identification keys and computer analyses of tissue proportions. Permineralized material, as it may yield full anatomical information (Figs 5b,c, 12, 13, 22b, 28, 36, 38 and 43b), can be considered the most valuable plant fossil material. However, investigation by sectioning is not without problems. Although the peel technique means that scarcely any material is lost during sectioning, the required three dimensional reconstruction is a laborious process. (Computer technology is currently being applied to this problem, e.g. Niklas and Boyd, 1987.) Also if surface cell shapes are important, as they often are in seeds (Figs 18 and 34) these can be difficult to reconstruct from sections.

Some of the well preserved compression material (e.g. Figs 7, 10, 15, 19, 26, 31, 32, 35c and 44), may therefore be as valuable as permineralized material. Compression fossils of charcoalified nature (Fig. 19); fruits and seeds (Fig. 35c); megaspores (Figs 7 and 10), etc. can best be studied using scanning electron microscopy after cleaning in hydrofluoric acid under a specially adapted

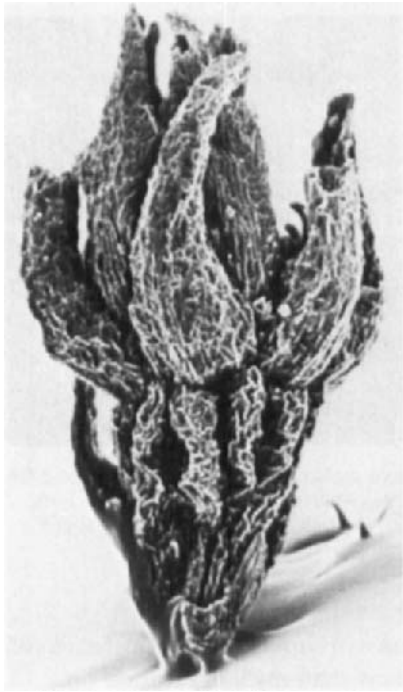

Fig. 19. A charcoalified flower, *Scandianthus* (Saxifragaceae) from the late Cretaceous, × 75 (Friis, 1985b). Illustration courtesy of E. M. Friis.

fume hood. Resin embedding and thin sectioning for light or transmission electron microscopy (Fig. 10) may also be utilized. Leaf cuticles may be studied using SEM and TEM but initially are investigated from preparations made for light microscopy (Figs 50a,b). Venation patterns are studied using cleared leaves (Figs 50d,g). Well preserved fossil leaves (often described as mummified) may reveal equally fine venation detail (e.g. Fig. 32).

Techniques used in the study of leaves are described by Dilcher (1974) and Basinger and Christophel (1985). Those used in the study of compression material of flowers are described by Daghlian *et al.* (1980) and Crepet and Daghlian (1980). A variety of techniques and conservation methods may be required in any one investigation (e.g. Collinson, 1983b; Schaarschmidt, 1985; Manchester, 1986). Further details on investigatory techniques, collecting, handling, conservation and curation are given in Scott and Collinson (1983); Collinson (1987, 1990); Kummel and Raup (1965); Wagstaffe and Fidler (1968) and Shute and Cleal (1987). Papers in DiMichele and Wing (1988) describe a variety of techniques for handling and analysing plant palaeoecological data. Papers in Gastaldo (1989) document taphonomic methodology. Other relevant references are cited in Section IV.B.

IV. MAJOR INNOVATIONS FROM RECENT STUDIES

A. "WHOLE PLANT" BIOLOGY

Reconstruction of fossil plants should ideally be based on organic connections between different fossil organs but this is sadly rare in the fossil record. Alternative approaches involve extrapolation from common occurrence of unusual anatomical features and from scars of former attachment. Perhaps the most reliable alternative is consistent, recurrent association of the different organs together at several, different fossil localities, and/or in thin or restricted fossiliferous horizons, combined with the absence of other organs likely to have been derived from the same group of plants. Gastaldo (1989, pp. 61–83) discussed taphonomic evidence which may affect this method. I still consider it reliable in the cases mentioned below where careful attention has been given to taphonomic bias.

All these approaches have been used on Early Tertiary floras in recent years. We now have more than ten reconstructed Tertiary flowering plants, and a few pteridophytes and conifers.

These rare reconstructed plants provide a much firmer basis for phylogenetic studies than the abundant isolated organs. They often demonstrate unexpected combinations of characters in one plant which are now segregated in modern plant groups (e.g. leaves like one modern genus and fruits like another). Alternatively they may confirm relationships with a single modern genus previously only inferred from isolated organs (e.g. leaf or seed). They also provide information on whole plant biology for use in functional studies, e.g. pollination and dispersal (see Section IV.E) and in reconstruction of ancient plant communities (see Section IV.C). Sadly it is unlikely that there will ever be sufficient "whole plant" information to supersede the data from isolated organs. No one would dismiss evidence from isolated fruits and seeds when investigating dispersal strategies; that from isolated flowers in pollination biology; or that from isolated leaves in physiognomic studies. The "whole plants", however, do provide vital "key data" with which studies of isolated organs may be linked to give a more complete evolutionary picture. "Whole plants" are especially crucial to our understanding of the segregation of diagnostic characters in monophyletic groups during early stages of diversification. In view of the rarity, importance and current increase in our knowledge of reconstructed Tertiary plants major examples are detailed below.

1. Monocotyledons

Although palms are frequently represented in the fossil record by *in situ* stumps (e.g. Fig. 38), leafy crowns and various leaves, fruits and seeds (Daghlian, 1981) I know of no reconstructed examples. Schaarschmidt and Wilde (1986) and Collinson (1988b and in progress) have good evidence for flowers

(see Fig. 44) and fruits in the Eocene lake of Messel, West Germany having been derived from the same plants.

Cevallos-Ferriz and Stockey (1988b, p. 1111) may have vegetative remains derived from the same parent plants as the Araceae subfamily Lasioideae fruits and seeds which they described. In the Princeton chert, a permineralized peat-bed, the chances of proving organic connection are extremely high (see also Section II.F.3, *Metasequoia* from the same locality).

Biradar and Bonde (1988; personal communication) described a permineralized fossil monocotyledonous plant with organic connection between a broad, rhizomatous stem (previously named *Cyclanthodendron*) and an upright leafy pseudostem with ensheathing leaf bases (named *Musocaulon*) and leaf petioles (named *Heliconiaites*). Anatomical similarity in the axes strongly suggested that an inflorescence axis bearing fruits (named *Tricoccites trigonum*) was also borne by this plant. This is the first reconstructed Tertiary monocotyledon and the plant apparently combines features of modern Pandanaceae and Cyclanthaceae. Aerenchyma and stellate pith across nodes in *Heliconiaites* may indicate a marginal aquatic habitat. The Deccan Traps permineralized floras, from which these organs have been described on many former occasions (see references in Section II.G) have considerable potential to provide further examples of "whole plants".

2. Trochodendrales and Cercidiphyllales

Four reconstructed plants have been assigned to these closely related orders. Three are "*Cercidiphyllum*-like" (Crane, 1984, 1989a; Crane and Stockey, 1985, 1986) and one belongs to Trochodendraceae (Crane *et al.* 1990; Crane 1989a).

Cercidiphyllum (in the monotypic family Cercidiphyllaceae) is today restricted to Japan and south east China. The *Cercidiphyllum*-like fossils are diverse and widely distributed in the Palaeocene of North America, Europe and the USSR, especially at high latitudes. The most completely known example is *Joffrea* from Canada (Fig. 20). This plant is reconstructed from staminate and pistillate inflorescences (bearing pollen and ovules, respectively), infructescences, seeds, seedlings and leafy shoots. It is particularly remarkable in the preservation of seedlings (Fig. 21) (Stockey and Crane, 1983) revealing not only crucial aspects of reproductive strategy but also ontogenetic growth stages. The southern English *Nyssidium* plant is known both from the Palaeocene and Early Eocene. Leaves, infructescences, fruits and seeds are preserved. *Trochodendrocarpus* from the USSR is known from leafy shoots with attached infructescences and dispersed fruits.

These three fossils plants differ from extant *Cercidiphyllum* and from each other in their shoot organization and in the way their leaves and pistillate inflorescences (hence also fruits) are borne. *Joffrea* (Fig. 20) has a monopodial short shoot (terminated by an apical bud) with axillary infructescences

Fig. 20. Reconstructed *Cercidiphyllum*-like plant, *Joffrea* from the Palaeocene of Canada (see also Fig. 21). (a) Pistillate inflorescences, (b) mature infrustescences, each on short shoot with attached leaves. Young carpel 9–11 mm in length, mature follicle 18–30 mm in length. From Crane and Stockey (1985) with permission.

and opposite decussate leaves whereas *Cercidiphyllum* has a sympodial short shoot bearing a single leaf, an axillary bud and a single terminal infructescence (often composed of only four fruits). *Nyssidium* and *Trochodendrocarpus* both lack short shoots. In the former the long shoot bears whorled leaves and a terminal inflorescence. In the latter it bears alternate leaves and an axillary inflorescence (Crane and Stockey, 1986).

In the three fossils, bicarpellate apocarpous flowers (giving rise to pairs of follicles) are frequent (Fig. 20) suggesting that this may be the primitive con-

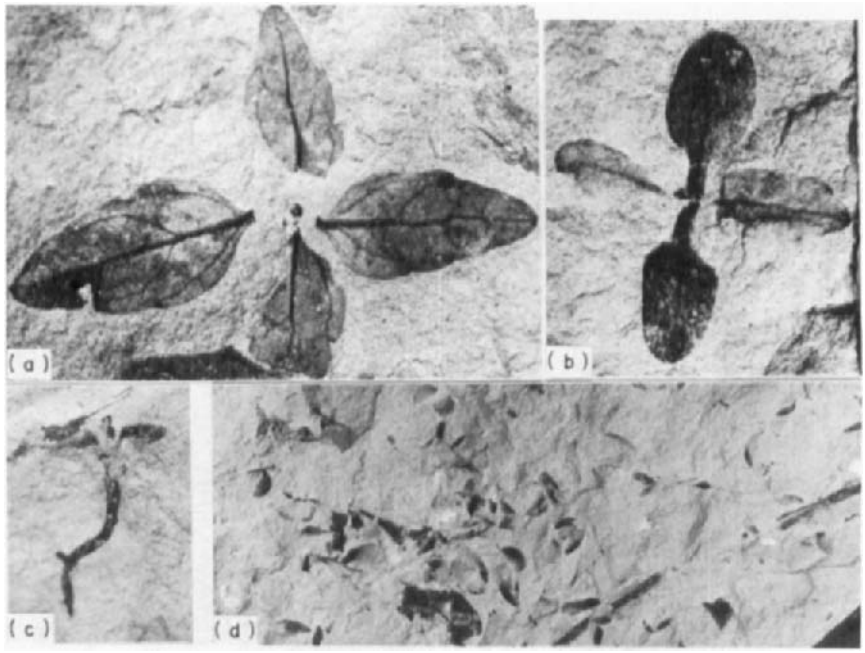

Fig. 21. Seeds and seedlings of a *Cercidiphyllum*-like plant, *Joffrea* from the Palaeocene of Canada (see also Fig. 20). (a) Seedling showing first and second pairs of adult leaves, ×4.464; (b) young seedling showing cotyledons and first pair of leaves, ×4.278; (c) seedling in lateral compression showing cotyledons, hypocotyl and lateral root, ×3.069; (d) rock slab bearing numerous, small, crescentic seeds with thin wing and small, dark seed body, ×0.93. (a),(b),(c) From Stockey and Crane (1983) with permission.

dition for the family. This strengthens the idea of a close phylogenetic relationship between Cercidiphyllaceae and Hamamelidaceae (Crane and Stockey, 1986).

These whole plant studies reveal something of the diversity of plants to which relatively similar isolated organs may have belonged. Assignment of the isolated leaves or fruits to modern *Cercidiphyllum* is clearly inaccurate. Floristic diversity and the role of *Cercidiphyllum*-like plants may be undervalued if all are assigned to a single species. According to interpretations from their seeds and seedlings (Crane, 1981; Crane and Stockey, 1985) these *Cercidiphyllum*-like plants were opportunistic colonizers of open and disturbed floodplain habitats (see also Section IV.C).

Nordenskioldia (Trochodendraceae) is known from fruits and infructescences (Fig. 22b,c; Crane *et al.*, 1990). Leaves (Fig. 22a) and shoots, probably of this plant, are found in association. This genus is also an important element in high latitude Northern hemisphere Palaeocene floras.

Increasing knowledge of these early representatives of the Hamamelidae (which show some characters intermediate between those of modern Hama-

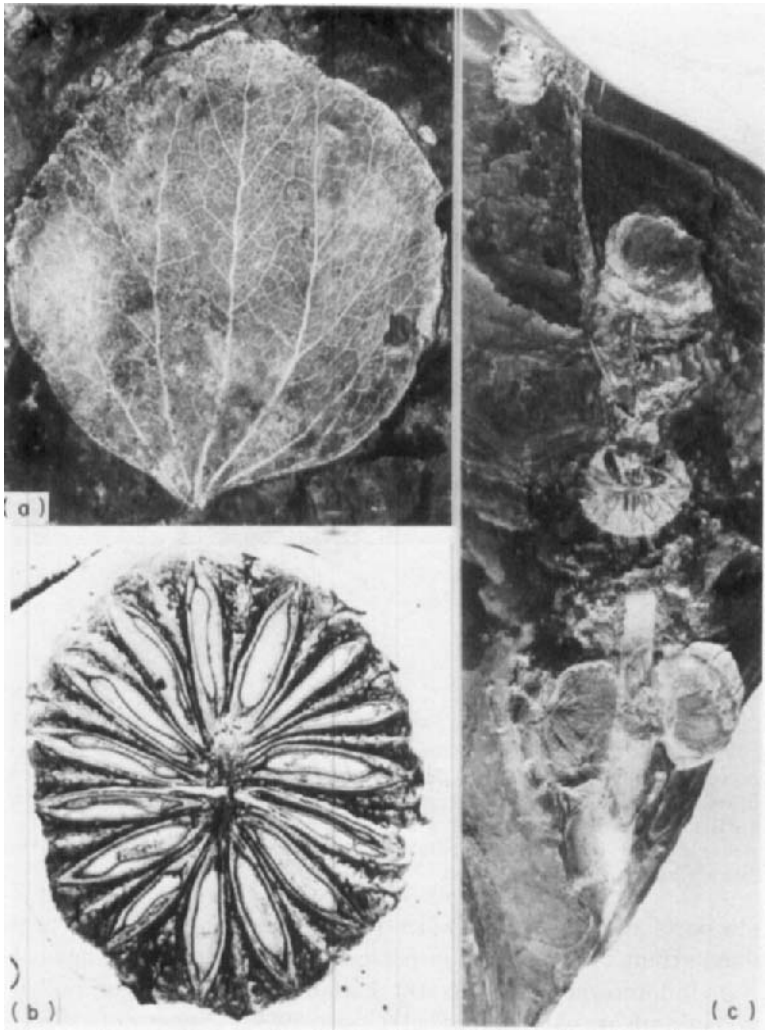

Fig. 22. Associated organs from a member of the Trochodendraceae, Palaeocene, USA (Crane *et al.*, 1990). (a) Leaf, × 1.5; (b),(c) *Nordenskioldia*. (b) transverse section of permineralized fruit, × 5; (c) fruiting axis, × 2. Illustrations courtesy of P. R. Crane.

melidae and Magnoliidae) is valued for understanding the early radiation of "higher" dicotyledons (see Crane, 1989a).

3. *Platanaceae*

The Platanaceae (plane tree family) had already differentiated by the end of the Early Cretaceous where they were represented by forms with insect pollinated, unisexual flowers, with five carpels or stamens, well-developed

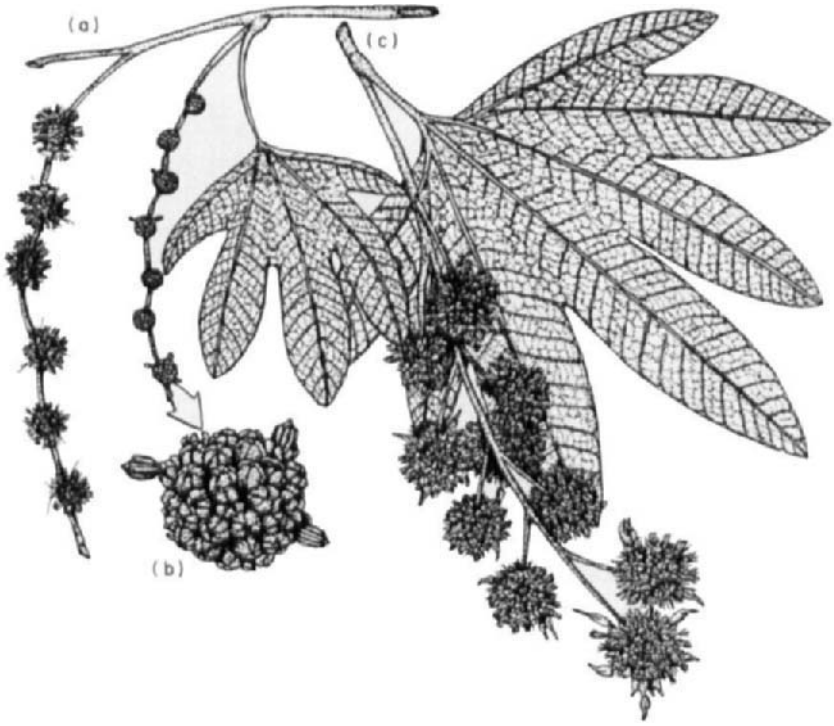

Fig. 23. Reconstructed plant, the Clarno plane or "*Macginitiea* plant" (Platanaceae), mid-Late Eocene, USA (see also Fig. 24). (a) Branch bearing pistillate (left) and staminate flowering axes; (b) staminate inflorescence beginning to shed stamen groups; (c) branch bearing mature fruiting axis, lowest fruiting head in process of fruit dispersal. Leaf lamina ranges from 15–35 cm in maximum length. From Manchester (1986) with permission of the publisher.

perianth parts and very small pollen (8–10 μm in length). These probably resembled extant *Platanus kerii* in possessing numerous sessile heads borne along an inflorescence axis. Extant Platanaceae with larger pollen and reduced perianth are wind pollinated (Crane, 1989a; Crane *et al.*, 1986, 1988; Friis *et al.*, 1988). Rare leaves are found associated with the Cretaceous pistillate flowers and the two share cuticular characteristics. The leaves are palmately lobed. They intergrade in venation and cuticular structure with pinnately lobed or compound *Sapindopsis* leaves. These factors imply a close phylogenetic relationship between early representatives of the subclasses Hamamelidae (including Platanaceae) and Rosidae (including *Sapindopsis*) (Crane *et al.*, 1986, 1990; Crane, 1989a).

The "*Platanites hebridicus*" plant, reconstructed from associated leaves and pistillate and staminate inflorescences occurs in the Palaeocene of Scotland (Crane *et al.*, 1988). It was very similar to extant Platanaceae, like *P. kerrii*, and was probably wind pollinated. However, the leaves are pinnately compound, thus further strengthening the relationships proposed above.

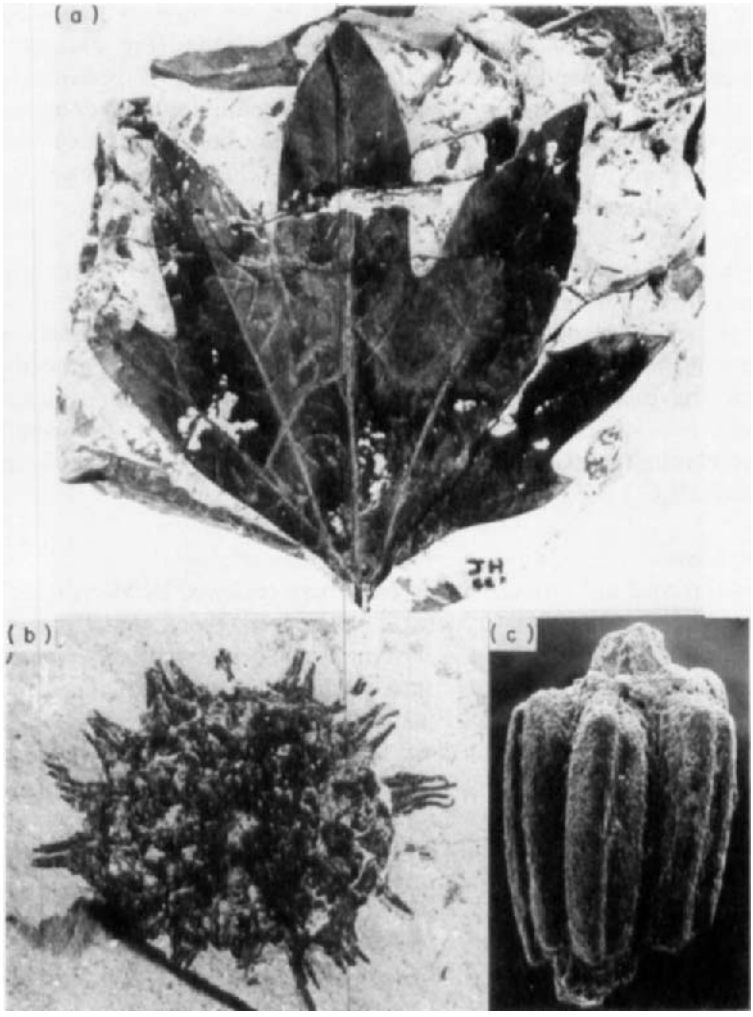

Fig. 24. Organs from the "*Macginitiea* plant" (Fig. 23). (a) Leaf, (*Macginitiea*), × ½; (b) fruiting head (*Macginicarpa*), × 3; (c) group of five stamens (*Macginistemon*), SEM, × 20. (a),(b) Compression fossils; (c) permineralized with silica. Illustrations courtesy of S. R. Manchester. From Manchester (1986) with permission of the publisher.

Amongst the diversity of Early Tertiary Platanaceae leaves, woods and reproductive structures summarized by Crane *et al.* (1988), there is another reconstructed plant (Manchester, 1986). This is certainly not only the most completely known fossil member of the Platanaceae but also the most thoroughly reconstructed fossil flowering plant.

The "*Macginitiea* plant" or Clarno plane (Fig. 23) from the mid–Late

Eocene of Oregon, USA, is reconstructed on the basis of repeated co-occurrence of organs at numerous localities. The leaves (Fig. 24a), petioles and stems, pistillate and staminate inflorescences (Fig. 24c), pollen, fruiting heads (Fig. 24b) and fruits are all known and a combination of compression and permineralized preservation has given additional anatomical details. This plant (Fig. 23) had palmately lobed leaves, elongate inflorescence axes (staminate with sessile heads), pollen 12–16 μm in length, flowers with well developed perianth and whorls of five stamens or free carpels, and achenes which lacked dispersal hairs. The suite of characters combines features of the two modern subgenera of *Platanus* and features unknown in the extant genus. Achenes of modern Platanaceae bear hairs which are a specialization for wind dispersal and the wind pollinated flowers lack a differentiated perianth and have larger pollen (16–22 μm in length). The Clarno plane was therefore less specialized for wind pollination and seed dispersal than modern Platanaceae. Other aspects of the ecology of this plant are discussed in Section IV.C.

4. Fagaceae

The fossil record of Fagaceae has recently been reviewed by Manchester and Crane (1983), Jones (1986), Crepet (1989), Romero (1986a) and Tanai (1986) the last two concentrating on southern hemisphere beeches (*Nothofagus*). The family probably included a late Cretaceous "ancestral complex" and radiated in the Early Tertiary. Fossils assignable to modern genera of the various subfamilies first appear in the Northern hemisphere Eocene.

One Early Tertiary member of the Fagaceae, *Fagopsis* (Figs 25 and 26) is represented by attached leaves, pistillate and staminate inflorescences (Fig. 26) and fruits (Manchester and Crane, 1983). *Fagopsis* is an important element in an Early Eocene flora from Washington State and in two Early Oligocene floras from Colorado and Montana. *Fagopsis* combines characters now segregated in subfamilies of modern Fagaceae. The organization of pistillate inflorescences and fruits resembles members of the Castaneoideae whereas that of the staminate inflorescence suggests *Fagus* (Fagoideae) and that of the anthers and pollen both Fagoideae and Quercoideae. Major differences from extant Fagaceae are the minute nuts, the cupule morphology and the spiral arrangement of units in the fruiting head (Fig. 25). Fruit wedges were regularly shed in strings, adhering at their apices, in groups of up to 12. These features may reflect a generalized wind dispersal strategy in contrast to the animal dispersal typical of modern Fagaceae (e.g. oaks, chestnuts, beeches).

Daghlian and Crepet (1983) described staminate inflorescences, leaves and fruits of *Quercus* from the Oligocene of Texas, USA. Although all these organs occur in association the authors were reluctant to consider them as representing one species because no organic connection could be demonstrated and other probable *Quercus* fossils occurred at the same site. The

Fig. 25. Reconstructed plant, *Fagopsis* (Fagaceae), Oligocene, USA (see also Fig. 26). At left branches showing pistillate inflorescence (top), young foliage (centre) and staminate inflorescence (base). At right branches showing fruiting heads, the lower example during fruit shedding. Fruiting head 12-18 mm in length. From Manchester and Crane (1983) with permission.

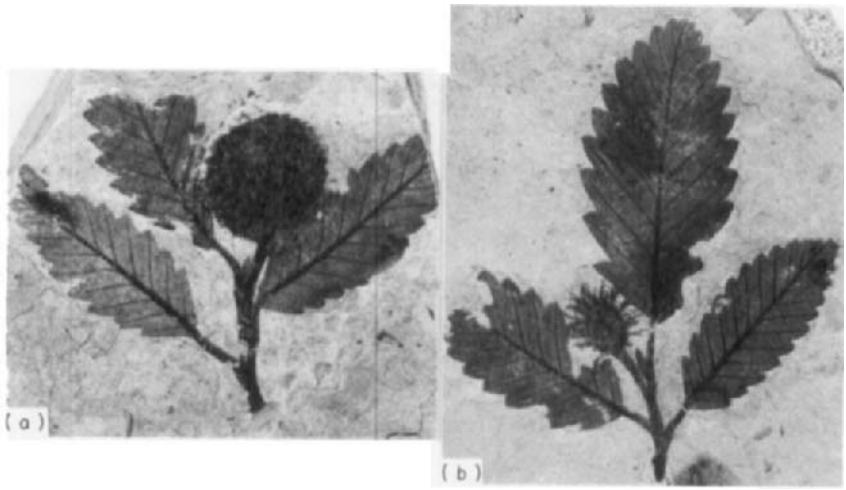

Fig. 26. Elements of the *Fagopsis* plant (Fagaceae), Oligocene, USA (see also Fig. 25). (a) Leafy branch bearing staminate inflorescence, ×1.35; (b) leafy branch bearing pistillate inflorescence, ×0.9. Illustrations courtesy of S.R. Manchester. From Manchester and Crane (1983) with permission.

Fig. 27. Reconstructed plant, *Palaeocarpinus* (Betulaceae), Palaeocene, southern England, Leafy branch bearing small nutlets each subtended by paired, lobed bracts. Bracts 8–15 mm maximum length. From Crane (1981) with permission.

suite of organs does show that *Quercus* was clearly differentiated by the Oligocene although, if the organs did belong to one species, this combined features now segregated in modern subgenera.

5. Betulaceae

There are three well-documented examples where betulaceous fruits are associated with betulaceous leaves, although none have organic connection (Crane, 1981; Manchester and Crane, 1987; Crane and Stockey, 1987). Crane

(1989b) reviewed the fossil history of the family. Fruits of *Palaeocarpinus* (Crane, 1981) have a small nutlet with two large lobed bracts (Fig. 27). They are known from southern England and North America (Crane *et al.*, 1990) in the Palaeocene. *Asterocarpinus* (Manchester and Crane, 1987) has a small nutlet borne on an involucre of four to seven radiating wings and occurs in three Early Oligocene floras from North America. Both genera can be assigned to the Coryleae but show novel combinations of features not present in any modern form. In particular *Palaeocarpinus* combines a small nutlet, like modern *Carpinus* (hornbeam), with two large bracts, like modern *Corylus* (hazel), probably a modification for wind dispersal. Retention of a primitive character may partially explain the unlikely combination of two large bracts (as expected in a wind dispersed form) with a large, animal dispersed nutlet in living *Corylus*. In *Asterocarpinus* the involucre represents yet another modification for wind dispersal no longer present in modern members of the family. Both fossil fruits are associated with coryleoid leaves which on the basis of systematic assignment and repeated co-occurrence (Manchester and Crane, 1987) may be from the same parent plants.

One member of the Betuleae is also known from associated fossil organs (Crane and Stockey, 1987). These include leaves, infructescences, fruits, staminate inflorescences and pollen from the Middle Eocene of Canada. Both vegetative and reproductive structures suggest that this is a species of *Betula* similar to extant forms.

Extant genera of Betuleae (*Alnus* and *Betula*) were clearly differentiated by the mid-Eocene whereas the Coryleae of the Eocene included few forms referable to modern genera but several extinct forms. *Carpinus* and *Ostrya* do not appear until the Late Eocene/Oligocene (Crane, 1989b).

6. *Ulmaceae*

Manchester (1989) describes multiple organ reconstructions from the Eocene of North America and Europe of species from two modern genera of Ulmaceae (*Zelkova* and *Chaetoptelea*) and one extinct genus, *Cedrelospermum*. These are represented by leafy branches bearing fruits or flowers. All three examples come from the Ulmoideae which have broad winged fruits and show that this subfamily underwent radiation in the Early to Middle Tertiary. Celtoideae (with drupe-like fruits) probably radiated earlier (evidence from fossil fruits) but there are no "whole plant" fossils to confirm this.

7. *Juglandaceae*

The major review of Juglandaceae (Manchester, 1987) (see Section IV.B.2) documents diverse examples of associated organs. These include leaves, fruits, fruiting heads and staminate inflorescences. In the Platycaryeae fruits and staminate inflorescences, very like those of the modern genus *Platycarya*, are associated with a rather distinctive foliage. One extinct genus is known

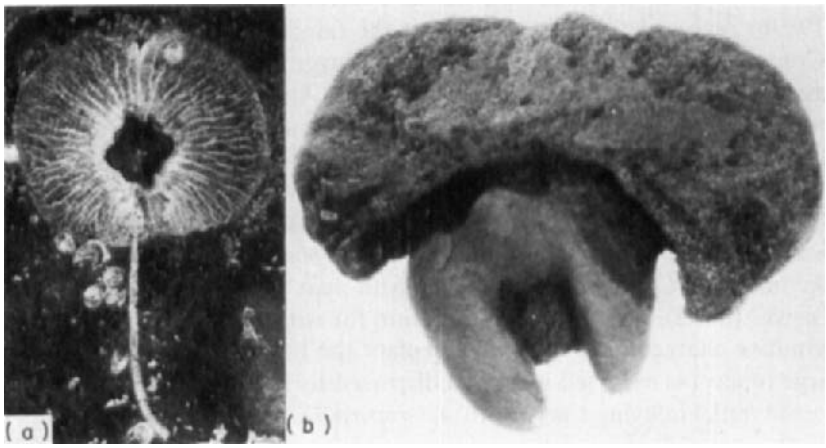

Fig. 28. Fruits of the tribe Juglandeae (Juglandaceae—walnut family) (Manchester 1987). (a) *Cyclocarya* winged nutlet, Palaeocene, USA, × 1.5. Illustration courtesy of S. R. Manchester. (b) *Juglandicarya depressa* from the Eocene, London Clay flora, southern England, probable fruit of *Cyclocarya* but with no trace of the wing remaining in the abraded fossil. Pyrite permineralization eroded to reveal internal locule cast, × 11.

from fruits and associated leaves. Pollen of *Platycaryapollenites* is associated with both taxa.

In the Engelhardieae several sets of organ associations are known. One includes staminate inflorescences, fruits and leaves; others, just leaves and fruits. Characters of the modern genus *Oreomunnea* are combined with those of *Alfaroa* (leaflets) or *Engelhardia* (fruits).

In the Hicoreae there is one example of associated fruits, leaves and staminate inflorescences from the Early Oligocene all of which are assigned to modern *Carya*. In Juglandeae, two Palaeocene species are known from associated foliage and fruits. One has fruits assigned to the modern genus *Cyclocarya* (Fig. 28), the other to a similar extinct genus *Polyptera* with a lobed, not entire, wing. The leaves both differ slightly from those of modern Juglandeae. A species of *Juglans* is known from leaves and fruits in the mid-Eocene of Clarno, Oregon.

8. Salicaceae

A single specimen of a species of *Populus* (Fig. 29) from the Middle Eocene Green River Formation, Utah, USA shows attached leaves and a fruiting raceme. Leaves of probable *Populus* have a fossil record extending back into the late Palaeocene but this is the first record of attached fruits (Manchester *et al.*, 1986). The fossil plant is very similar to modern *Populus* except for the tendency to palmate leaf venation (living species are pinnately veined) and the presence of the fruiting raceme on the same young shoot as the leaves (those of living species occur on old twigs). The authors do not consider these

Fig. 29. *Populus* (Salicaceae) showing leafy branch bearing fruiting raceme, mid-Eocene, USA, ×0.425. Illustration courtesy of S. R. Manchester. From Manchester *et al.* (1986) with permission.

differences sufficient to distinguish the fossil from *Populus* and indeed specifically relate it to the single extant species of the section *Abasco*. Although it differs in having trivalvate (compared with bivalvate) capsules this may merely represent the primitive condition for the genus (Manchester *et al.*, 1986).

These authors also refer (p. 160) to a specimen bearing leaves and a fruiting raceme which can be assigned to the modern genus *Salix*, also from the Green River Formation. The Salicaceae was therefore represented by members of both modern genera by the Middle Eocene.

9. Ebenaceae

Fossil staminate flowers containing pollen (Figs 30 and 31) and leaves (Fig. 32) found in association in the Late Eocene of southern Australia share a characteristic cuticular structure, indicating that they are derived from a single species of plant (Basinger and Christophel, 1985). Both organs are very similar to those of extant species of *Diospyros* (ebony and persimmon) of the Ebenaceae. Knowledge of floral features is crucial for familial and generic assignment.

The presence of this plant (named *Austrodiospyros*) in the Australasian Late Eocene, well before this continent connected with Malesia (see Fig. 2 and Section II.D), supports proposals that Ebenaceae originated and were widespread in Gondwanaland prior to the final separation of continents probably in the Later Cretaceous (Basinger and Christophel, 1985).

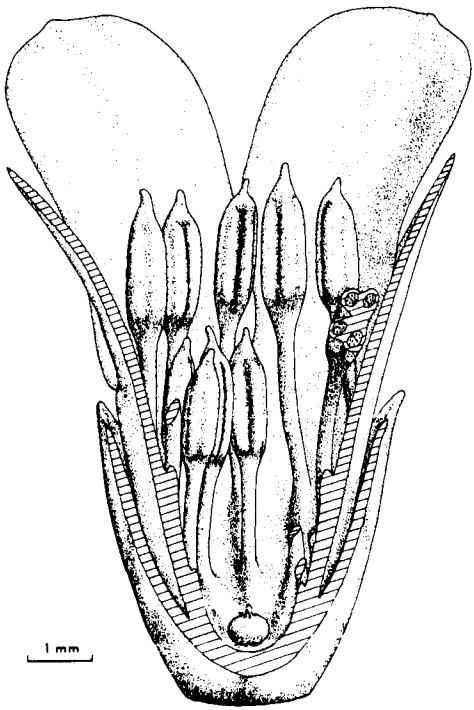

Fig. 30. Reconstruction of *Austrodiospyros* (Ebenaceae) flower (see also Figs 31 and 32), mid-Eocene, Australia, hairs on all organs omitted. From Basinger and Christophel (1985) with permission.

10. Aceraceae

In their extensive revision of the family Wolfe and Tanai (1987) list 13 Early Tertiary *Acer* species represented by associated fruits and leaves. The extinct genus *Bohlenia* (Wolfe and Wehr, 1988) is also known from associated fruits and leaves. The isolated samaras are indistinguishable from *Dipteronia* (the only other modern genus of Aceraceae) but the fruit is trilocular like those of tribe Paullinieae of the related family Sapindaceae. This is taken by the authors as a strong indication of shared ancestry for this tribe and Aceraceae. The "'*Acer*' *arcticum* complex", which occurs in the Palaeocene and Eocene, has fruits which differ from those of *Acer* in the distal rather than proximal position of the coalesced veins on the wing. Leaves associated with these samaras also differ from *Acer* in their tooth morphology. The authors consider this an extinct genus of Aceraceae.

11. Pteridophytes and Conifers

Significant studies of whole plant biology in these groups include those on Schizaeaceae (Fig. 6), *Azolla* (Fig. 9), *Isoetites* (Fig. 11b) and *Metasequoia* (Fig. 12). For details see Section II.F.

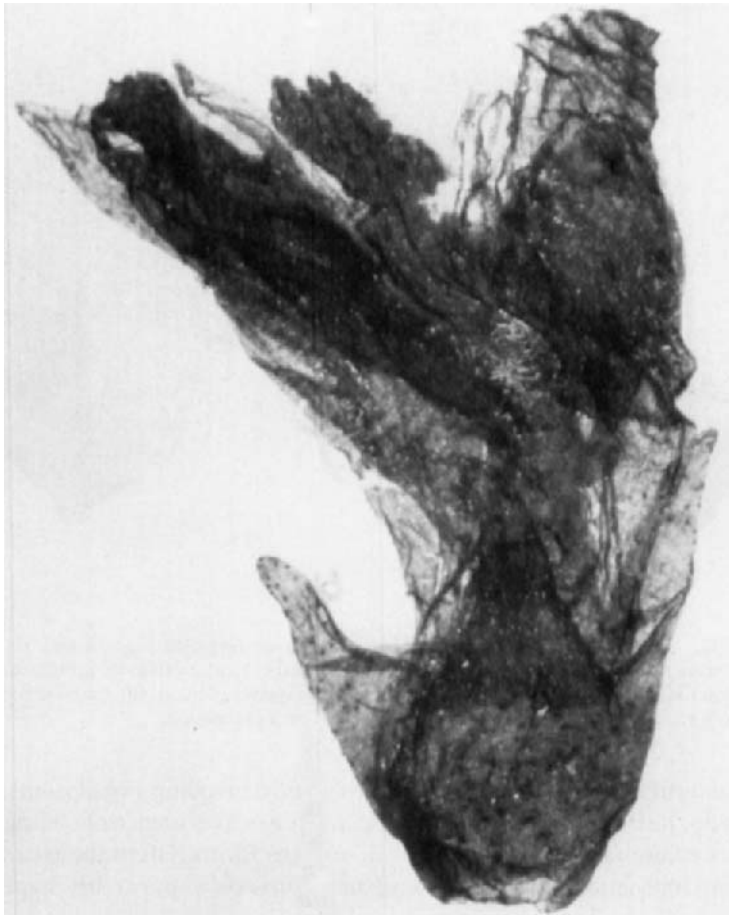

Fig. 31. *Austrodiospyros* (Ebenaceae), "mummified" flower, mid-Eocene, Australia, × 15 (see also Figs 30 and 32). Illustration courtesy of J. F. Basinger. From Basinger and Christophel (1985) with permission.

B. SYSTEMATIC AND EVOLUTIONARY STUDIES

1. Cladistic Approaches

Cladistic methodology has been scarcely applied in studies by Tertiary palaeobotanists, although it has found favour with those investigating the origin of angiosperms and the phylogenetic relationships of seed plants (see Crane, 1985; Doyle and Donoghue, 1986). See Stein (1987) for a review of cladistic methodology and its application to fossil plants. Much of the reluctance of palaeobotanists to use cladistics can probably be related to the limited number of fossil "whole plants" (Section IV.A) where an extensive suite of diagnostic characters can be deduced. With the more extensive fossil record of isolated organs characters are limited in number and may not be

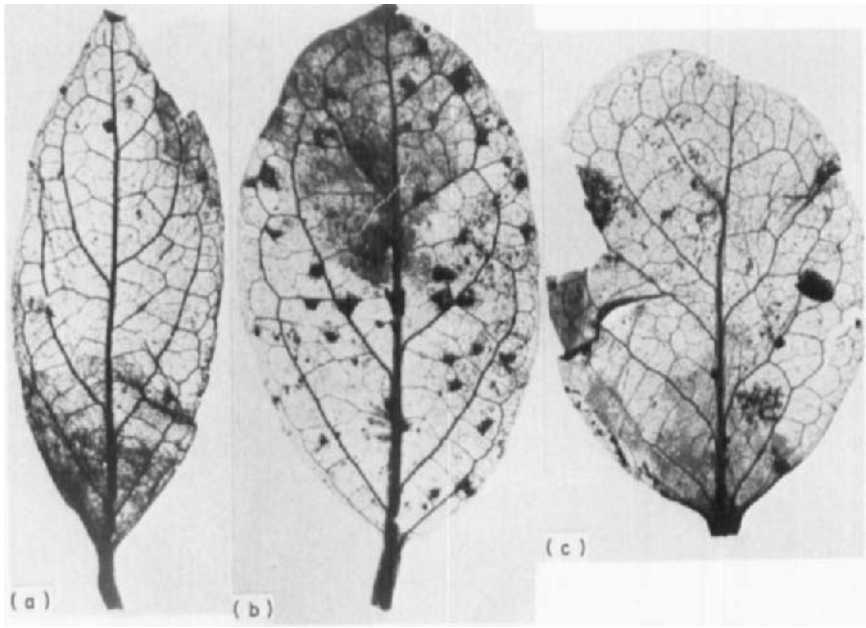

Fig. 32. Leaves associated with the Ebenaceae flower (see also Figs 30 and 31). (a)–(c) Three leaves showing variation in form, × 1.8. Unusual cuticular anatomy present on all the leaves and the flowers indicates derivation from a single species of plant. Illustrations courtesy of J. F. Basinger. From Basinger and Christophel (1985) with permission.

representative of the entire plants or their interbreeding populations. Plant fossils do, however, frequently provide unequivocal evidence of characters or character combinations not present in modern forms. Often these can elucidate phylogenetic relationships and may provide support for hypotheses regarding the polarity of characters. Examples from "whole plants" have been given in Section IV.A. Isolated organs, e.g. seeds of Nymphaeaceae (Figs 18 and 33), may also provide this type of information. Cell surface shapes (Fig. 18) imply relationship with one or more modern genera but features of the germination cap (Fig. 33) may exclude a close relationship with any modern genus, as for *Sabrenia* (Collinson, 1980b). As fossil relatives for various modern plants become better known it is hoped that both palaeo and neo-botanists will incorporate these data into phylogenetic analyses. Papers in Crane and Blackmore (1989) give several examples of such integrated approaches. Other examples of cladistic methodology are given below.

Crane and Manchester (1982) used cladistic analysis to show that the juglandaceous fruit *Casholdia* displayed generalized engelhardioid morphology. The Late Palaeocene fruit predates the mid-Eocene evidence of fruits with trilobate bracts, typical of modern members of the tribe. Collinson (1982) attempted a cladistic analysis, based on fruit characters alone, for fossil and modern Potamogetoneae (including modern *Potamogeton* and

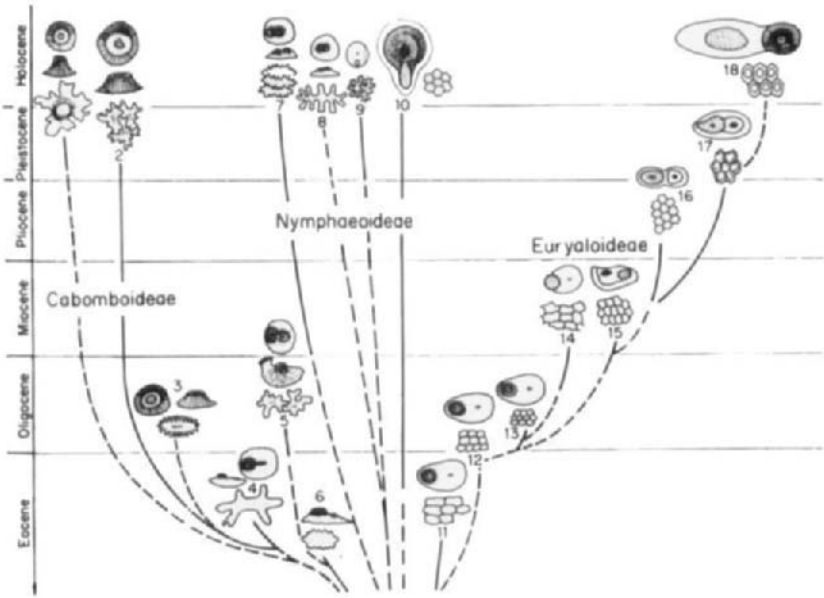

Fig. 33. Suggested evolution of the Nymphaeaceae (water-lilies) based on seed surface cells and germination caps (see also Fig. 18). From Mai (1985) with permission of the publisher, VEB Gustav Fischer Verlag, Jena.

Ruppia). Although the resultant cladogram was very poorly resolved and incorporated much homoplasy it did support the recognition of certain fossils as representatives of extinct taxa of equivalent rank to those containing modern *Potamogeton* and *Ruppia*.

Wolfe and Tanai (1987) have produced a cladistic analysis of the numerous extinct and extant sections of the genus *Acer* using 94 characters. Character polarization was based on outgroup comparison mainly with Sapindaceae. According to these authors Sapindaceae and Aceraceae probably share a common ancestor and the fossil *Bohlenia* (with associated fruits and leaves) combines features of Aceraceae and the tribe Paullinieae of the Sapindaceae. From a cladistic standpoint Aceraceae should be reduced to tribal rank within Sapindaceae, otherwise they are a paraphyletic group. Paullinieae (rather than Harpullieae) are considered to be the extant tribe of Sapindaceae most closely related to the Aceraceae. Fossils assigned to the "'*Acer*' *arcticum* complex" may represent an extinct genus of Aceraceae. The authors proposed classification involves both cladistic and phenetic evidence (Wolfe and Tanai, 1987, p. 25). Their fig. 3 for the suggested cladistic relationships does not include characters and has extinct sections indicated in ancestral positions. Furthermore certain characters, e.g. the development of tricolpate pollen, have been assumed to have arisen only once (Wolfe and Tanai, 1987, p. 19), rather than subjecting the entire character distribution to parsimony analysis. These factors may unfortunately detract from the atten-

tion that this significant paper will gain amongst those applying cladistic analyses in problems of plant phylogeny.

The thorough compilation of *Acer* fossils (leaves and fruit) from western North America is an elegant data base for future analyses. The first fossils assignable to modern sections of *Acer* occur in the Palaeocene but the major diversification occurred during the Eocene apparently in volcanic upland regions. In the early Middle Eocene 10 extinct sections are recorded but maximal diversity of 15 sections with 34 species is in the Late Eocene, by which time over half the modern sections can be recognized. Maximum abundance, however, occurred later following the spread of microthermal vegetation after the terminal Eocene climatic deterioration. Many Asian sections of *Acer* seem to have originated in North America during the Eocene implying a number of dispersal events. Extinction of various sections in North America occurred in the Late Eocene and Early Oligocene and Miocene. Several long-distance dispersal events are suggested for sections related to modern Eurasian forms but which appear in North America in the Miocene.

The southern hemisphere beeches (*Nothofagus* species) are the subject of extensive debate. Their biogeography has been regarded as the clue to the general distribution of life in the Southern hemisphere and the sequence of the break up of Gondwanaland. Phylogenetic analysis has been applied to this problem using vicariance biogeography (Humphries, 1981, 1983). In this methodology a cladistic analysis of the modern species is used to infer the likely sequence of separation of the various continents (i.e. dispersal events isolating populations) and fossils are accorded a minimal role. Tanai (1986) and Romero (1986a) have subsequently re-emphasized the value of the fossil record in understanding *Nothofagus* distribution. All authors reach the same conclusion of a southern origin for the genus (i.e. in south Gondwanaland). One critical aspect of the debate is the significance of fossil "*N. brassii*"-type pollen. This pollen is restricted today to species occurring in New Guinea and New Caledonia (subsection Bipartitae). In the past however, from the Late Cretaceous onwards, it is found in south Australia, New Zealand and South America (Romero, 1986a; Tanai, 1986). The two pollen types "*menziessii*" and "*fusca*" also occur in the Cretaceous but they cannot be so clearly related to any one modern group. The significance of "*N. brassii*" pollen has been disputed at least partly because of the absence of correlated macrofossils. However, Hill (1987) has described cupules of section Bipartitae (in association with this pollen) from a Late Oligocene flora in Tasmania. This places the previously wider distribution of the section beyond dispute and account must be taken of this factor when considering evolution and dispersal of the genus. In this respect the model provided by Tanai (1986, fig. 14) with a more recent common origin for the Australian and Tasmanian species with those of New Guinea and New Caledonia (and hence implying a more recent separation of these regions) may therefore be more appropriate. However, Tanai's model separates South American members of subsection Quadri-

partitae from those of Australasia, whereas these are shown to be closely related in the cladistic analysis of Humphries (1981).

Future discoveries of fossil cupules and nutlets, preferably in association with well defined leaf material, should help to place the fossil record of *Nothofagus* on a firmer footing. Cladistic analyses including fossil material may then help to clarify the evolution and biogeography of *Nothofagus*.

2. *Other Approaches*

Family level reviews. Those studies on *Acer* and *Nothofagus* mentioned above are fine reviews of the respective fossil records. Undoubtedly many more such studies are needed. Of equal importance are similar studies at the family level, e.g. Collinson (1989c)—Moraceae, Cannabaceae and Urticaceae; Crane (1989b)—Betulaceae; Manchester (1989)—Ulmaceae; Crepet (1989)—Fagaceae. These studies attain their greatest value when based on a variety of organs and with global coverage. Such work is rarely undertaken owing to the enormity of the task involved. One example is the excellent review (Manchester, 1987) of the Juglandaceae (walnut family), which has an extensive fossil record of leaves, wood, pollen, inflorescences and fruits. Many of the fossils are represented by organ association (see section IV.A.7) thus making this family probably the best known in the flowering plant fossil record.

The Juglandaceae probably evolved from plants of the Normapolles complex, with bisexual flowers and tiny nutlets, during the latest Cretaceous. Their major diversification occurred in the Early Tertiary when the centre of diversity was North America and Europe. The present day centre is in eastern Asia but the fossil record argues against an Asian centre of origin. *Cyclocarya* (Fig. 28A), for example, is restricted to Asia today but occurred in North America and probably Europe (Fig. 28B) in the Early Tertiary. All four modern tribes of the family can be recognized in the Eocene, though there is a greater diversity of extinct forms amongst the tribes with wind dispersed fruits. Tribe Engelhardieae is usually considered the most primitive representative on the basis of modern comparative studies but this is not borne out by the fossil record. The trilobate bracts of the nutlets are readily preserved but do not occur in the Palaeocene whereas fruits of Juglandeae do (e.g. Fig. 28). Many Palaeocene and Eocene genera, with restricted distributions, became extinct by the Oligocene whereas those which had by then established broader ranges survived to the present day. The climatic cooling at the end of the Eocene may have been partly responsible for the extinctions (Manchester, 1987).

Application of image analysis to evolutionary studies of seed assemblages. A number of studies of the fossil record of flowering plants have been based only on single organs. Whilst lacking the diversity of information provided by studies involving material with attachment or association evidence, they have the advantage of potentially offering a very closely spaced set of samples

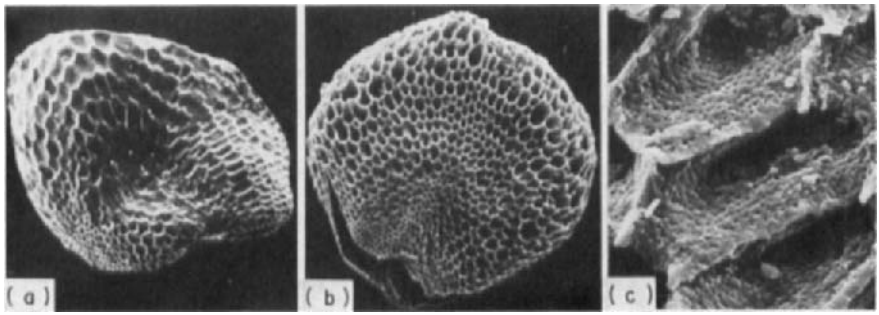

Fig. 34. Diagnostic seed morphology of *Eurya* (Theaceae—tea family). (a) Modern *Eurya japonica*, × 25; (b) fossil *Eurya stigmosa*, × 20; (c) surface cells from (b) (outer periclinal wall lost during decomposition), × 500. All SEM.

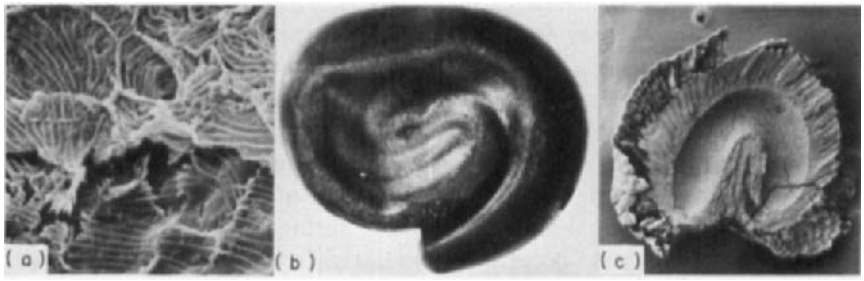

Fig. 35. Diagnostic internal anatomy of seeds. (a) Spirally thickened cells from the inner testa of Rutaceae, Eocene, Germany (Collinson and Gregor, 1988), SEM, × 405; (b) internal cast of Sapindaceae seed showing embryo, pyrite permineralization, London Clay flora, Eocene, southern England (Collinson, 1983b) × 6.4; (c) longitudinal section of seed from an extinct Theaceae showing embryo cavity and anatomy of the seed coat, Palaeocene, southern England (Collinson, 1986a), SEM, × 13.5.

if the organ is readily fossilizable. In particular, fruits and seeds are not only durable, but also frequently diagnostic to modern generic or specific rank (Figs 18 and 33–36). Fruits and seeds of wetland species have a particularly good fossil record (Collinson, 1988a; Mai, 1985). Figure 37 for *Stratiotes* and Fig. 33 for Nymphaeaceae demonstrate the nature and quality of the potential information for such material. It is unfortunate that both methods of illustration imply evolutionary lineages which are purely speculative. Figure 8 for *Azolla* and fig. 11 of Mai (1985) for Neogene *Trapa* (which is like that for *Stratiotes* (Fig. 37), but without link lines) are preferable at this stage of our knowledge.

Current work in my laboratory (Collinson *et al.*, 1987) has begun an investigation of *Stratiotes* seeds using image analysis with a microcomputer. Three assemblages were chosen for a preliminary analysis undertaken by Robert Scotland. One of these is new Palaeocene material from southern

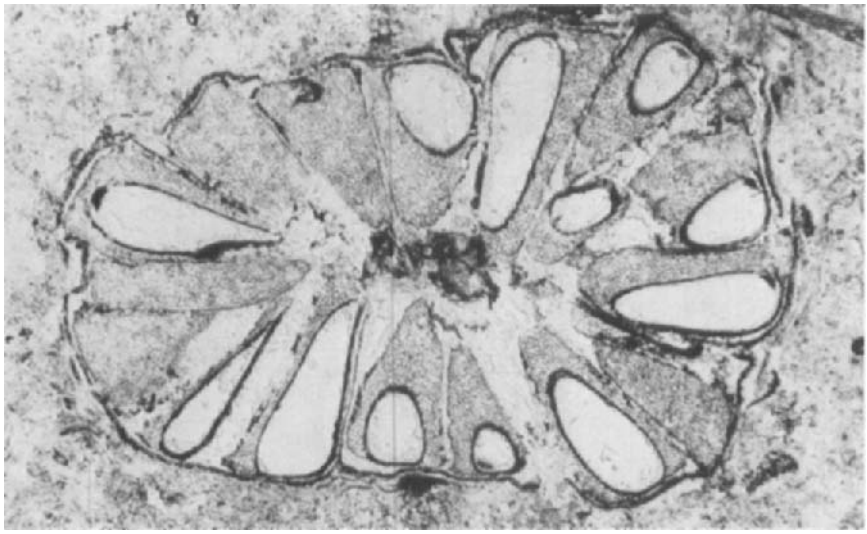

Fig. 36. Transverse section of permineralized fruit of *Decodon* (Lythraceae), containing numerous seeds, mid-Eocene, Canada, × 25. Illustration courtesy of S. Cevallos-Ferriz. From Cevallos-Ferriz and Stockey (1988a) with permission.

England (Collinson, 1986a) (thus corresponding with the "origin" of the proposed lineages on Fig. 37). The others are assemblages which would be assigned to *S. headonensis* (1) and *S. neglectus* (6) in terms of Fig. 37. We used only left or right valves of germinated (hence fully mature) seeds. The seed valve features were digitized into a BBC microcomputer using a graphics tablet and a video camera mounted on a stereo microscope. The computer programmes were written by Tony Brain. Various measurements were made and subjected to multivariate statistical analysis, with the help of Martin Ingrouille. These preliminary analyses failed to distinguish the three assemblages but rather showed a continuum of morphological variation with no obvious subdivisions. The morphological similarity between the new Palaeocene material and the "*S. headonensis*" assemblage was more pronounced than that between "*headonensis*" and the "*S. neglectus*" assemblages. The former are separated by 16 million years and the latter by only 1.5 million years demonstrating differential rate of change. The "*S. headonensis*" assemblages split on cluster analysis into two clusters one of which falls into *S. headonensis* (1) on Fig. 37 the other into *S. hantonensis* (3) on Fig. 37; two "species" which had previously been recognized by Chandler (1961) at one stratigraphic level. The *headonensis* morphology is a more appropriate forerunner of *neglectus*, whereas Fig. 37 places *hantonensis* in this position. This split may represent a cladogenesis but no consistent segregation of any one assemblage could be achieved by any of the methods we used.

The preliminary results thus show gradual anagenetic change at varying

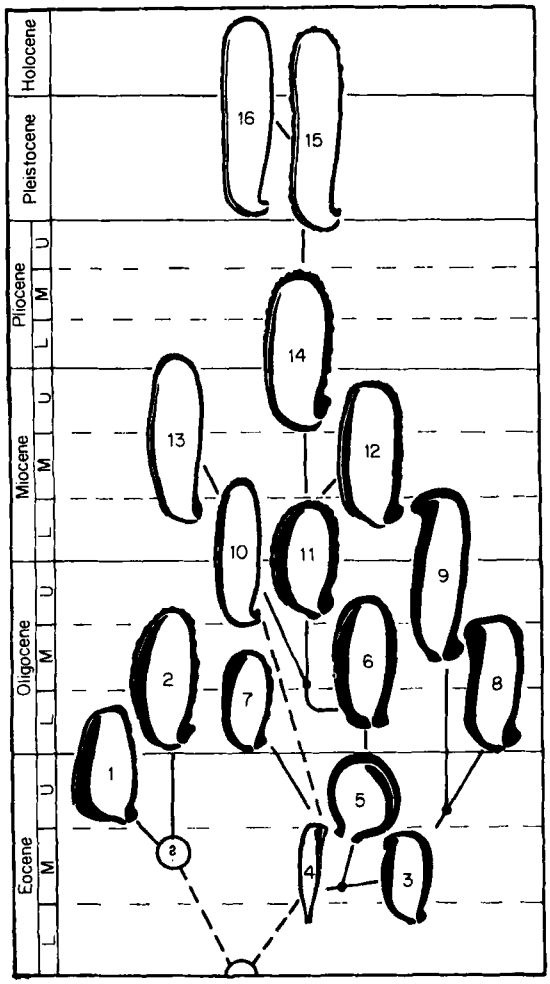

Fig. 37. Stratigraphic distribution and proposed evolutionary scheme for *Stratiotes* seeds. Each number refers to a named species. From Mai (1985) with permission of the publisher, VEB Gustav Fischer Verlag, Jena.

rates and include one possible cladogenesis. Continued application of this methodology should help to clarify patterns of evolution in these well represented fossil seed assemblages.

Morphological systematics and numerical taxonomy. Plant fossil assemblages often present a bewildering array of variation of systematic, evolutionary and environmental significance, but often masked by standard systematic methods. This is especially true for leaf fossils, as leaves can exhibit a range of environmentally controlled, phenotypic variation (note the variation for example on Fig. 32 and Fig. 50f and h). Burnham (1986a,b) applied an alternative systematic method which she termed "morphological system-

atics". Assemblages of modern and fossil leaves were scored for various characters including aspects of size, shape, venation, marginal serration, etc. and these were subjected to multivariate statistical analyses. Critical characters can then be recognized which define the foliage of the modern genera and these can be applied to fossils. On this basis she recognized three modern genera of Ulmoideae in Early Tertiary floras. One or more morphotypes (which might or might not be equivalent to modern species, i.e. interbreeding populations) could be recognized within each genus. Moreover, no smooth transitions were observed between fossil and/or modern genera. This suggests that stem species must have been rapidly isolated, and so provides a basis for discussion concerning the evolutionary patterns within the group. Morphological systematics represents one case where application of Linnean binomials to plant fossil assemblages may be inappropriate (see papers in Spicer and Thomas, 1986). Numerical taxonomy and alternative nomenclature was applied to leaves from the Eocene of Australia by Blackburn (1981) and Hill (1982). Hill grouped leaf OTUs (Operational taxonomic units) into parataxa based on 47 characters scored for over 500 specimens; 25 parataxa were defined from this without the implication that these represented biological species. Hill (1982) considered that "there is little doubt that those parataxa represented by a large number of specimens represent a natural taxonomic unit". This is not necessarily true for the smaller groupings.

Reconstructed whole plants (see Section IV.A) demonstrate that very similar leaf forms may be borne by plants whose other organization (e.g. of fruits, fruiting heads or leaf arrangement) was rather different. This is well exemplified by the extinct *Cercidiphyllum*-like plants. This knowledge argues strongly in favour of the application of such rigorous morphological systematic methodology to fossil leaf assemblages. The task is time consuming even when digitized images are used (e.g. Blue and Jensen, 1988, for modern *Quercus*). Improving computer technology, such as automated image analysis being developed by Tony Brain for our *Stratiotes* studies, should help with this problem. Unlike *Stratiotes*, many seeds, fruits and flowers do not lend themselves to easy delimitation of statistically quantifiable morphological characters. This is compensated for partly by their highly diagnostic nature and limited phenotypic plasticity.

C. COMMUNITY RECONSTRUCTIONS

Reconstructions of Early Tertiary plant communities have tended to rely heavily on extrapolation of ecological tolerances from nearest living relatives. Recent approaches have differed in several ways (see discussion in Scott and Collinson, 1983; Collinson and Scott, 1987a,b). Information from ecological anatomy (section IV.D) and palaeophysiology (Section IV.I) should become increasingly important in addition to taphonomic evidence.

Taphonomic studies on modern vegetation have provided clues concern-

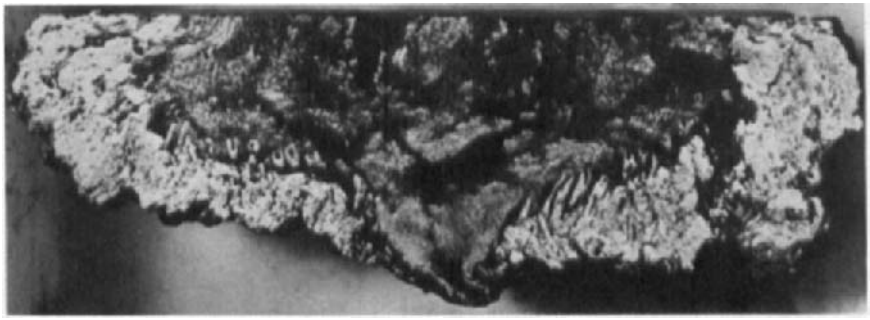

Fig. 38. Polished surface of a median longitudinal section of the basal portion from a previously *in situ* palm stump showing the main and adventitious roots departing from the stump base. Pyrite permineralization, Palaeocene, England, × 0.3.

ing many aspects of bias in the fossil record (e.g. Spicer, 1980, 1981; Collinson, 1983c; Scheihing and Pfefferkorn, 1984; Ferguson, 1985; Rex, 1986; papers in Broadhead, 1986; Gastaldo, 1989). These indicate, for example, that many leaf floras may be of very local origin and need not reflect regional climax vegetation (see Section IV.F). Assemblages that are deposited following storms, vulcanicity or after considerable transport from their sites of growth, may have diagnostic characteristics which will permit recognition of these facts. Simple interpretation of such assemblages as if they represented a single local community can thus be avoided. A great deal more modern taphonomic work remains to be done before reliable diagnostic features of all aspects of bias will be understood. This should be a high future priority for plant palaeocologists. Much greater care is now taken to consider the sedimentological context of Early Tertiary floras along with evidence from associated biota.

Collinson and Hooker (1987) attempted to integrate a range of evidence prior to reconstructing the general pattern of community change through the Early Tertiary of southern England. Late Palaeocene communities included reedswamp with palms (known from *in situ* stumps, Fig. 38) and disturbed fluvial floodplains with pioneering shrub/tree communities and patches of more stable vegetation. Early and Middle Eocene times were characterized by widespread forest of tropical aspect and with a fringing coastal *Nipa*-dominated mangrove including some Rhizophoraceae, e.g. *Ceriops* (Wilkinson, 1981). Changes began in the early Late Eocene culminating in the latest Late Eocene when a *Typha/Acrostichum*-dominated mire with a few isolated tree islands became established over a wide area. Certain aspects of this reconstruction, e.g. the *Typha/Acrostichum*-dominated mire, are better established than others, e.g. the nature of the tropical aspect forest of the Early Eocene London Clay flora. Wetland vegetation is naturally more easily reconstructed so that the marginal mangrove of London Clay and early Middle Eocene time is more clearly understood than the forest on the land-

ward side. Sedimentological studies are essential but do not assist reconstruction of communities when the fossils were accumulated, after considerable transport, in a marine shelf deposit like that of the London Clay. The southern English Tertiary is particularly well suited to attempts to unravel the intricacies of community evolution. A superposed, richly fossiliferous sequence of strata, mainly deposited in coastal floodplain environments, spans the latest Palaeocene to earliest Oligocene (for further discussion see Collinson and Hooker, 1987). Current work in my laboratory by Ros Singer on palynofacies aims to provide additional evidence on the ancient plant communities and depositional environments. In future we hope to incorporate new evidence from palaeosols with our data along with expanded information on other faunal remains.

Elsewhere only scattered sites may be available for study and their interpretation may be complicated by orogeny, vulcanicity, problems of correlation, etc. Careful assessment of sedimentological evidence helps to permit ecological interpretations of fossil floras from these individual sites. In the Middle Eocene of Messel, West Germany, additional evidence from exceptional preservation of the biota (Figs 44 and 47, and Section IV.E) should eventually allow reconstruction of much of the ancient ecosystem around this lake (see Collinson, 1988b, and other papers in the volume; Schaal and Ziegler, 1988). Other examples of preliminary analyses of Early Tertiary floras in their sedimentological context include Potter and Dilcher (1980), Basinger (1984) and earlier references; Basinger and Christophel (1985); Hill and Macphail (1983); Holy (1978).

A more detailed example is provided by Bown *et al.* (1982) where a combination of evidence from many disciplines refuted previous suggestions of a sparsely vegetated sahelien environment for the Oligocene Fayum of Egypt. The landscape was actually a forest, bordered by a coastal mangrove.

In another example Christophel *et al.* (1987) examined microfloras, megafloras and their sedimentological context in the Eocene of Victoria, Australia. As a result of this careful study a possible modern model for the ancient vegetation (extant lowland rainforest of Noah Creek, northeast Queensland) was recognized. Taphonomic studies in the modern vegetation supported the model (see Section IV.G.1 for a further discussion).

Manchester (1986) used a variety of evidence to interpret the Palaeoecology of the Clarno plane (see Section IV.A.3). This, and *Cercidiphyllum*-like plants were co-dominants in a pioneering tree community developing on terrain devastated by an earlier ash fall. *In situ* trees are widely spaced and have low stature, poorly differentiated soils are present and leaves and wood of the two dominant trees are abundant in the sediments. Rarer associates of this community were palms and Lauraceae. A similar pioneering community, though dominated by the *Cercidiphyllum*-like plants, is indicated by numerous seedlings preserved in the Palaeocene of Canada (Stockey and Crane, 1983; Crane and Stockey, 1985; see also Section IV.D)

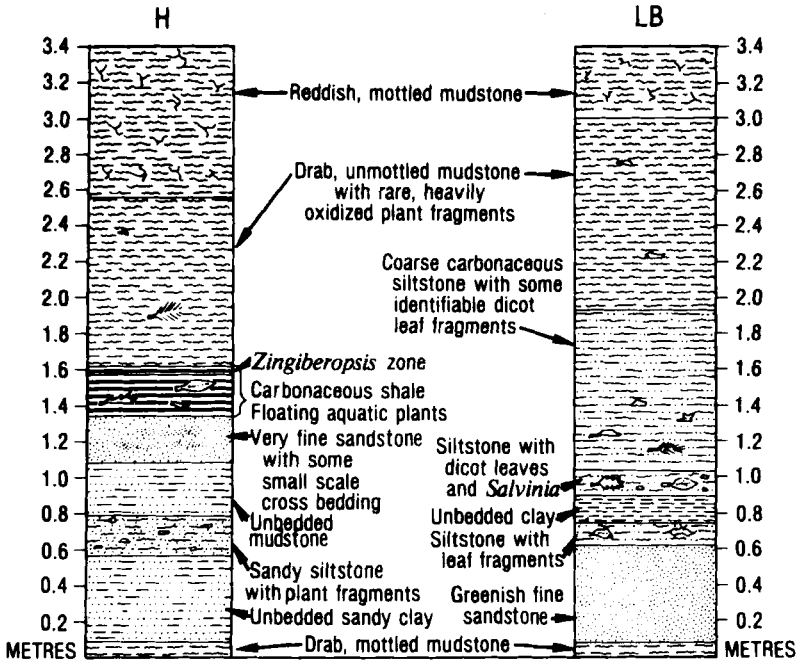

Fig. 39. Examples of stratigraphic sections, from two localities (L and HB) in the lower Eocene Willwood Formation, Rocky Mountains, USA. The sections pass through lenticular units deposited during the infilling of abandoned channels. Contained plant remains are interpreted in their sedimentological context (along with evidence from one other locality) to produce the reconstruction in Fig. 40. From Wing (1984) with permission of the author and publisher, The Society of Economic Paleontologists and Mineralogists.

Wing (1984) used sedimentological and palaeobotanical evidence to reconstruct vegetational succession in several sub-communities from the Early Eocene of the Bighorn Basin, USA. Figure 39 shows the stratigraphic sections, logging sedimentary features and plant occurrences, of one of the units. Figure 40 shows the reconstructed vegetational succession of the infill of an abandoned channel pond. Other sedimentary units demonstrate the succession over a larger area from floodplain marsh to swamp forest and drier ground floodplain forest (Wing, 1984). Wing and Bown (1985) show how this approach might be further exploited.

The petrified forests of Yellowstone National Park, USA, have long been regarded as examples of Eocene forests preserved *in situ* (Dorf, 1964). Numerous levels containing upright tree stumps can be observed in an exposure at Specimen Ridge. However, following the recent eruption of Mount Saint Helens many observations were made concerning tree stump and trunk deposition in comparable modern volcanogenic sediments (e.g. Fritz, 1980; Fritz, 1983; Fritz and Harrison, 1985). These showed that upright tree stumps could be transported and re-deposited so as to appear as if they had been buried *in situ*. A palaeoecological analysis of the woods and pollen from

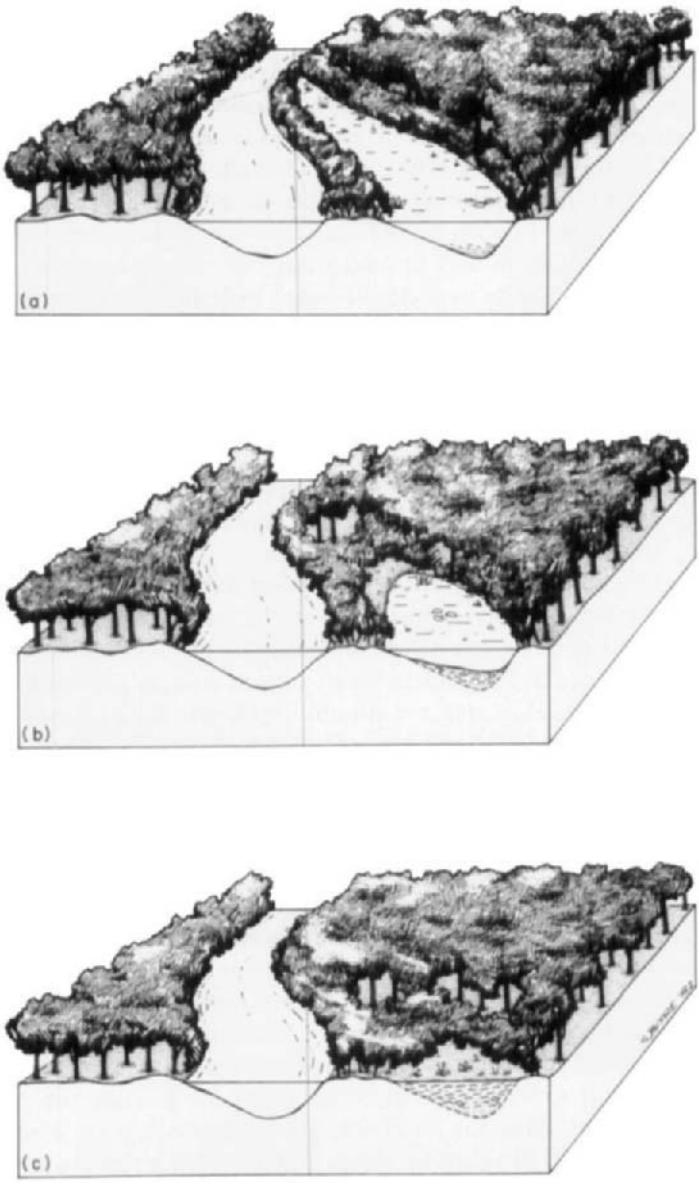

Fig. 40. Reconstruction of vegetation succession in an abandoned channel using information from Fig. 39. (a) Following channel abandonment the open waters are colonized by floating plants such as *Salvinia*. Riparian shrubs and trees occur around the channel and the margins support marsh vegetation; (b) the hydrosere proceeds with wetland vegetation, dominated by *Zingiberopsis*, encroaching across the channel; (c) finally the infill sediments dry out and are colonized by a floodplain forest. Succession in the general floodplain area has been interpreted from other sedimentary units to include a floodplain marsh; swamp forest and drier ground floodplain forest. From Wing (1984) with permission of the author and publisher, The Society of Economic Paleontologists and Mineralogists.

the sediments at Yellowstone (Chadwick and Yamamoto, 1984) emphasized earlier observations of "mixed" floras in single levels. Growth ring evidence and leaf and pollen floras all implied that mixtures of tropical and temperate plants existed at individual stratigraphic horizons (see also Fritz, 1981). These factors seemed to support suggestions that the "fossil forests" were not preserved *in situ*. Retallack (1981) and Yuretich (1984) responded to these observations by demonstrating that some stumps were unquestionably in place as shown by fine roots embedded in fine grained sediments below conglomerates which flowed around the trunk bases. Furthermore, well-differentiated soil profiles were developed at these horizons. One example of these data and associated stratigraphy is given in Fig. 41. Other tree logs were clearly horizontal and obviously had been transported (Fig. 41). Ammons *et al.* (1987) have provided further support for the *in situ* nature of some stumps by documenting cross identification of growth ring signatures at some levels. Karowe and Jefferson (1987) have documented incipient silicification in trees buried *in situ* on Mount Saint Helens. Present evidence suggests therefore that the Yellowstone forests are much more complex than previously thought. They seem to include trees buried *in situ* and others probably transported from elsewhere (for further discussion see Wing, 1987). They represent an excellent resource for future study combining multidisciplinary approaches in order to reconstruct the ancient forest communities.

Further evidence of Palaeogene forest communities is provided by exceptional preservation of *in situ* tree stumps and forest floor litter in the Late Eocene of Axel Heiberg Island in the Canadian Arctic (Basinger, 1987a,b,c; Francis and McMillan 1987; B. A. Lepage, personal communication, 1988). Preliminary analyses suggest that a floodplain forest was dominated by Taxodiaceae (*Metasequoia* and *Glyptostrobus*) with subordinate *Cercidiphyllum*-like plants, Betulaceae and *Osmunda*. Regional vegetation included many conifers especially Pinaceae (Fig. 15) and deciduous dicotyledons of the Fagaceae, Juglandaceae and other Betulaceae.

Some modern communities have a very poor fossil record. Grasslands, with herbaceous plants, shedding few organs except pollen and fruits, and these only near ground level, stand little chance of good preservation in the megafossil record. Thomasson (1985, and references therein) has provided the only unequivocal evidence of fossil grassland grasses, the earliest of which, if correctly dated at 23 mybp, are earliest Miocene. They do not become diverse until the Late Miocene. Other evidence (briefly reviewed by Collinson and Scott, 1987a) including pollen analysis implies a Miocene or later origin for extensive grasslands (see also Axelrod, 1985). Retallack (1986) has suggested that fossil soils may provide an alternative line of evidence. In Badlands National Park, South Dakota, USA, he has documented a progressive opening up of vegetation based on palaeosols. Wooded habitats in the Early Oligocene gave way to savannah (open wooded habitats) in the early Late Oligocene and to open grasslands appearing in the mid Late

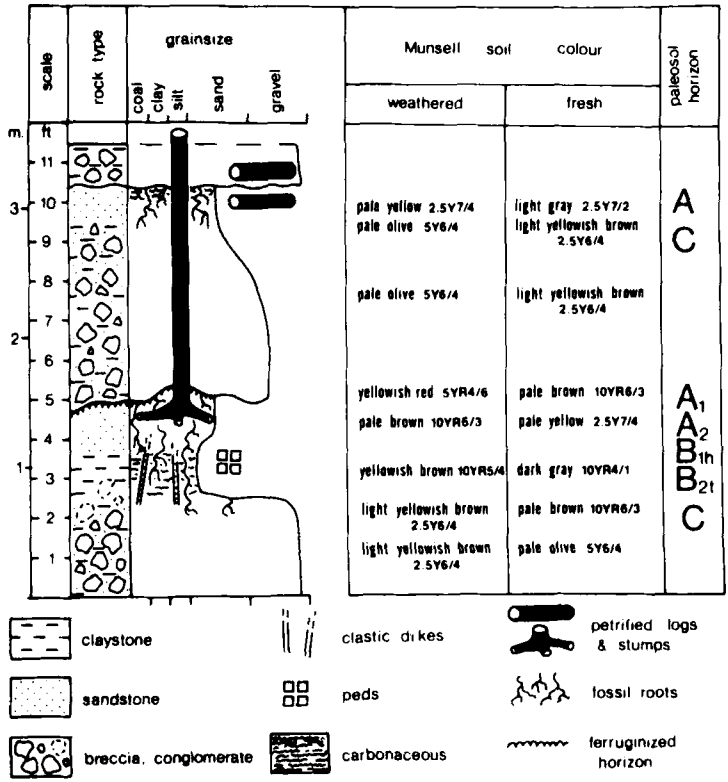

Fig. 41. Well-differentiated palaeosol, with *in situ* tree stump, Eocene, Specimen Ridge, Yellowstone National Park, USA. From interval 90–94 m of Fritz (1980). From Retallack (1981) with permission of the author and publisher, The Geological Society of America.

Oligocene and dominating in the latest Late Oligocene. This evidence indicates that grasslands existed prior to specialized mammalian grazers and hence that grazing pressure was not the primary reason for the evolution of grasslands. Climatic change is offered as a possible explanation with grasses more able to withstand drier conditions. Retallack (1988) further emphasizes the value of an integrated approach utilizing palaeosols, vertebrate and plant fossil occurrences to interpret the deposits of the Badlands area.

D. ECOLOGICAL ANATOMY

Various features of plant anatomy and morphology may provide clues of an ecological nature, though these are relatively rarely applied to plant fossils. Examples include: r- versus K-selectivity and biotic versus abiotic dispersal deduced from fruit and seed morphology (Tiffney, 1985c, 1986b); pollination

vectors and biology deduced from floral anatomy (Crepet, 1985; Crepet and Friis, 1987; Friis and Crepet, 1987); wood anatomy and growth rings indicating growth conditions (Creber and Chaloner, 1984a,b, 1985; Wheeler *et al.*, 1987; Wolfe and Upchurch, 1987a); leaf physiognomy and cuticle structure indicating growth conditions (Upchurch and Wolfe, 1987; Wolfe and Upchurch, 1987a,b; Wolfe, 1987); internal anatomy indicating hydrophytic adaptation (Collinson, 1988a); and anatomical features which indicate other aspects of physiology (Sections IV.G.3 and IV.I).

Most of these relationships are complex and care must be taken when extrapolating from morphology to function, e.g. ornamented and sculptured epidermis (seeds and leaves) may act to (a) increase water repellency, (b) reduce contamination (by dust and pathogens), (c) control surface temperature (by modifying turbulence and heat exchange with surrounding air) or (d) limit attack by microinvertebrates (Barthlott, 1982; Juniper and Southwood, 1986). Nevertheless as increasing experimental data accumulate for modern plants, application of ecological anatomy in interpretation of fossil plants and their role in communities should expand. Collinson and Scott (1987a,b) and Knoll and Niklas (1987) provide some general discussion on aspects of this topic. A few examples from Tertiary floras are cited below as an indication of the potential value of the method.

Tiffney (1985c) and Friis and Crepet (1987) reviewed the evolution of fruit and seed characteristics in flowering plants. Early Late Cretaceous fruits were mainly dry with apparently no special modifications for dispersal. The variety of dry fruits increased in the latest Cretaceous with some special modifications for wind dispersal. Fleshy fruits did not become common until the Early Tertiary. Cretaceous disseminules or propagules were smaller than those of the Early Tertiary when size increase implies an increased range of dispersal methods. More detailed studies of some flowering plant families including the Juglandaceae (Manchester, 1987), Platanaceae (Manchester, 1986) and Betulaceae (Crane, 1981; Crane *et al.*, 1990; Crane, 1989b) show an increasing trend towards modification for animal dispersal during the Tertiary (for further discussion see Section IV.E.2).

The smaller seed size of early flowering plants (Tiffney, 1985c) has lent support to their interpretation as early successional colonizers. An elegant example of this is in the Early Tertiary is found in the Palaeocene of Canada (Stockey and Crane, 1983; Crane and Stokey, 1985). Here in one narrow horizon numerous seedlings (Fig. 21a,b,c) have been preserved. Seeds of the parent *Cercidiphyllum*-like plant (Fig. 21d) were small, produced in large numbers and exhibited apparently synchronous epigeal germination which has been preserved over an extensive area. *Platanus*-like seedlings are also present in the same association. Other plant fossils include herbaceous and woody wetland species (e.g. *Azolla*, *Equisetum* and taxodiaceous conifers). The *Cercidiphyllum*-like plant can therefore be interpreted as an opportunistic "weedy" colonizer of disturbed floodplain habitats. A similar situation

can be inferred for occurrences in the Palaeocene of England (Crane, 1984) and in the Eocene of Oregon (Manchester, 1986).

Ecological aspects of the wood anatomy of modern plants have only recently been investigated (see references in Wheeler *et al.*, 1987; Wolfe and Upchurch, 1987a), and have rarely been applied in palaeobotany. A thorough survey of these aspects of wood evolution as a whole and/or in individual families or genera would be of considerable value. In addition, aspects of wood anatomy could become accepted elements in community reconstructions. Features which can be of value in angiosperm woods include parenchyma distribution, vessel element diameter and number, vessel element length, and vessel distribution (e.g. ring porosity). A "vulnerability value" (vulnerability to freezing or drought) and a "conductivity capability" (reflecting water need of the plant) can also be calculated (Wolfe and Upchurch, 1987a). Evidence to date suggests that several features of angiosperm woods, e.g. simple perforation plates, paratracheal parenchyma, short vessel elements and ring porosity, were rare or absent in the Early Tertiary. Most of these may be related to growth conditions implying reduced seasonality and more tropical conditions. In at least one example, the Clarno plane (Manchester, 1986, p. 208), the wood (*Plataninium*) is widespread in the North American Eocene. This wood has scalariform not simple perforation plates but is otherwise very similar to modern *Platanus*. A climatic explanation would be consistent with climatic conditions at that time deduced from other evidence. However another explanation would be that scalariform perforation plates are merely primitive for *Platanus*. Comparative studies of wood from contemporaneous deposits of known temperate climate would be the only reliable means to resolve this question. Bande and Prakash (1984), in their extensive review of Indian Tertiary woods, did document trends of increasing specialization. They considered that all the floras studied existed under similar warm, humid, tropical to subtropical conditions and hence discarded environmental influences as explanations for the changing anatomy.

One of the major aspects of ecological wood anatomy is that of growth rings. Their main use in palaeobotanical work is to infer climates and seasonality. Leaf physiognomy is also used to infer climates and both topics are discussed in Section IV.F.

Ecological aspects of floral biology are most frequently used to infer pollination vectors. These are considered in Section IV.E below.

E. ANIMAL–PLANT INTERACTIONS

Several aspects of animal–plant interaction may potentially be recorded in fossil floras. These involve both direct and indirect evidence. Major lines of evidence in Early Tertiary floras concern floral organization and pollination vectors, fruit and seed biology and dispersal vectors, and animal diet in its

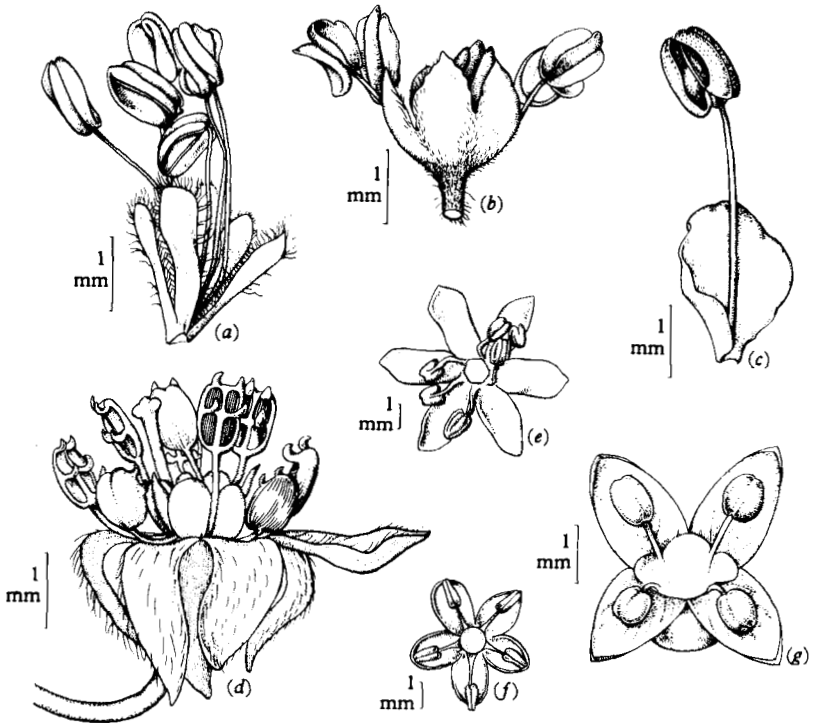

Fig. 42. Flowers from the Eocene Baltic Amber. (a)–(c), Various staminate flowers with simple perianth; (d) Lauraceae with valvate anther dehiscence; (e)–(g) isolated corollas with fused petals and attached stamens. From Friis and Crepet (1987) with permission.

broadest sense (which inevitably partly overlaps with pollination and dispersal biology).

1. Floral biology and pollination vectors

Until comparatively recently little was known about fossil flowers. Tertiary flowers are now well known from the Indian Deccan Traps permineralized flora (latest Cretaceous–Eocene); the late Eocene/Oligocene Baltic amber (Fig. 42); and from a range of sites with compression fossils (Fig. 43a) in the Early Tertiary of North America (Crepet, 1985; Friis and Crepet, 1987; Taylor, 1988a). In addition flowers are known from the permineralized cherts of the Canadian Eocene but few (e.g. Fig. 43b) have been published (Basinger, 1976; Basinger and Rothwell, 1977; Stockey, 1987). A few flowers (e.g. Figs 30 and 31) are recorded from the Australian Eocene (Basinger and Christophel, 1985; Christophel *et al.*, 1987). Some flowers associated with "whole plants" are discussed in Section IV.A. An exciting assemblage of flowers occurs in the Middle Eocene site of Messel, West Germany. Here exceptional preservation has occurred in an algal rich oil-shale. Over 50 flowers have been collected but most are represented by very few specimens

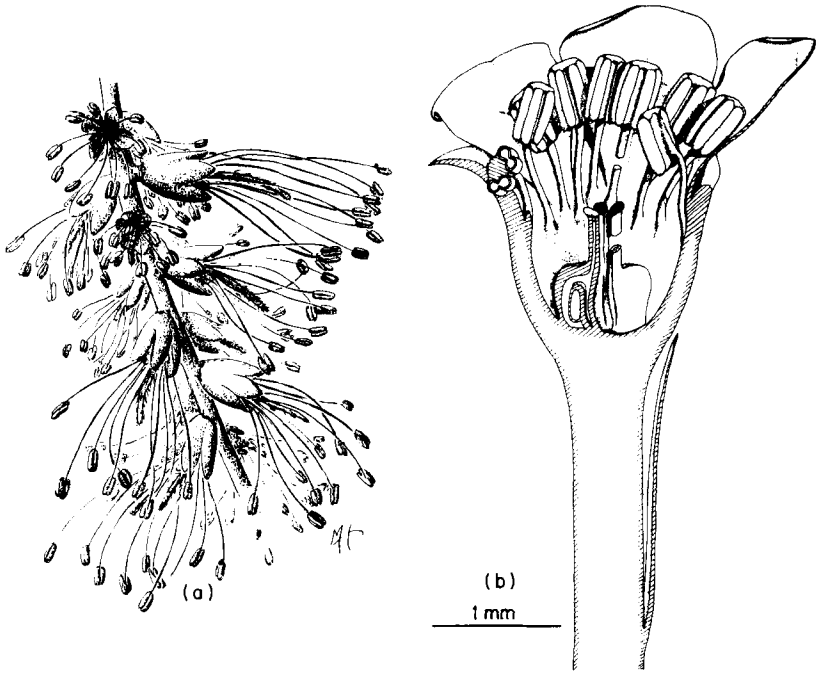

Fig. 43. Reconstructed flowers from the Eocene of North America. (a) *Eomimosoidea* (Leguminosae, Mimosoideae) a compressed inflorescence from the USA, average length of corolla 1.95 mm. From Crepet and Dilcher (1977) with permission. (b) *Palaeorosa* (Rosaceae) a permineralized flower from Canada. From Basinger (1976) with permission.

(Schaarschmidt, 1985, 1986, 1988; Schaarschmidt and Wilde, 1986). Only the palms have yet been described in detail (Schaarschmidt and Wilde, 1986). One form (Fig. 44) is represented by more than 1000 specimens. Compaction in the oil-shales has made details of the gynoecium difficult to observe but *in situ* pollen can be studied using fluorescence microscopy. The fruits developed from these flowers are also preserved and are the most abundant fruit type at the site (Collinson, 1988b, and in preparation). Unfortunately no assemblages of charcoalified flowers, such as those reviewed by Friis (1985b) which have provided so much information on Late Cretaceous flowers (e.g. Fig. 19), have yet been found in the Tertiary.

Friis and Crepet (1987), Crepet and Friis (1987) and Crepet (1985) have reviewed the Early Tertiary floral record. The types of floral organization which had already evolved by the Late Cretaceous are shown in Fig. 45. The general pattern for the Early Tertiary may be described as follows. In the Late Palaeocene several flower types are recorded which reflect a variety of innovations important in advanced insect pollination. These include (Fig. 45i) brush flowers (cf. Fig. 43a) of the Mimosoideae which suggest pollination by a faithful pollinator with a single kind of pollen in the appropriate

Fig. 44. Palm flower from the Mid-Eocene oil-shales of Messel, West Germany (Schaarschmidt and Wilde, 1986), × 10. Illustration courtesy of F. Schaarschmidt.

spot on its body. Papilionoid flowers (Fig. 45j) also occur at the same locality. These are the first record of strongly zygomorphic flowers, although weak zygomorphy occurred earlier. They have ornamentation on the wing petals which is associated with bee pollination in modern members of the tribe. The first record of unisexual insect pollinated flowers (Euphorbiaceae) occurs in the same deposit.

In the Early Eocene the first flowers with a tubular corolla occur. One of these forms is referable to the Gentianaceae and contains pollen of the type referred to the previously enigmatic dispersed pollen genus *Pistillipollenites*. (However, another flower form (Stockey and Manchester, 1988) with uncertain family affinities (not Gentianaceae) also contains this pollen.) The Gentianaceae flower had an open funnel form with free lobes of the corolla flattened into a landing platform. Other flowers of the same age have longer, narrower corolla tubes with narrower openings. Brush type flowers (Fig. 43a) also occur. Flowers from the Eocene Baltic amber (Fig. 42) are very diverse. The most common form (Fig. 42d) has anthers with valvate dehiscence. There are also numerous isolated fused corollas with attached stamens (Fig.

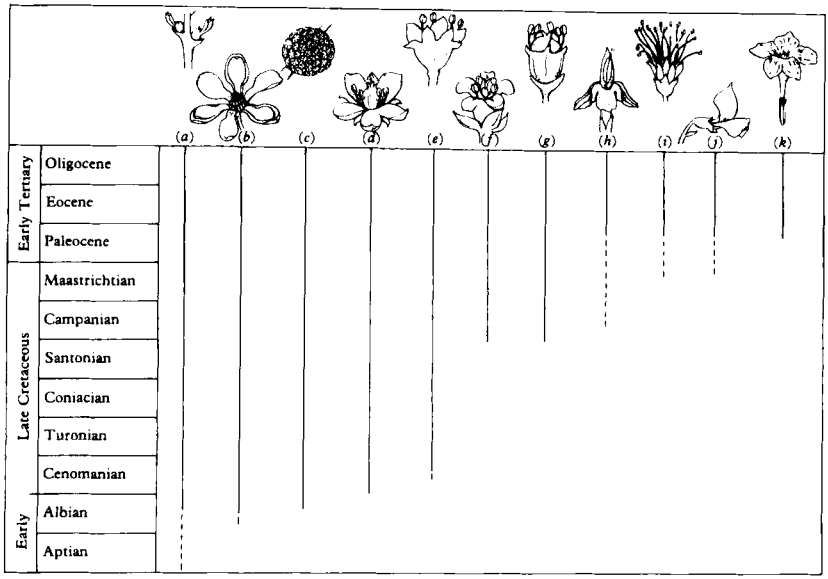

Fig. 45. Time of appearance of major floral types. Solid lines based on flower evidence, dashed lines inferred, usually from pollen. (a) Chloranthoid flowers; (b) magnolialean flower; (c) platanoid flowers; (d)–(g) actinomorphic, hypogynous and epigynous flowers with reduced numbers of floral parts, (f) and (g) sympetalous; (h) zygomorphic flowers; (i) brush flowers; (j) papilionoid zygomorphic flowers; (k) deep funnel-shaped flowers. Reproduced (with explanation modified) from Friis and Crepet (1987) with permission.

42e,f,g). Nectaries are known from Cretaceous polypetalous flowers but in the Deccan flower *Sahnianthus* they are within a floral tube. By analogy with living flowers, nectaries are assumed to have been present in the tubular corollas mentioned above.

This combined floral evidence suggests that advanced lepidopterans and bees were important pollinators during the Early Tertiary. Flowers with long tubular corollas were probably pollinated by long-tongued lepidopterans. Flowers with more open funnel-shaped corollas were probably visited by bees. Mimosoideae and Papilionoideae also had flowers closely adapted to bee pollinators. An even more specialized example has been found in the Middle Eocene. This malpighiacean flower has oil glands on the sepals and clawed petals. In modern representatives three tribes of anthophorid bees pollinate such flowers while reaching around the clawed petals to collect oil.

A further tantalizing glimpse of insect–plant pollinator interactions can be seen in the Middle Eocene of Messel. Many mammals are preserved as whole skeletons with stomach contents (Richter, 1987; Schaal and Ziegler, 1988; see also Section IV.E.2). Stomach contents of one of the bats showed that it fed exclusively on Lepidoptera and in one example the stomach contents also included a pollen clump (Richter and Storch, 1980) presumably from the plant which one of the insects pollinated.

This diverse range of advanced insect pollination mechanisms indicates that insect pollination was a very important factor in the Early Tertiary. The advantages conferred on angiosperms by the receptive stigma and closed carpel became fully exploited through co-evolution with these advanced pollination vectors. Persistence of small isolated populations must have decreased extinction rates yet cross fertilization was facilitated, increasing variation and hence speciation rates (see Crepet, 1983, 1985).

Other biotic pollinators including birds, bats and other mammals may not have been important in the Early Tertiary. Their fossil record implies a later involvement in pollination biology (Crepet, 1985).

Abiotic wind pollination was also very widespread in Early Tertiary plants. The Baltic amber includes several staminate flowers with a simple perianth (Fig. 42a,b,c). Members of the Betulaceae, Juglandaceae, Fagaceae and Platanaceae documented above (Section IV.A) were all important wind pollinated elements in Early Tertiary floras.

2. Fruit and seed dispersal and vertebrate diet

Fruit and seed diversification has been reviewed by Tiffney (1985c, 1986a,b) and Friis and Crepet (1987) and important aspects have been noted earlier (Section IV.D). In several well-documented families, Juglandaceae, Betulaceae and Fagaceae (see Section IV.A), the Early Tertiary members seem to have been less specialized for animal dispersal than are their modern relatives. However, the large dry, often animal-dispersed, nuts in these families do occur in Early Tertiary strata. Manchester (1987) considered that, in Juglandaceae, evolution of animal dispersed fruits could be related to evolution of rodents. Rodents which might have been capable of dispersing such nuts had evolved by the latest Palaeocene (Luckett and Hartenberger, 1985). An exciting discovery in the Tertiary of southern England (M. E. Collinson and J. J. Hooker, in preparation) are seeds of *Stratiotes* (Fig. 46c) which have been gnawed by a small rodent.

According to Tiffney (1985c) large propagules adapted for animal dispersal were rare prior to the Early Tertiary which saw a diversification of propagule size and form. Fleshy fruits were also rare prior to this time. Fleshy fruits are largely inferred in the fossil record from stones and seeds whose affinites are with near living relatives with fleshy fruits. In view of some of the differences in reproductive biology known from Early Tertiary members of modern groups this may be an unreliable and undesirable extrapolation. The exceptional preservation at the Eocene site of Messel has, however, provided conclusive evidence of former soft fruits in at least two members of the Vitaceae (grape vine family) and one Menispermaceae (Collinson, 1988b). The Vitaceae are particularly exciting in view of the preservation of Vitaceae seeds in the gut contents of a *Propalaeotherium* (cf. Fig. 47), an early horse relative. Several specimens of this mammal are known with gut contents preserved which include various leaves and Vitaceae seeds (Richter, 1987; Schaal

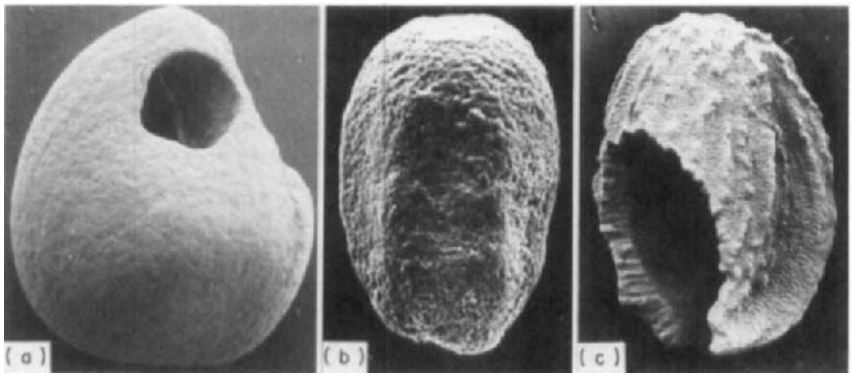

Fig. 46. Evidence of animal–plant interaction. (a) *Rutaspermum* seed with insect exit hole (Collinson and Gregor, 1988), mid-Eocene, Germany, × 11.16; (b) termite coprolite, Palaeocene, southern England, × 116.25; (c) *Stratiotes* seed gnawed by small rodent, Late Eocene, southern England, × 8.37. All SEM.

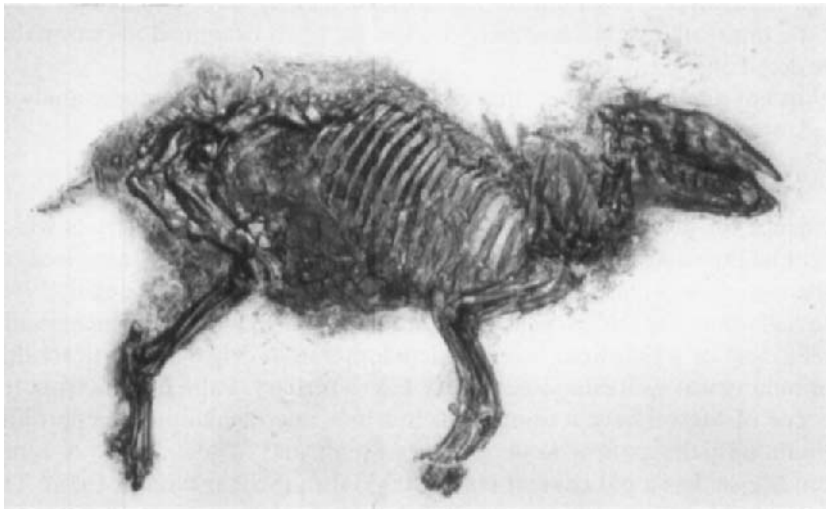

Fig. 47. Skeleton with gut contents containing plants (Richter, 1987). *Propalaeotherium parvulum* an early horse relative from the mid-Eocene of Messel, Germany. Shoulder height approximately 30 cm. Senckenberg Museum collection 1982. Illustration courtesy of J. L. Franzen. Reproduced with permission from J. L. Franzen and Natur-Museum Senckenberg. Photograph E. Pantak/U. Wegmann, Senckenberg Museum.

and Ziegler, 1988). The teeth of this mammal, on the basis of modern dental analogy (see Collinson and Hooker, 1987), indicate a diet of soft fruit and leaves (in contrast to the coarser diet of modern grazing horses with high crowned teeth). It is valuable to have the inferred diet confirmed by the gut contents and the documentation of soft tissues in fossil Vitaceae fruits. A number of other Messel mammals contain gut contents (Richter, 1987; Schaal and Ziegler, 1988) but most have not yet been determined in detail.

Messel gut contents were used by Collinson and Hooker (1987) to confirm other inferences from modern dental analogy. Mammalian diets were then examined in the context of vegetational change in the Early Tertiary of southern England. A broad correlation was established between mammals adapted for eating soft fruit and soft browse in forested environments of tropical aspect and those adapted for fibrous browse in more open habitats including large tracts of herb-dominated wetlands. The opening up of these habitats occurred gradually during the Late Eocene.

Wing and Tiffney (1987a,b) have recently considered the reciprocal interaction of tetrapod herbivory (meaning all aspects of plant eating) and angiosperm evolution. They note the near absence of large herbivores in the Palaeocene and their low diversity until the Late Eocene and see the interval as one of stasis in this interaction following the K/T boundary upheaval. The stasis was further interrupted by the opening of habitats following the terminal Eocene climatic deterioration, resulting again in the dominance of large herbivores. As Collinson and Hooker (1987) have documented, the "static interval" exhibits a variety of changes when examined in reasonably fine detail on a local scale.

Ancient diet may also be inferred from geochemical and isotopic analyses of plant and animal material. This is discussed in Section IV.I.

3. Other insect–plant interaction

Evidence for insect–plant interaction such as eaten leaves and fruits which might be expected amongst Early Tertiary floras is surprisingly rare. Seeds of Rutaceae show small escape holes (Fig. 46a) (Collinson and Gregor, 1988) and leaf mines are also recorded (Crane and Jarzembowski, 1980; Rozefelds, 1988) most of which were probably lepidopteran in origin and indicate that leaf mining was well established in the Early Tertiary. Palm flowers from the Eocene of Messel have feeding trails in which microlepidopteran coprolites containing palm pollen occur (Schaarschmidt and Wilde, 1986). A beetle from Messel has a gut content containing pollen (Schaarschmidt 1988). The absence of a more extensive record of these and similar interactions is probably due more to lack of recognition than any other factor.

One interesting example (M. E. Collinson, in progress) is provided by small hexagonally faceted cylinders (Fig. 46b) which occur in the Early Tertiary of southern England (Palaeocene and Eocene). These are indistinguishable from faecal pellets of modern termites which utilize a gut flora to digest wood. Such coprolites occur from the Wealden onwards consistent with the known megafossil record of the group (Jarzembowski, 1981).

F. PALAEOCLIMATIC RECONSTRUCTIONS AND INTERPRETATION OF LARGE SCALE VEGETATIONAL PATTERN

In the past interpretations of climatic conditions from Tertiary fossil floras

have relied heavily on extrapolation from the ecological tolerances of nearest living relatives. Over the last 20 years the approach has shifted towards physiognomic methodology. Anatomical and morphological features of woods and leaves reflect the conditions under which they have developed and thus appear in the same environment regardless of the systematic affinity of the plants. Other aspects of plant palaeophysiology are potential, but largely unexploited, indicators of past climate and atmospheric composition. These include the $^{13}C/^{12}C$ isotope ratio of fossil material and possibly stomatal density (Moore, 1983; Raven and Sprent, 1989). These are discussed in Section IV.I.

1. Leaf Physiognomy

Methodology. Leaf physiognomy has been used extensively to interpret ancient climate and vegetation pattern by Wolfe (1971, 1978, 1985, 1986, 1987, 1990), Wolfe and Upchurch (1986, 1987a,b) and Upchurch and Wolfe (1987). These studies have been based largely on the documentation (Wolfe, 1979) of relationships between leaf form, climate and vegetation in eastern Asia. Forest types could be classified according to mean annual temperature (MAT) and mean annual range of temperature (MART). Some slight modifications were made to the 1979 work and the most recent interpretation was given by Wolfe (1985, 1986, 1987, fig. 1 in all cases) and is reproduced here as Fig. 49. As would be expected the relationships are complex and further influenced by rainfall variation but may be generalized as follows:

1. Megathermal vegetation. MAT > 20°C. Tropical and paratropical rain forest. (Fig. 48a–c).

Leaves entire-margined; large (notophyllous to typically mesophyllous*); many with enlongated apices (drip tips); high density of fine venation; diversity of liana leaves (which are often broad, with a cordate base and palmate venation); most leaves are coriaceous (indicative of evergreen habit).

2. Mesothermal vegetation. MAT 13–20°C. Broad-leaved evergreen forest. Subdivided according to MART into microphyllous broad-leaved evergreen forest, notophyllous broad-leaved evergreen forest and mixed broad-leaved evergreen and decidous forest with, increasing MART.

Leaves intermediate. Percentage of entire-margined forms correlates with decreasing temperature. In eastern Asia megathermal/mesothermal boundary about 60% entire-margined decreases about 3%/°C. (In Southern hemisphere boundary at 68–70% and decreases 4%/°C.) Serrate margins also typical of deciduous leaves.

3. Microthermal vegetation MAT < 13°C. Mainly mixed coniferous and mixed northern hardwood forest. Includes mixed broad-leaved evergreen

* Leaf size classification. Length: < 8 cm, microphyllous (MI); 8–12 cm, notophyllous (NO); > 12 cm, mesophyllous (ME) (includes larger sizes (MC) macrophyllous, > 25 cm, and megaphyllous).

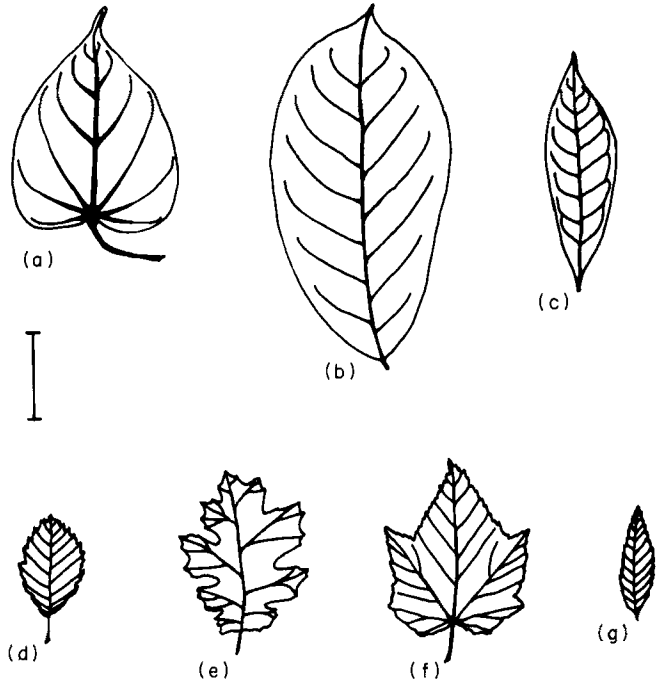

Fig. 48. Leaf physiognomy characteristic of vegetational types. Only primary and secondary veins are shown. (a)–(c) Leaves from megathermal vegetation, tropical rain forest; (d)–(g) leaves from microthermal vegetation, e.g. mixed northern hardwood forest and/or mixed broad-leaved deciduous forest. Redrawn following Wolfe (1978, 1985). Scale bar, 5 cm.

and coniferous forest; mixed mesophytic forest and mixed broad-leaved deciduous forest.

Leaves of mixed northern hardwood forest (Fig. 48d,e,f,g). Leaves have non-entire margins, many are lobed, most are serrate; leaves small (notophyllous to microphyllous); drip tips absent, lianes not diverse; fine-venation density low; leaves typically thin (indicative of deciduous habit).

There are some differences between these relationships established in eastern Asia and comparable patterns elsewhere, especially North America (Wolfe, 1979; Wolfe, 1987, fig. 2). The most obvious difference is that a large area of eastern North America that might be expected to have broadleaved evergreen forest has broad-leaved deciduous forest. This is now considered to be the result of selection for deciduousness at the K/T boundary (Wolfe, 1987; Wolfe and Upchurch, 1986, 1987a,b). Dolph (1979) and Dolph and Dilcher (1979) found a much less reliable correlation between climate and leaf physiognomy in Costa Rica. Their criticism was dismissed by Wolfe (1985, p. 359) as based largely on misinterpretations or inadequate sampling of modern vegetation.

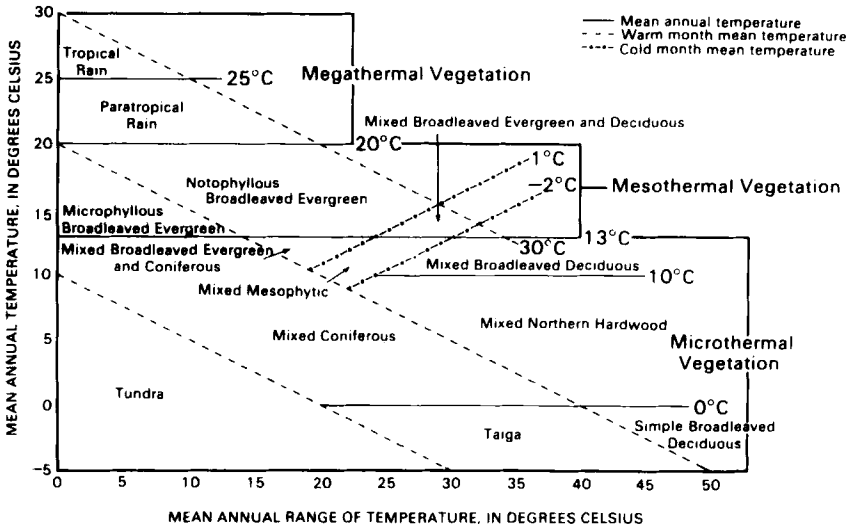

Fig. 49. Temperature relations of major forest types based on extant vegetation in eastern Asia. From Wolfe (1987) with permission of the author and publisher, The Paleontological Society.

Application. Wolfe and Upchurch (1987a) have recently presented their latest refinement on application of the method in their work on Cretaceous floras. Discussion of the method and potential problems are given here and in the following section. Further refinement (increased modern data base and multivariate analytical method) is given by Wolfe (1990).

The leaf margin (percentage entire versus serrate) criterion is used to provide temperature estimates. It is necessary to decide which scale to use. The Southern hemisphere scale (4% increase in entire-margined species indicates 1 C increase in MAT; megathermal/mesothermal boundary 68–70% entire) exists in modern mainly evergreen vegetation whereas the Northern hemisphere scale (3%/°C; boundary 60% entire) exists in largely deciduous vegetation. Wolfe and Upchurch (1987a) and Upchurch and Wolfe (1987) chose the Southern hemisphere scale for their Cretaceous work as Cretaceous vegetation was also low in deciduous forms and included many conifers like the modern southern hemisphere. This scale also gives a more conservative estimate of temperatures.

Leaf size reflects the available water during the growing season and hence is used to infer rainfall. Leaf size for fossil assemblages is expressed as a leaf-size index for dicotyledonous leaves. The index used by Wolfe and Upchurch (1987a) is as follows: $4 \times \%MC + 3 \times \%ME + 2 \times \%NO + 1 \times \%MI - 100$ divided by 2. For extant megathermal rain forests this index approaches 75. The largest leaves are found in the understory of multistratal closed-canopy forests today.

Other physiognomically useful features include: attenuated apices (drip

tips) which are common in understory rain forest leaves; liana leaves, with cordate-base of the lamina (Fig. 48a), lianas are particularly abundant in multistratal closed-canopy forests; and the thickness or coriaceous nature of the evergreen versus the thin deciduous leaf, deciduous plants occurring in disturbed habitats, areas which have pronounced cold or dry periods and in areas with low light levels (for further details see Wolfe and Upchurch, 1987a). Cuticles also exhibit physiognomically useful features (Wolfe and Upchurch, 1987b), e.g. being thicker and more pubescent in drier or colder situations.

Requirements for reliable use. Successful application of this approach requires that:

(a) Vegetation was in equilibrium with climate, i.e. there was climax vegetation.

Vegetation of disturbed habitats may give an erroneous physiognomic signal. Stream sides present one potential problem (see (b) below). Furthermore, when studies are made in areas of known intense volcanism or other catastrophic disturbance (e.g. much of the North American Early Tertiary and the K/T boundary) this is also a problem. Wolfe and Upchurch (1987a, pp. 62–64) note that use of the leaf margin criterion in Palaeocene megathermal and mesothermal vegetation of the USA produced very low temperature estimates. These were incorrect because the vegetation was anomalously deciduous, hence serrate-margined, during recovery after the K/T event (see Sections IV.F.1 and II.B.1).

(b) The sample is fully representative of the ancient climax vegetation. Taphonomic or collecting bias must be minimal.

In practice this largely rules out analyses based on past literature or museum collections. Furthermore, modern taphonomic studies (e.g. Spicer, 1981; Ferguson, 1985; Gastaldo, 1989) have shown that representation of a vegetation in a leaf assemblage may be strongly biased. The most obvious bias is in favour of streamside vegetation. Physiognomy of leaves may indicate their streamside origin (Wolfe, 1985, 1986, 1987; Wolfe and Upchurch, 1987a,b), but these are of little or no value for climatic interpretation as their morphology does not reflect climate. Wolfe (1985, 1986) noted that North American Palaeocene vegetation is of little value in climatic interpretations because most North American Palaeocene floras represent floodplain (i.e. stream-influenced) vegetation. Vegetation outside this local influence, e.g. on interfluves, should be analysed so the sedimentological data and reliable community reconstructions discussed in Section IV.C become crucial. Overbank deposits may provide the most reliable assemblages for physiognomic studies (Wolfe and Upchurch, 1987a; Burnham, 1989). Channel assemblages and lacustrine assemblages are generally biased in such a manner as to make them inappropriate for physiogonomic analyses unless supplemented by data from other depositional environments (Burnham, 1989; Gastaldo, 1989; Spicer and Wolfe, 1987). Lacustrine deltas in low-energy environments may

preserve two discrete leaf-beds. A lower bed which reflects lakeside vegetation and an upper bed which reflects vegetation growing upstream (Spicer and Wolfe, 1987).

Other more subtle bias may also occur, e.g. in favour of smaller sun leaves from high in the canopy from where they may be more easily blown to depositional environments. The relative timing of flood events and leaf fall in deciduous plants will influence preservation of the more delicate deciduous leaves. Individual, tougher evergreen leaves may have a higher fossilization potential than thinner deciduous leaves. However, seasonal shedding of large numbers may aid survival of deciduous forms (perhaps through "saturation" of the decomposer niche). A given leaf assemblage from an area of mixed evergreen and deciduous vegetation could well be strongly biased in favour of deciduous forms.

Many more studies are needed to identify bias introduced in potential fossil plant accumulations, such as leaf litter, in a variety of depositional environments from a range of vegetation types. Studies in tropical sites (e.g. Burnham, 1989) are particularly needed. Such work is currently being undertaken in the Australian rain forests (D. R. Greenwood, in preparation, personal communication, 1988) and preliminary results are reported by Christophel and Greenwood (1988, 1989).

(c) A large sample size (at least 20–30 leaf forms (species)) is essential (Wolfe, 1971; Wolfe and Upchurch, 1987a, p. 35) for reliable leaf margin analyses. In addition, large sample size will partly reduce the effects of any bias from (b) above and helps to ensure as full a representation of the ancient vegetation as possible.

(d) A number of sample localities are required to reduce the effects of local variation, both in the original vegetation and in the depositional environment. These should be closely contemporaneous as possible and should include a range of lithologies (see (b) above).

(e) Variation in palaeolatitude and palaeoaltitude must be taken into account when comparing floras from different ages or localities to interpret large scale climatic and vegetational change. In the former, continental movement may have shifted the site and the leaf floras merely reflect this shift, not a change in regional climate. In the latter, floras from higher altitudes would be likely to produce different physiognomic signals, reflecting only local conditions. Controlling factors, such as local rain shadow zones, must also be considered.

(f) Some understanding of the influence of evolutionary instability on foliar physiognomy would be necessary in order to interpret assemblages from periods of rapid diversification such as the Early Tertiary.

(g) Problems of the "species concept" in fossil material must be taken into account. See Section IV.B for some recent approaches.

These requirements represent the "ideal" situation which is unlikely to exist frequently, if ever, in the fossil record. Several factors suggest that, in

spite of the apparent problems, leaf physiognomic analyses do provide a general indication of climatic and hence vegetational change on a large scale. Firstly, the palaeoclimates inferred from the analyses broadly correlate with those inferred from independent physical means (see Section II.C; Wolfe, 1978, 1986; Upchurch and Wolfe, 1987).

Secondly, consistent patterns often emerge from samples in different areas (Wolfe, 1971, 1978, 1985, 1986; Upchurch and Wolfe, 1987). Recently the study of dispersed cuticles has been added to that of overall leaf form as an additional source of palaeoclimatic information (Wolfe and Upchurch, 1987b). Such cuticles have features (e.g. thickness and hairiness; but see Section IV.D) which may reflect climatic conditions. They partially solve some of the problems mentioned above and contribute additional data in the following manner: they can be recovered in large numbers from a relatively small sediment sample; they may occur in horizons where entire leaves are absent or difficult to extract whole (these may be crucial horizons deposited during a period of disturbance or change); where leaves and cuticles can both be studied, they provide mutual confirmation as the dispersed cuticles can be attributed to specific whole leaves; leaves not represented by dispersed cuticles were probably from very local plants and hence did not break up prior to sediment coverage; cuticles not represented on whole leaves may be derived from plants growing in more distant vegetation.

Results from the K/T boundary. Wolfe and Upchurch (1986, 1987b) have utilized leaves and dispersed cuticles from a variety of lithologies in their detailed study of the boundary in the western interior of North America. They recognize five phases of vegetational change in the southern part (mid-palaeolatitudes).

Phase 1, pre-boundary, is characterized by broad-leaved evergreen plants with a high percentage of entire-margined leaves indicating megathermal vegetation. Very few drip tips are present and leaves are small, often hairy, all characters which imply dry conditions and a warm, only subhumid climate is inferred.

Phase 2, immediately above the boundary, is dominated by ferns and probably represents early recolonization following mass kill.

Phase 3, is the early phase of angiosperm recolonization. Angiosperms are again dominant but in low diversity and typical of early successional forms. A warm, wet climate is indicated.

Phases 4 and 5 show increasing diversity. Phase 5 is indicative of megathermal rain forest vegetation, with many large entire leaves with drip tips.

The overall vegetation throughout the succession (except phase 2) is broad-leaved evergreen. No temperature differences are indicated. Changes in features such as leaf size, apical attenuation and hairiness of cuticles indicate a major increase in precipitation at and above the boundary. Most of the evergreen dicots from phase 1 (63 out of 75) do not reappear after the bound-

ary. Most deciduous plants do. Most evergreen conifers also became extinct to be replaced by deciduous Taxodiaceae.

In the higher palaeolatitudes (north of approx. 60°N) changes were slightly different. In phase 1, vegetation was of large-leaved deciduous forest. Phase 5 shows an increase in the proportion of large leaves indicating increased precipitation similar to phase 5 in the south. However, there are few entire-margined species and far more phase 1 forms (i.e. deciduous forms) survived into phase 5. Vegetation remains deciduous forest.

Taken together these changes document a selection for deciduous taxa which was most striking in mesothermal vegetation but also ocurred in megothermal areas. No similar selection occurred in the southern hemisphere. This selection left a lasting effect on northern hemisphere vegetation accounting for the dominance of broad-leaved deciduous trees (Wolfe, 1987; Wolfe and Upchurch, 1986, 1987b). The authors considered that selection for deciduousness at the K/T boundary can best be explained by a short period of either dark or cold which favoured dormancy mechanisms. Drought is eliminated as a possible cause because of the inferred increase in precipitation. They considered this explanation consistent with models of an impact winter (see Section II.B). Wolfe (1990) reinterpreted temperature changes to indicate a marked increase following the K/T boundary.

Global vegetational and climatic patterns in the Early Tertiary.

1. Northern hemisphere high latitudes.

During the Late Cretaceous, Palaeocene and Eocene mesothermal, broad-leaved deciduous forest or a mixed forest of broad-leaved deciduous and coniferous plants occurred northward of 65–70°N (cf. a maximal extent to 50°N today). Land surface temperatures may have been up to 30°C warmer than today. These floras include *in situ* tree stumps (fossil forests) from several areas and stratigraphic levels with growth rings which show that conditions were favourable for forest growth (Axelrod, 1984; Francis and McMillan, 1987). The floras have been discussed by Wolfe (1980, 1985, 1986, 1987); Axelrod (1984); Spicer *et al.* (1987); Francis and McMillan (1987) and Basinger (1987a,b,c) using data from Greenland, Alaska, Spitsbergen, northern Siberia and the Canadian Arctic.

These forests were unique in physiognomy; deeply-lobed leaves and liana-like leaves were rare or absent and many leaves were large but thin and deciduous, often with serrate margins. The deciduous nature of these floras can be attributed to absence of winter light (i.e. they were adapted to a period of dormancy). The anomalously large leaf size may reflect the low angle of incidence of light coupled with warm growing season and long day length in summer (Wolfe and Upchurch, 1987a, p.36). Large leaves would maximize photosynthesis but would not overheat. (Note also that in this anomalously deciduous vegetation (with serrate-margined leaves) erroneous temperature values would be obtained from percentages of entire-margined species.)

Important elements in these forests were deciduous Taxodiaceae, *Cercidiphyllum*-like plants, Trochodendraceae, Hamamelidaceae, Betulaceae, Juglandaceae, Fagaceae, Platanaceae, Aceraceae, Ulmaceae, Salicaceae, etc. At latitudes up to 70° or 75°N some broad-leaved evergreens, including palms, were also present. New elements tended to invade the area via unstable habitats, e.g. along streams, from the south (Spicer *et al.*, 1987). Diversity increased in the Eocene with about 30 dicotyledonous families represented by the Late Eocene.

As noted in Section IV.A, many of these families included representatives very similar to modern genera in the Palaeocene or Early Eocene. Their major diversification had thus been accomplished. Subsequent diversifications were at lower taxonomic levels (e.g. species level). Climatic deterioration at the end of the Eocene led to a reduction in diversity of these floras but with a recovery in the later Oligocene due to diversification of groups which had persisted.

The coniferous forests which cover part of this area today originated in the Late Tertiary and Quarternary (Basinger, 1987a,b,c; Wolfe, 1985, 1986). A different, mixed coniferous forest, poleward of the broad-leaved deciduous forest, is reconstructed on the maps of Wolfe (1985, 1986) for the Middle Eocene onwards. However, as he stated (1985, p. 366) actual megafossil evidence for this is generally lacking. Basinger (1987c) documented a range of conifers, especially Pinaceae (Fig. 15) from the Late Eocene of Axel Heiberg Island. He commented that the floras are somewhat intermediate between those of the Early and Late Tertiary.

Early Tertiary high latitudes were thus occupied by forests of unique composition and physiognomy with no modern equivalent. Their maximum northward extent probably occurred during the climatic maxima of the Early Eocene when, according to the maps of Wolfe (1985, 1986), they covered much of the pole to the exclusion of microthermal vegetation. In the Early Palaeocene they reached south to contact with megathermal paratropical rain forests probably as a result of extinction amongst evergreen forms at the K/T boundary. By the Late Palaeocene evergreen forms had again attained dominance south of 65°N in megathermal vegetation but deciduous forms remained a major element in mesothermal vegetation (Wolfe, 1987; Wolfe and Upchurch, 1986, 1987a,b).

2. Northern hemisphere lower latitudes.

Except in the Early Palaeocene (see above) three vegetation belts occupied lower latitudes; from north to south these were: a mesothermal broad-leaved evergreen forest with many deciduous forms, megathermal paratropical rain forest, and megathermal tropical rain forest (Wolfe, 1985, 1986, 1987). Paratropical forests reached almost to 60°N during Eocene temperature maxima. Land surface temperatures were from 5–10°C warmer than the present day.

At certain times land bridges (see Section II.D) may have allowed direct migration between the northern hemisphere continents. The Eocene paratropical forests across the area do have many genera in common (Wolfe,

1985, 1986; Collinson, 1983b) but were certainly not uniform in composition. They also included a variety of taxa whose nearest living relatives are now "temperate" plants. Many of the genera they contained are now extinct while others were apparently very similar to modern forms. Nearest living relatives are scattered across a variety of geographic (e.g. South East Asia, North America) and ecological (e.g. tropical rain forest, mixed mesophytic forest) settings.

Wolfe and Upchurch (1986, 1987a,b), Wolfe (1985, 1986, 1987), Upchurch and Wolfe (1987) and Crane (1987) all emphasize the significance of the origin of these multistratal rain forests for land biota. A variety of new or greatly expanded habitats would have been created, which spread with the diversification and expansion of these forests. Certainly in the Early Eocene a highly diverse rain forest flora existed. Many fossils whose nearest living relatives are lianas (Menispermaceae, Icacinaceae, Vitaceae) were important elements in these floras.

In North America seasonally dry climates had developed by the late Middle Eocene in the mid-latitudes of the continental interior and in the south-east (Wolfe, 1985, 1986; Upchurch and Wolfe, 1987). Vegetational change is also documented in southern England (Collinson and Hooker, 1987) which could be due to increased seasonality or cooling climate. Wolfe (1985, 1986, 1987) also documents some aspects of upland vegetation in volcanic terrains during the Middle Eocene in North America. Wing (1987) gives detailed documentation for the Rocky Mountain area. Diversification of lineages which were subsequently to occupy microthermal vegetation (see below) may have begun in these upland areas in the Eocene.

Fluctuations in climate during the Eocene (Wolfe, 1978; and see Section II.C) would have resulted in shifting vegetational belts. Opportunities were thus provided for the introduction of new taxa into previously established vegetation. Many more detailed studies are necessary in order to increase our understanding of local vegetational changes within the overall global pattern. Wing (1987) noted the development of local floral provinciality in the Rocky Mountain area of North America during the Eocene.

Some details of the Eocene/Oligocene transition have been documented in the John Day Basin in Oregon, western USA (Manchester and Meyer, 1987). Here Eocene near tropical broad-leaved forests are replaced by deciduous northern hardwood forests in the Oligocene.

The terminal Eocene temperature deterioration involved a shift from low to high mean annual ranges of temperature and a major temperature decline. The megathermal rainforests became restricted to latitudes below 20°N and the northern mixed coniferous forests spread south of 50°N. A microthermal broad-leaved deciduous forest (which may have originated in upland areas during the Eocene (Wing, 1987)) developed to occupy areas south to 35°N with mesothermal broad-leaved evergreen forests between here and 20°N. Changes much later in the Tertiary involved deforestation with woodlands, grasslands, steppe, taiga and tundra all originating in the Oligocene or later

(Wolfe, 1985, 1986; Upchurch and Wolfe, 1987). These changes may be related to physical factors including Antarctic glaciation, circum-polar currents and sea level fluctuations (see Section II.A–D) which may have led to seasonal and drier climates. Axelrod (1987) suggested that the Late Oligocene Creede flora (Colorado, USA) showed essentially modern aspect whereas Wolfe and Schorn (1989) considered that the Creede forest communities have no modern analogue. European floras still exhibited mixed composition (with nearest living relatives in North America, eastern Asia, the subtropics and Europe) in the Middle Miocene (e.g. Friis, 1985a).

3. Southern hemisphere.

Many of the floral data for the southern hemisphere are based on extrapolations from near living relatives, often from pollen, sometimes from leaf assemblages (Axelrod, 1984; Wolfe, 1985). Leaf physiognomic work has however, been undertaken for several Eocene floras from southeastern Australia (Christophel, 1981, 1984; Basinger and Christophel, 1985; Christophel *et al.*, 1987; Christophel and Greenwood, 1987, 1988, 1989). These imply the presence of either simple notophyll vine forest or complex notophyll vine forest *sensu* Webb (1959), both lowland rainforests, the former cooler in aspect. Wolfe's (1985) terminology interpreted these as indicating (1985, p. 364) either warm notophyllous broad-leaved evergreen forest (at the Nerriga locality) or paratropical rainforest (Anglesea and Maslin Bay localities). This means that during parts of the Eocene large-leaved, broad-leaved evergreen forest vegetation extended to about 50–55°S. Christophel *et al.* (1987) used physiognomic, floristic and taphonomic methods to compare the Anglesea flora with modern vegetation. They recognized a modern model in lowland rainforest (complex mesophyll vine forest of Webb, 1959) vegetation of northeast Queensland where streamside elements introduce notophyllous tendencies.

Evidence from Tasmania (Hill and Gibson, 1986) shows that a cooling, comparable to that in the northern hemisphere, occurred during the late Early and later Tertiary. A lineage of *Nothofagus* shows a leaf size decrease and tropical Casuarinaceae forms are replaced by temperate ones (Hill, 1984b). These data suggest that cold- and dry-adapted floras were in place by the latest Oligocene/Early Miocene.

Romero (1986b) used percentages of entire-margined leaves to assess vegetation and climate in the Early Tertiary of South America. Only the southernmost floras from Patagonia had sufficient species to give significant counts (see Section IV.F.1) as he recognized, and heavy reliance was placed on just the margin character. However, evidence from near living relatives and from vertebrates was in general agreement with conclusions from the physiognomic evidence. These suggested paratropical rain forest in Patagonia in the Early Eocene changing through subtropical in the Middle Eocene, mixed mesophytic forest in the Upper Eocene to mixed northern hardwood forest in the Oligocene.

According to Romero (1986b) and Wolfe (1985) the paratropical rain-

forest extended onto the Antarctic peninsula in the Early Eocene although this suggestion is based on extrapolation from implied climatic gradients rather than on direct megafossil evidence. Case (1988) documented Palaeogene leaf floras from Seymour Island, Antarctica. Three small floras (one Palaeocene, one Middle and one Late Eocene) are of low diversity, dominated by podocarp and araucarian conifers, *Nothofagus* and ferns. The Middle Eocene *Nothofagus* is a notophyllous form implying amelioration of climate compared to earlier and later microphyllous forms. Evidence from Antarctic fossil woods suggests a forest dominated by Southern hemisphere conifers (Podocarpaceae and Araucariaceae) and including *Nothofagus* (Francis, 1986) growing under a warm to cool-temperate climate in the Early Tertiary. Evidence from Antarctic pollen (Askin, 1988a,b) suggests cool-humid podocarpaceous conifer-dominated vegetation with an understory of ferns and endemic angiosperms in the Palaeocene with a mixed *Nothofagus*/conifer forest in the Eocene. More abundant and diverse floras combined with refined stratigraphy and absolute dating in this area are essential before climates and vegetation can be reconstructed in detail. However, a coniferous and/or *Nothofagus* forest probably existed over southern South America, part (possibly most) of Antarctica and into Tasmania (these areas were geographically close, see Section II.D) during much of the Early Tertiary (Tanai, 1986; Case, 1988). This has implications for our understanding of the dispersal of marsupial, but not placental, mammals into Australia (Case, 1988).

These combined data strongly suggest that vegetational belts extended almost as far south in the Southern hemisphere as they did north in the Northern hemisphere. At temperature maxima tropical or paratropical vegetation reached 55–65° of palaeolatitude in both hemispheres. The differences between Southern and Northern hemisphere vegetation today include the general absence of broad-leaved deciduous forest from the former. This may be explained in part by invoking restriction in the influence of the K/T boundary event (Wolfe and Upchurch, 1986, 1987b; Wolfe, 1987). Geographic and tectonic factors (see Section II.D) of shifting continental positions and the origin of circum-polar currents, etc., would also have influenced evolution of vegetation.

2. Wood anatomy

Wolfe and Upchurch (1987a) summarized ecological features of wood anatomy and tabulated their occurrence in some modern plants. In angiosperm woods, vessel diameter and frequency in transverse section together provide an estimate of vulnerability to air embolism. This is caused by freezing or transpirational stress and thus reflects growth conditions. A vulnerability value can be deduced as $V = d/D$ where d is the mean or average vessel diameter and D is the mean or average vessel number per square millimetre. Large trees typically have higher V values than smaller trees and shrubs but the highest values occur in megathermal trees. A conductivity value (C) can

also be calculated based on vessel number per unit area, reflecting the amount of water which can be transported and hence the water need of a plant. High conductivity values occur in the tallest trees of both humid and dry habitats.

The most common features of wood anatomy used as an indication of growth conditions are growth rings (Fig. 12e–h). Where there is little seasonal variation in temperature or precipitation woods usually lack growth rings. In areas with strong seasonal variations both angiosperm and coniferous woods have well-defined growth rings. Relative uniformity of rings reflects variations in growth conditions over the years. Relative width of early and late wood indicates the relative lengths of the favourable and unfavourable growth periods. Width and structure of late wood indicates the rapidity with which the growing season ended. Features such as tracheid diameter in conifers and vessel distribution (e.g. ring versus diffuse porosity) in angiosperms are also useful. Creber and Chaloner (1984a,b, 1985) and Francis (1986) have applied this methodology in studies of fossil woods. Wolfe and Upchurch (1987a) integrated wood and leaf evidence in their study of Cretaceous floras. However, I am not aware of any study integrating wood anatomical evidence with foliar physiognomic evidence for Early Tertiary floras. The two methods would offer both mutually supporting information and some potential to resolve the various parameters of growth control, e.g. evidence of growth rings in paratropical climate would suggest seasonal precipitation. Further information may be obtainable from isotopic analyses of woods (see Section IV.I).

The only tentative evidence for Early Tertiary wood ecology so far available (summarized in Wheeler *el al.*, 1987) indicates a general absence of widespread seasonal climates prior to the Oligocene. Wolfe and Upchurch (1987a) gave data for four Palaeocene and one Eocene (Yellowstone, USA) wood assemblage. They noted that this evidence is very limited but suggested that K/T boundary events selected against plants whose woods had high V and C values (cf. selection against evergreen leaves). Resultant trees of the Early Palaeocene, even in megathermal rain forest areas, did not show the wood anatomical features typical of those areas today.

At high latitudes Late Cretaceous and Early Tertiary woods show pronounced seasonal growth (Upchurch and Wolfe, 1987; Axelrod, 1984, p. 129; Francis, 1986). This evidence is consistent with that from other sources (see Sections IV.F.1, II.C). Wide and relatively uniform growth rings in Late Cretaceous and Early Tertiary Antarctic woods from 59–62°S palaeolatitudes reflect a mild temperate climate favourable for forest growth (Francis, 1986). Woods (including those from *in situ* tree stumps) from the Early Tertiary of the Canadian Arctic (Ellesmere and Axel Heiberg Islands) at palaeolatitudes similar to their present latitude of 80°N (Fig. 2), also have wide growth rings indicating conditions favourable for tree growth (Axelrod, 1984; Francis and McMillan, 1987; Basinger, 1987c).

G. THE AUSTRALIAN EARLY TERTIARY

Over the last 5–10 years a resurgence of interest in Australasian Tertiary floras have provided a striking wealth of new information. Previously most of our knowledge of this area was based on palynological studies or on older literature in which many megafossils were misidentified. The recent studies include examples of several of the approaches which I consider of particularly high value and hence have already been referred to in this chapter (Sections II.F.2,3,4; III; IV.A.9; IV.B.2; IV.F.1). Hill (1988a) provided a catalogue of Australian Tertiary angiosperms and gymnosperms including lists of revised and rejected records. He emphasized that much remains to be done. He was particularly concerned that too much reliance should not be placed on the relatively small numbers of well-understood fossils. Even in the best-known cases (see later) many leaf species remain unidentified. It is especially important to take account of this when reconstructing the evolution of Australian vegetation. Nevertheless, I feel it is useful to draw these studies together in this section in order to demonstrate the significant results which have been achieved over a comparatively short time. This should serve to encourage others embarking upon revisionary or new research programmes.

1. Mainland Australia

Four Eocene floras from southern-eastern Australia have been the subject of recent study. The Nerriga flora (New South Wales) consists mainly of leaf compressions which can often be removed intact (termed mummified by authors); 26 angiosperm taxa have been recognized of which one belongs to the Casuarinaceae (Christophel, 1980), 12 to the Lauraceae (Hill, 1986) and the others remain undetermined (Hill, 1982). Three cycads (see Section II.F.4; Hill, 1978, 1980) have also been described from this site.

The Maslin Bay flora (South Australia) includes over 200 leaf forms (Christophel and Blackburn, 1978). Some of these were subjected to numerical taxonomic analyses and members of the Proteaceae were identified (Blackburn, 1981). Christophel and Greenwood (1987) describe another South Australian Eocene flora from Golden Grove. This includes some 35 leaf types (including Proteaceae, Elaeocarpaceae, Lauraceae, Myrtaceae and Podocarpaceae) and several flowers and fruits.

The Anglesea flora (Victoria) is by far the best known. It includes both leaves and reproductive structures, many of which can be removed intact (Figs 31 and 32). Flowers and leaves of *Austrodiospyros* (Ebenaceae) are the best-documented example (see Section IV.A.9; Basinger and Christophel, 1985). Proteaceae are also represented by inflorescences (Christophel, 1984) and by several leaf forms (Christophel *et al.*, 1987; Hill and Christophel, 1988). *Gymnostoma* (formerly *Casuarina*, Casuarinaceae) is abundant and includes both foliar and reproductive material. Many other dicots occur

including members of the Lauraceae, Elaeocarpaceae, Cunoniaceae, Sterculiaceae and Saxifragaceae (Christophel *et al.*, 1987) and Myrtaceae (Christophel and Lys, 1986). *Lygodium* (a climbing fern), cycads (including *Bowenia*; Hill, 1978, 1980) and conifers (*Podocarpus*; Greenwood, 1987) also occur.

The Anglesea fossils occur in numerous lenses which represent confined depressions (e.g. oxbow lakes) within a meandering river system. The lenses contain a similar microflora but differ considerably in their megaflora. Different species, e.g. *Gymnostoma*, *Austrodiospyros* and *Brachychiton* (Sterculiaceae) dominate different lenses. This is probably due to patchiness in the parent vegetation. A possible modern model for the Anglesea flora has been found in the Noah Creek area of northeast Queensland. Here floristic, physiognomic, sedimentological and vegetational features are all similar to those of Anglesea. Furthermore the patchiness is represented not only in modern vegetation but in potential fossil accumulations in the sediments of Noah Creek (Christophel *et al.*, 1987). One noteworthy feature of the pollen assemblages is the abundance of *Nothofagus* pollen when megafossils of the genus are entirely absent. This can be explained by high production and long distance wind dispersal of the pollen (Christophel *et al.*, 1987).

Banksia (Proteaceae) reproductive structures have been described from the Eocene of Western Australia (McNamara and Scott, 1983). Leaves similar to *Banksia* are well-represented in the Tertiary of south-eastern Australia (Hill and Christophel, 1988). There are two reports of *Nothofagus* leaves from the Early Tertiary of mainland Australia (Christophel, 1985; Hill, 1988b). A. C. Rozefelds (personal communication, 1988) is currently studying an extensive mid-Tertiary flora from Central Queensland. Leaves, woods and fruits and seeds from a diverse range of taxa suggest a rainforest community with near living relatives in Queensland today.

2. Tasmania

A number of Early Tertiary floras have recently been described from Tasmania. Many are dated by palynological correlation and their ages are not known precisely. Eocene floras include Regatta Point (probable Early Eocene), Hasties (probable mid-Late Eocene) and Cethana (probable Late Eocene–Early Oligocene). Oligocene floras include Pioneer and Little Rapid River (both likely to be Late Oligocene; Hill, 1987; Hill and Gibson, 1986; Hill and Bigwood, 1987).

These floras are at present distinct from the mainland Australian examples in that they contain abundant *Nothofagus* leaf material (Hill, 1983a,b, 1984a,b; Hill and Gibson, 1986) and also the first fossil record of cupules of section Bipartitae associated with *Nothofagus brassii*-type pollen (Hill, 1987; see Section IV.B.1). Cupules similar to those of the extant Australian species *N. cunninghamii* and *N. moorei* and another form (Fig. 50c) similar to species now living in South America, also occur in the Little Rapid River flora (Hill, 1987, and personal communication, 1988).

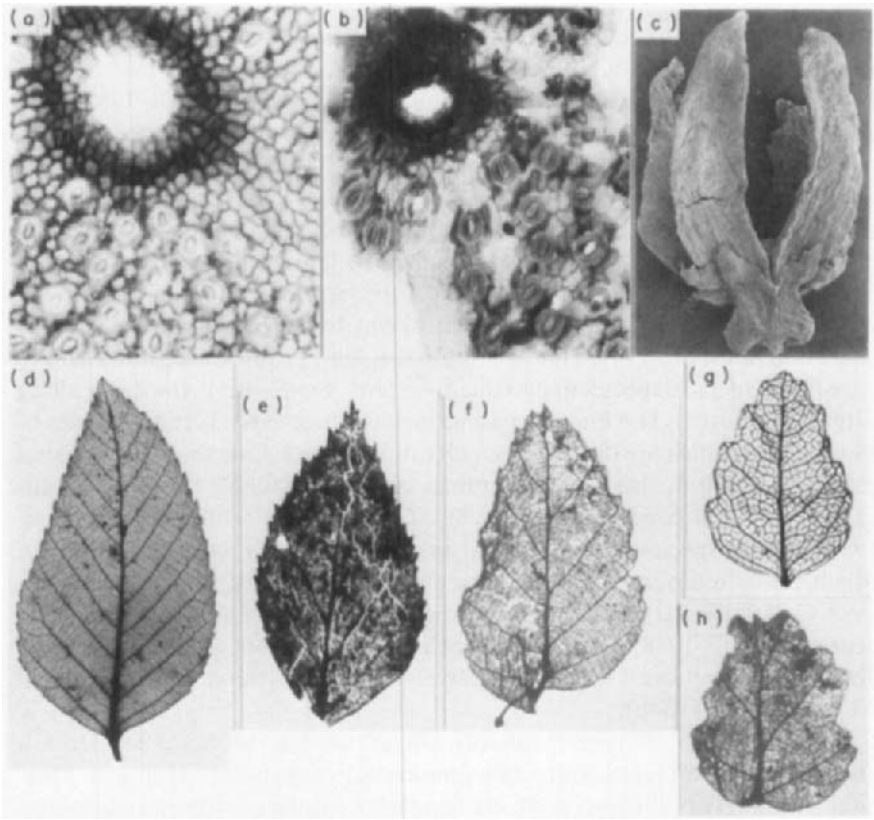

Fig. 50. Modern and Tasmanian fossil *Nothofagus* leaves and cupule (see Hill and Gibson, 1986; Hill, 1987). (a) Modern *N. moorei* leaf cuticle, × 124.8; (b) cuticle of *N. johnstonii*, Oligocene, × 124.8; (c) *Nothofagus* sp. cupule (undescribed form similar to those of modern S. American species), Oligocene, × 8.9; (d) modern *N. moorei* cleared leaf, × 1.16; (e) fossil *N. johnstonii* leaf, × 1.16; (f),(h) fossil leaves from a single Miocene assemblage showing intergradation between earlier *N. johnstonii* and modern *N. cunninghamii*, × 2.5; (g) modern *N. cunninghamii* cleared leaf, × 1.96. Illustrations courtesy of R. Hill.

Leaf fossils of *Nothofagus* from the latest Eocene or Oligocene floras include two forms (one is shown in Fig. 50b,e). Each is similar to, and partly intergrades with, modern *N. cunninghamii* (Fig. 50g) from mainland Australia and Tasmania and modern *N. moorei* (Fig. 50a,d) which occurs in temperate rainforest of Queensland and New South Wales (Hill, 1983a,b, 1984a, 1988b; Hill and Macphail, 1983; Hill and Gibson, 1986). The earliest of these fossils, *N. tasmanica* are close to *N. moorei* in morphology but slightly later forms (*N. johnstonii*; Fig. 50b,e) show a range from leaves similar to *N. moorei* to forms resembling *N. cunninghamii*. The evidence implies possible common ancestry for the two modern species (Hill, 1984b) with the *N. moorei* form being ancestral to *N. cunninghamii* (Hill, 1983a). The leaves show a progressive size reduction (Hill and Gibson, 1986) through the Late

Tertiary (Fig. 50f,h) culminating in extant *N. cunninghamii*. This can probably be explained by adaptation to decreasing temperatures (see Section IV.F.1). Fossil *Nothofagus* leaves from mainland Australia (Hill, 1988b) are also intermediate between these two extant species. One of these occurs close to the site where extant *N. moorei* grows in New South Wales showing that a migration model alone could not explain the leaf occurrences. Other leaves assignable to *N. gunnii* (a modern Tasmanian endemic) and forms similar to modern New Zealand species also occur in the latest Oligocene or Miocene floras of Tasmania (Hill, 1984a; Hill and Gibson, 1986).

Several other megafossils have recently been described. The Araucariaceae (Bigwood and Hill, 1985; Hill and Bigwood, 1987) from Eocene and Oligocene floras include species assignable to extant *Agathis* and *Araucaria* along with extinct forms. The Podocarpaceae include three early Tertiary species of *Phyllocladus* which are distinct from all extant species. One shows an unusual partial webbing of the planated branch system; a possible primitive or advanced, reduced condition (Hill, 1989). Cupressaceae (Hill and Carpenter, 1989) include species of *Libocedrus*, *Austrocedrus* and *Papuacedrus* none of which occur today in Australia. In contrast, *Lomatia* (Proteaceae) foliage from Cethana is very similar to a modern Tasmanian endemic species (Carpenter and Hill, 1988). The records of Casaurinaceae (not yet documented in detail) are significant when considered along with those from mainland Australia (Hill, 1984b).

The flora from Pioneer (probably Oligocene) has been studied from a microfossil, macrofossil and sedimentological standpoint (Hill and Macphail, 1983). This suggests a closed temperate rainforest vegetation dominated by *Nothofagus*, similar to, but more complex than, modern Tasmanian *Nothofagus cunninghamii* rainforests. Some niches were clearly occupied by different plants in the past, e.g. a fern similar to *Cyathea* filled a riparian niche now occupied by *Dicksonia*.

3. Summary and significance

These Australian floras document unequivocally the former Eocene occurrence of families very significant in modern Southern hemisphere vegetation especially Proteaceae, Casuarinaceae, Podocarpaceae and Araucariaceae. Proteaceae were clearly already diverse in the Eocene and included extinct forms and forms referable to modern groups. Several leaf fossils similar to *Banksia* (Hill and Christophel, 1988) and one *Lomatia* (Carpenter and Hill, 1988) show xeromorphic features. The fossil *Banksia*-like leaves have thick cuticles and hairs but superficial stomata unlike modern species, many of which have stomata sunken in hair-filled pits. The fossil *Lomatia* has reduced leaf form and thick cuticle similar to the modern forms. These xeromorphic features are interesting as many of the fossils occur in assemblages which have a physiognomic signature (see Section IV.F) of rainforest or cool wet climate. This supports hypotheses that the xeromorphic features of Proteaceae

evolved initially in wet conditions, perhaps in response to low fertility soils. The plants were then "preadapted" to developing aridity in the later Tertiary and to fire in more recent times (Carpenter and Hill, 1988).

Casuarinaceae are clearly also an ancient group in spite of their apparently derived features. Modern *Gymnostoma*, now restricted to tropical areas and occurring in Australia only in northeast Queensland, was an important element in the Early Tertiary. It was replaced by forms similar to those of modern south Eastern Australia during the later Tertiary. Similar replacement of warm-loving *Nothofagus* is also documented in Tasmania. The *Nothofagus* fossils are also valued for the understanding of the biogeography of this important Southern hemisphere genus.

The Middle Eocene communities in Australia have been carefully investigated using several lines of evidence. Paratropical rainforest, similar to modern analogues in northeast Queensland, extended beyond 50°S palaeolatitude in the Eocene. Cold adapted floras were already in place in Tasmania in the earliest Miocene. These sources of evidence are valuable indicators for the evolution of Australian vegetation. Hill (1984), Hill and Gibson (1986) and Christophel and Greenwood (1989) discussed this in more detail. Hill and Read (1987) have applied these data in discussions of the origin of endemism in Tasmania cool-temperate rainforest. They concluded that many of the endemics evolved relatively recently *in situ* as a response to Tertiary climatic changes.

H. NORTHERN HEMISPHERE PALAEOCENE FLORAS

Whereas many floras have been described from the Northern hemisphere Palaeocene (see Crane *et al.*, 1990) significant gaps have remained. Fruit and seed floras were extensively known from southern England and continental Europe but Palaeocene examples have only recently been documented (Collinson, 1986a; Collinson and Hooker, 1987; Mai, 1987b). In southern England these proved crucial in the interpretation of vegetational and related mammalian faunal changes. With the inclusion of Palaeocene from East Germany a fruit and seed record, from Late Cretaceous through the Early and much of the Late Tertiary, now exists in continental Europe. When time correlation is refined and the scattered sites can be placed in context these data will be of considerable value in evolutionary studies.

In addition, the Palaeocene site of Almont in North America (Crane *et al.*, 1990) provides evidence for whole plant reconstruction. An extensive fruit and seed flora is also present which, when examined in detail, may permit comparisons with the European floras. This has always been complicated by the dominance of leaf floras in North America and fruit and seed floras in Europe. Revision of the mainly leaf flora from Mull, Scotland (Kvaček and Boulter, 1989) will also be of value for this comparison. On the basis of land

connections and similar mammalian faunas a strong similarity might be expected between the floras (see Tiffney, 1985a,b).

I. PALAEOPHYSIOLOGY AND THE PALAEOATMOSPHERE: ISOTOPE AND GEOCHEMICAL STUDIES

Several aspects of plant physiology are reflected in plant morphology and these have already been mentioned (Sections IV.D and IV.G.3). Documentation of changes in stomatal indices during the recent rise in atmospheric CO_2 levels (Woodward, 1987) suggests another possible way in which plant morphology may reflect the environment, in this case the palaeoatmosphere (Raven and Sprent, 1989, p. 164). Numerous Tertiary angiosperm-dominated leaf floras could be exploited in this regard although considerable care must be given to selecting floras from comparable microhabitats; comparing fossils of the same taxon; and considering the influence of evolutionary instability (see discussion, Section IV.F.1 and IV.B). Furthermore, refined understanding of genetic control and the influence of microhabitat (external and internal to plant tissues) on stomatal indices in modern plants may be necessary before the fossil material can be fully exploited.

Tree rings also preserve evidence of historically recent atmospheric changes in CO_2. Increased growth rates have been demonstrated in boreal conifer forests (Moore, 1989, p. 184; see also references in Francey *et al.*, 1988). As Hudson (1989, p. 156) noted, the extensive evidence of Cretaceous and Early Tertiary polar forests (see Section IV.F.1) apparently demands high atmospheric CO_2 levels (producing global warming; a "greenhouse effect") as otherwise global models predict freezing temperatures at high latitudes.

The modern "greenhouse effect" (Hall, 1989), may result in sea-level rise and changing rainfall patterns which will effect world biota. An understanding of similar events in the past may help to predict the nature and severity of these changes.

The isotopic composition of plant material may possibly also reflect external atmospheric changes. However, such composition has been more frequently exploited as an indicator of the plant photosynthetic pathway (Moore, 1983, 1989; Raven and Sprent, 1989). Extrapolations from this have included interpretations of ancient vegetation belts hence climate and also diet (Holmes *et al.*, 1987; Brocherens *et al.*, 1988; Goodfriend, 1988).

The $^{13}C/^{12}C$ isotope ratio ranges from -22 to -33 in the more widespread, primitive C_3 photosynthetic pathway but from -10 to -18 in the more advanced CAM and C_4 pathways (Moore, 1983). The latter pathways do not suffer from photorespiration (where oxygen competes with carbon dioxide for RuBP carboxylase, the enzyme involved in fixation), because they either temporally (CAM) or spatially (C_4) isolate the fixation process from

high oxygen levels. They are thus efficient at higher temperatures where respiration is accelerated. The extra control over the time and place of fixation also results in more efficient use of CO_2 and water. These CAM and C_4 plants are generally more abundant today in high temperature and high light regimes which tend to occur in low latitudes and in more arid zones. Isotopic evidence of their presence in fossil material can therefore be regarded, to some extent, as indicative of latitudinal and climatic conditions. However, we know relatively little about the detailed control of microclimate on the distribution of these plants and even less of their ancient history. There is one C_4 plant fossil where isotopic and anatomical evidence (Kranz anatomy) were present in a Pliocene grass. Some ancient plant material has been analysed isotopically and evidence of the C_3 pathway has been found except in very recent material (Moore, 1983, p. 21; Holmes *et al.*, 1987; Raven and Sprent, 1989, pp. 163, 165).

There are clearly possibilities in Tertiary fossil plant material to trace the origin and/or diversification of the C_4 and CAM pathways. Most of the modern plant groups which exploit them (Moore, 1983, table 2; additional references in Raven and Sprent, 1989) either originated or diversified in the Tertiary though some, like isoetaleans, have a much more ancient history. Certain modern biomes, e.g. tropical grasslands, which may be dominated by C_4 plants, leave few determinate macrofossils and our knowledge of their fossil history is poor (see Section IV.C). Isotope evidence from organic deposits, or possibly from organic material in palaeosols (e.g. Guillet *et al.*, 1988) may provide further information.

Goodfriend (1988) used the carbon isotope ratio in Holocene fossil land snail shell as an indication of past diet of either C_4 or C_3 plants. As the distributions of the different modern plant associations are under strong climatic control (C_4 in arid zones) the shell ratios could be used to document former shifts in climatic belts.

Brocherens *et al.* (1988) analysed the carbon isotope ratio of bone collagen from a Late Cretaceous dinosaur. They obtained a ratio of -15 (comparable with modern C_4 and CAM plants) and inferred a diet of terrestrial plants some of which were adapted to arid conditions. They noted that an aquatic plant diet might also have resulted in this isotopic ratio (for further discussion and references on aquatic plant isotopic composition see Raven and Sprent, 1989).

Both bone and dental collagen may preserve unaltered organic material from which isotope ratios can be obtained. If this could be coupled with analysis of dental microwear (Solounias *et al.*, 1988), linked in some cases to fossils with plants in gut contents (e.g. in the Eocene of Messel; Schaal and Ziegler, 1988) rigorous testing of the method would be possible. Subsequently, sequences of isotope data could be obtained which could help to interpret ancient life in the absence of determinate macrofossils and help to confirm hypotheses advanced on other evidence (e.g. Sections IV.C and IV.E).

Variation within the carbon isotope ratio ranges documented for each major category of photosynthetic pathway may also prove useful in analysis of the palaeoenvironment. The interactions of the many competing causal factors are currently poorly understood but future work on modern plants should help to resolve these. Tree rings, for example, show a latitudinal dependency with more negative ^{13}C with increasing latitude. Controlling factors may include light intensity, temperature and humidity as well as genetic make-up (Francey *et al.*, 1988). Holmes *et al.* (1987) noted that changing parent plant composition of Tertiary coals (from *Glyptostrobus*-dominated to broad-leaf-dominated) resulted in more negative isotope ratios.

Two other applications of plant fossil material in studies of the palaeoatmosphere utilize charcoal and amber. Charcoal, the result of ancient forest fire combustion of plants, constrains the oxygen levels between 13 and 35% of the atmosphere (Chaloner, 1989) below which plant material would not burn and above which all land vegetation would be destroyed by fire. Amber, fossilized plant resin, encloses gas bubbles which contain ancient air (Berner and Landis, 1987). Ironically, the amber suggests oxygen levels in the Late Cretaceous which are so high as to conflict with evidence on plant combustibility.

One further approach to palaeoenvironmental analysis is the use of geochemical studies, particularly of biomarkers (chemical fossils with a demonstrable biological origin). The main research using these at present is directed towards hydrocarbon exploration in identification of source materials and depositional environments which have resulted in commercially exploitable oils and coals (e.g. Murchinson, 1987). A terrestrial, and/or higher plant source, can be inferred in several cases. As biochemical knowledge (of modern plants) and geochemical techniques become more refined diagnostic markers may well be found. Of interest, for example, are markers from the cutin and cuticular waxes (Juniper and Southwood, 1986) of different plants or vegetation types, e.g. conifers, tropical trees and grasses. If such markers can be reliably recognized then this will provide valuable evidence of ancient vegetation and the evolution of plant groups in situations where no recognizeable megafossils are preserved. Meanwhile, geochemical evidence regarding depositional environment (Murchison, 1987) should be more frequently included with other evidence (see Section IV.C) in palaeoenvironmental analyses including reconstructions of ancient plant communities.

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Origin and Evolution of Angiosperm Flowers

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I. INTRODUCTION

The time is ripe for a close collaboration between palaeo- and neobotanists on early flower evolution. During the last few years there has been a surprising number of spectacular discoveries of well-preserved Early and mid-Cretaceous flowers. At the same time, the study of floral development and biology of living archaic angiosperms has been intensified. It is necessary to integrate this wealth of new information, which has considerably modified our picture of early flower evolution. The present contribution envisages the problem equally from both sides: the fossil and the living plants. It is our aim to encourage synthesis of palaeo- and neobotanical discoveries.

The angiosperms are distinguished from all gymnosperms by their *angiospermy*, the inclusion of the developing seeds (the ovules) in carpels. As a consequence, the pollen grains do not germinate directly on the ovule but on the outer surface of the carpel (on the stigma), and the pollen tubes reach the ovules only inside the ovary. The carpel not only provides protection to the ovules—a trait that is also present in various gymnosperms by other means—but it also opens new possibilities for fine-tuned interactions between the male and the female gametophytes. *Intraspecific incompatibility systems* that function in the stigma and the pathway to the ovules are important new acquisitions of the angiosperms (Whitehouse, 1950; Knox, 1984; Zavada and

Taylor, 1986). They prevent fertilization of egg cells by sperm cells with too similar genotypes. At the same time, the intervention of the style enhances *pollen tube selection* among the entire pollen portion that has been transferred to the stigma in the process of pollination, and therefore, fertilization by the most vigorous male gametophytes (Mulcahy, 1979).

II. EXTANT PRIMITIVE FLOWERS

Comparative studies of living plants indicate that members of the Magnoliidae retain the largest number of primitive floral characters among extant angiosperms. This has subsequently been reinforced by the fossil record of angiosperms. In the following we consider selected families of the Magnoliidae to illustrate floral organization and diversity within the group.

A. MAGNOLIACEAE, ANNONACEAE

The flower of *Magnolia* is often used as a model for a typical euanthial flower (cf. Section IV.A). It is big (up to 20–30 cm diameter) and fairly multi-parted (comprising ca. a hundred organs) (Fig. 1a). The organs are spirally arranged; however, there is a tendency to form three trimerous whorls in the perianth (Tucker, 1961). Erbar and Leins (1982) found that the primordia of the first whorl are sometimes arrested in early development and are no longer apparent in the mature flower. There is, thus, a sharp gap between the vegetative and the floral region. The outer perianth whorl is sometimes more calyx-like, the inner two whorls more corolla-like. Stamens have long, basifixed anthers and short filaments (Canright, 1952; Endress and Hufford, 1989). Carpels are free but coalesce more or less in fruit development. They have two to several lateral, crassinucellar, anatropous, bitegmic ovules (Canright, 1960). They are slightly peltate (with the flanks congenitally fused over the ventral side at the base) or epeltate (with the flanks not fused over the ventral side at the base) (Leinfellner, 1969; van Heel, 1981; Erbar, 1983). Nectar is produced on the petal-like organs or on carpels (Daumann, 1930; Thien, 1974). Flowers are protogynous. Self-compatibility prevails (Bernhardt and Thien, 1987). Pollinators are mostly beetles. Pollen is monocolpate, boat-shaped, more or less tectate-columellate (Praglowski, 1974). Flowers of most Magnoliaceae are terminal on longer shoots, while the smaller flowers of *Michelia* and *Elmerrillia* are lateral (cf. Nooteboom, 1988; Weberling, 1988).

The flowers of the tropical Annonaceae are more diverse and more specialized in many respects. The perianth, however similar in the basic plan, often opens long before anthesis or forms a more or less closed chamber to protect small pollinating beetles (Fig. 1b) (Gottsberger, 1970, 1988). The number of stamens and carpels is considerably increased in several groups. Stamens

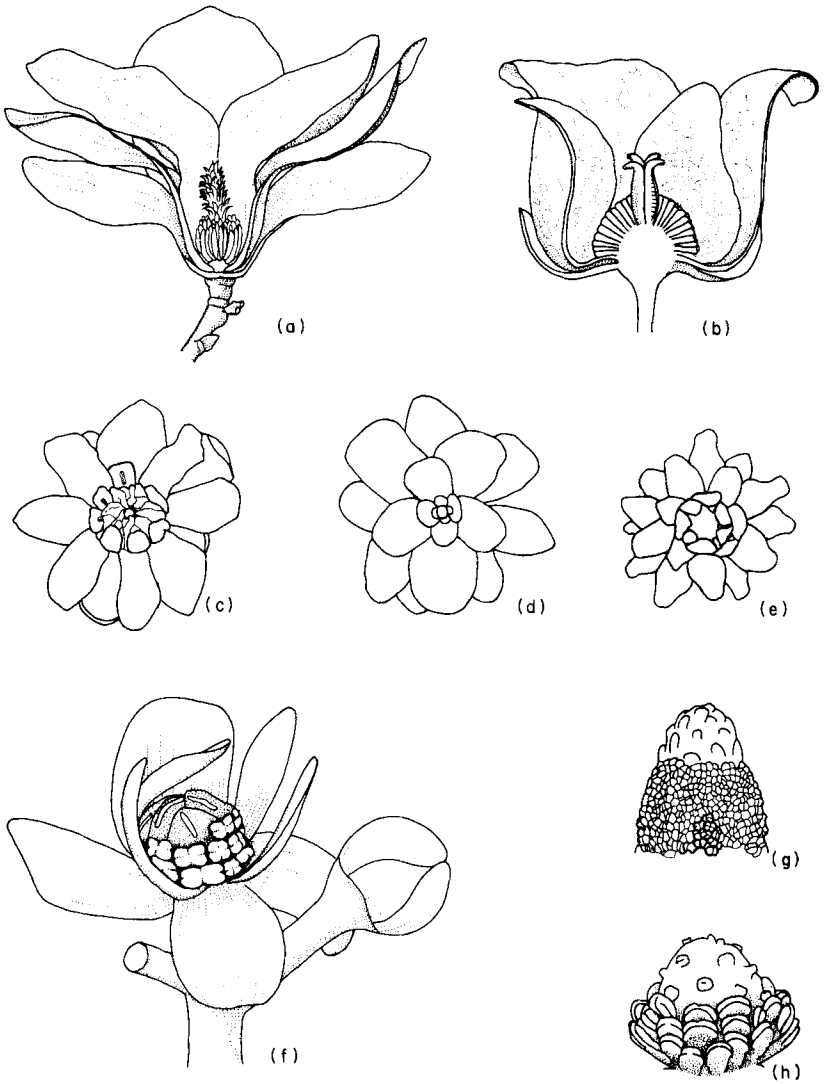

Fig. 1. Extant flowers of Magnoliaceae (a), Annonaceae (b), Austrobaileyaceae (c, d), Eupomatiaceae (e) and Winteraceae (f–h). (a) *Magnolia heptapeta*; (b) *Uvaria triloba*; (c, d) *Austrobaileya scandens* from above (c) and below (d); (e) *Eupomatia laurina*; (f) *Bubbia howeana*; (g) *Zygogynum pomiferum*; (h) *Zygogynum vinkii*. Redrawn from Wettstein, 1907 (a); Baillon, 1868 (b); Endress, 1980c (c, d); Endress, 1984b (e); Vink, 1985 (f); Endress, 1986a, based on Sampson, 1983 (g, h).

have almost sessile anthers but often enlarged, broadened, contiguous sterile apices that form a tight roof over the fertile parts of the stamens and carpels before anthesis (Endress, 1975). Stamens tend to abscise during anthesis but to be retained by the isolated and stretched tracheidal spiral thickenings of the vascular bundles for some time (Endress, 1985).

Carpels are mostly epeltate (Leinfellner, 1969; van Heel, 1981; Deroin, 1988). They contain from one to numerous ovules. Carpels mature into isolated fruitlets (most genera) or they coalesce after anthesis to form a pseudosyncarpous fruit (cf. Deroin, 1988; e.g. *Annona*). In *Isolona* and *Monodora* a syncarpous gynoecium occurs possibly derived from a monocarpellate form (Leins and Erbar, 1980, 1982; Deroin, 1985). The innermost tepals often contain food bodies (cf. Schatz, 1987) or are nectariferous (Kessler, 1988). Flowers are protogynous. Pollen is extremely various (Walker, 1971; Le Thomas, 1981, 1988; Hesse *et al.*, 1985; Waha, 1985; Le Thomas *et al.*, 1986; Morawetz and Waha, 1986).

Archaeanthus (Dilcher and Crane, 1985) and *Lesqueria* (Crane and Dilcher, 1985) are mid-Cretaceous flowers of magnoliaceous and annonaceous affinities (cf. Section III.B).

B. EUPOMATIACEAE, AUSTROBAILEYACEAE

Eupomatiaceae and Austrobaileyaceae are two small, isolated, eastern Australian families (Eupomatiaceae also Malesian) with some relationships with the Annonaceae (Endress, 1977b, 1980c). Both have medium-sized flowers with about 100 or less spirally arranged organs (Fig. 1c–e). Both have conspicuous inner staminodia that have several important functions in floral biology. In *Eupomatia* a perianth is completely lacking, only a saccate bract covers the floral bud. In *Austrobaileya*, small decussate bracts gradually transgress into the larger, spirally arranged tepals. Stamens are laminar and introrse in *Austrobaileya* (Bailey and Swamy, 1949), less so but still broadly based in *Eupomatia* (Hiepko, 1965). The carpels are free, strongly ascidiate (extremely peltate so that the form is flask-like and the rim is restricted to the uppermost region of the organ) and laterally bilobed in *Austrobaileya* (Endress, 1983), thus resembling some Annonaceae and the fossil mid-Cretaceous *Lesqueria* (Crane and Dilcher, 1985; cf. Section III.B); but they are fused, essentially without forming a compitum (a common, centralized pollen tube transmitting tract), in *Eupomatia*. The inner staminodes are osmophores (scent producing organs) in both groups; in *Eupomatia* they are further provided with mucilage-producing hair tufts (Endress, 1984a,b). The flowers are protogynous. *Austrobaileya* is pollinated by flies, *Eupomatia* by beetles.

The floral biology is best known in *Eupomatia* (Hamilton, 1898; Hotchkiss, 1959; Endress, 1984a; Armstrong and Irvine, 1989). In *E. laurina* plants flower in flushes. All flowers are synchronized during anthesis. They are

visited by curculionids of the genus *Elleschodes*. The pollinators are protected by the inner staminodes after the female phase of anthesis and lay their eggs into the massive synandrium consisting of the basally fused stamens and staminodes. The synandrium is shed on the second day of anthesis but persists on the ground for several days. The flowers are self-compatible but their strong dichogamy (temporal separation of female and male phases) and herkogamy (spatial separation of male and female organs; here by inner staminodes) prevent self-pollination. Similar flushes of synchronized flowers that lead toward functional dioecy are known from certain Annonaceae (Murray and Johnson, 1987; Deroin, 1988) and from other groups of the Magnoliidae (survey in Cruden, 1988; Endress, 1989b).

Pollen is tectate-columellate and monocolpate with a well-delimited colpus in *Austrobaileya* and thus bears some resemblance to *Clavatipollenites* (p. 107; Endress and Honegger, 1980). Pollen of *Eupomatia* is zonicolpate with a homogeneous exine (Woodland and Garlick, 1982).

C. WINTERACEAE

The flowers of the mainly southern hemisphere family Winteraceae vary from small to large. Variation in organ number is from few (around five or less) in some *Tasmannia* species, up to several hundred in some *Zygogynum* species (Fig. 1f–h). There is a considerable range of variation even within certain species, e.g. *Tasmannia piperita* (cf. Vink, 1970; Endress, 1986a). Floral phyllotaxis is spiral, whorled or chaotic (Hiepko, 1966; Vink, 1970, 1988; Erbar and Leins, 1983; Endress, 1987a).

The outermost parts of the floral envelope are often more or less fused. The stamens are differentiated into a filament and anther (cf. Sampson, 1987); they present their pollen by thickening of the filaments, which extends the spaces between the anthers (Carlquist, 1983). The carpels are mostly free and more or less strongly ascidiate with often numerous ovules (Tucker, 1959; Sampson, 1964; Sampson and Tucker, 1978; Leinfellner, 1969; van Heel, 1983; Erbar, 1983).

In *Zygogynum* the carpels are fused but lack a compitum (Vink, 1977; Endress, 1982). The gynoecium of (the almost extinct) *Takhtajania* (Leroy, 1980) is possibly bicarpellate and syncarpous (or monocarpellate, Tucker and Sampson, 1979).

Floral biology is diverse; beetles, flies and moths seem to be the main pollinators (e.g. Thien, 1980; Thien *et al.*, 1985; Gottsberger *et al.*, 1980; survey in Gottsberger, 1988; Endress, 1989b).

Pollen is tectate columellate and monoulcerate, mostly in tetrads. Very similar tetrads have been found from the Lower Cretaceous of Israel (Walker *et al.*, 1983). The unspecialized flowers together with homoxylous wood and the old pollen fossils render the Winteraceae a candidate for one of the most

primitive extant angiosperm families (cf. Gottsberger, 1974, 1988; Gottsberger *et al.*, 1980).

D. MONIMIACEAE, LAURACEAE

These two mainly tropical families are characterized by mostly small flowers (less than 1 cm diameter), carpels with a single median ovule, valvate anthers (among Monimiaceae *sensu lato* only in the less advanced groups), and often floral cups (Endress, 1972, 1980a,b; Philipson, 1986). In the Lauraceae the floral construction is highly constant: mostly trimerous whorls (two perianth whorls, three to four stamen whorls) and a single carpel (Fig. 2l). In the Monimiaceae floral construction is much more plastic with regard to phyllotaxis and number of floral organs. Whorled, spiral and chaotic phyllotaxis occur. In the most extreme and highly advanced genus *Tambourissa* some species have flowers with up to 2000 organs and reach a diameter of up to 8 cm (Endress and Lorence, 1983; Lorence, 1985) while in others the flowers have a diameter of less than 1 cm and contain less than 50 organs (Fig. 2a–k). A most unusual evolutionary trend is exhibited by the elaboration of the floral cup into an urceolate structure that completely encloses the carpels in the female flowers at anthesis with concomitant perianth reduction. Pollination does not take place at the stigma of the carpels but at a "hyperstigma" formed at the upper rim of the floral cup. Pollen grains germinate on a mucilaginous substance secreted at the entrance of the floral cup. The pollen tubes grow through the floral pore mediated by the secretion and eventually reach the carpels inside the closed floral cup (Endress, 1980a). Interestingly, this kind of evolutionary advancement, "angiocarpelly", has not been reached by any of the more advanced angiosperm groups. But it is known now from five genera of the Monimiaceae where it has probably arisen in parallel several times (Endress, 1980a,b; Endress and Lorence, 1983; Philipson, 1986). Apparently it represents an evolutionarily dead end whose success was only minor.

Floral fossils of the Monimiaceae are not known from the Cretaceous whereas flowers with distinct lauraceous characters are present in mid- and Late Cretaceous floras of eastern North America (cf. Section III.B,C). The unusual evolutionary pathway and plasticity of the monimiaceous flowers as contrasted with the stability of those of the Lauraceae seems important in the present discussion.

E. CHLORANTHACEAE

This mainly tropical family with a scattered distribution has small to very small flowers that are aggregated in spikes or spike-like thyrses (Fig. 2m–u). Most characteristic for the flowers is their extremely low organ number. In *Hedyosmum* and many *Ascarina* species the male flowers are made up of but

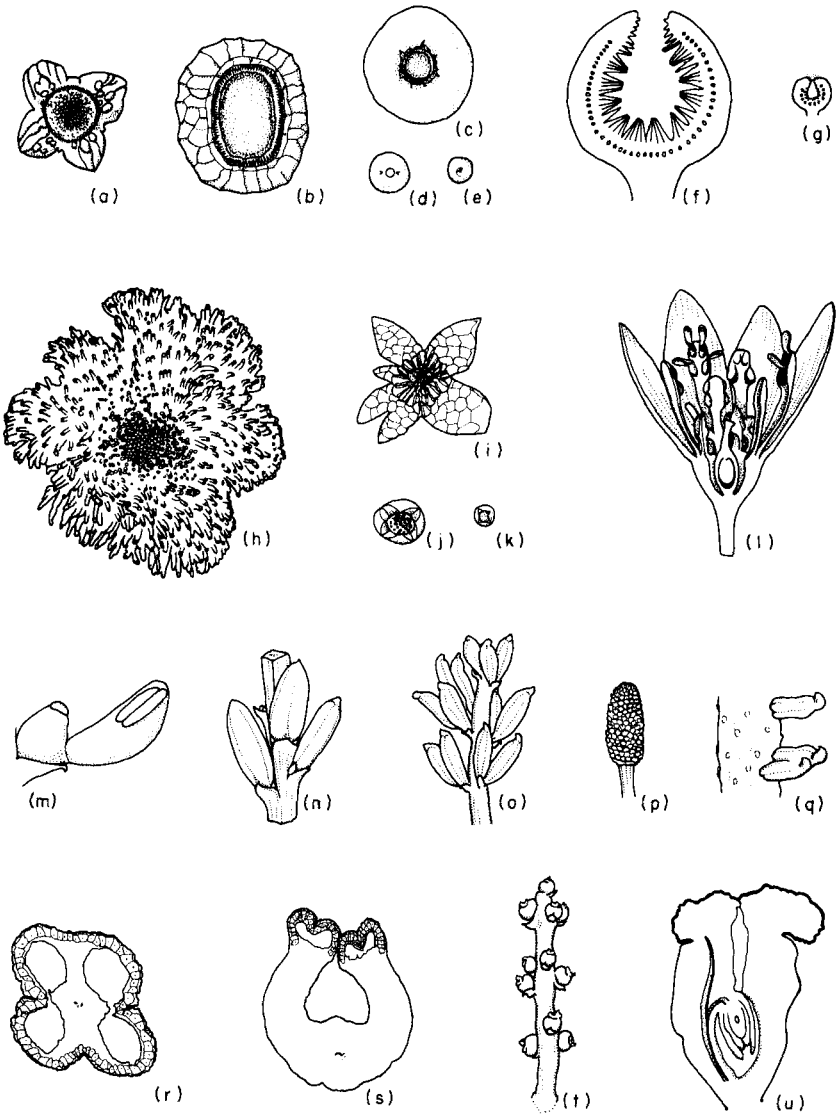

Fig. 2. Extant flowers of Monimiaceae (a–k), Lauraceae (l) and Chloranthaceae (m–u). (a–g) Female flowers of *Tambourissa*: (a) *T. peltata*, (b) *T. ficus*, (c, f) *T. sieberi*, (d) *T. leptophylla*, (e, g) *T. purpurea*, (h–k) male flowers of *Tambourissa*: (h) *T. ficus*, (i) *T. sieberi*, (j) *T. pedicellata*, (k) *T. purpurea*; (l) *Cinnamomum zeylanicum*; (m) *Sarcandra chloranthoides*, bisexual flower; (n, o) male flowers of (n) *Ascarina rubricaulis* and (o) *A. philippinensis*; (p, q) male flowers of *Hedyosmum orientale*; (r, s) transverse sections of chloranthaceous anthers: (r) *Ascarina rubricaulis* and (s) *Chloranthus spicatus*; (t) female flower of *Ascarina philippinensis*; (u) section of female flower of *Ascarina lucida*. Redrawn from Endress and Lorence, 1983 (a–k); Baillon, 1868 (l); Endress, 1987b (m, s, u); Jérémie, 1980 (n); Verdcourt, 1986 (o–q, t).

one stamen and lack a perianth: in *Ascarina* the female flowers consist of but one carpel. *Chloranthus*, on the other hand, has bisexual flowers consisting of a carpel and a one-sided androecium with three fused stamens, one bithecate and two monothecate (Swamy, 1953). In the insect-pollinated *Chloranthus* and *Sarcandra* the sterile anther parts are extended (Fig. 10a) and attractive by their colour and scent, while in the wind-pollinated *Hedyosmum* and, probably, *Ascarina* they are more restricted (Endress, 1986a). In *Sarcandra* and possibly *Chloranthus* anther dehiscence is slightly valvate, while in the other genera it is by simple longitudinal slits (Endress, 1987b). In *Hedyosmum* the subtending bracts of the male flowers have disappeared (Endress, 1987b) so that the inflorescence has been falsely interpreted as a single polyandric flower by Leroy (1983).

The carpel is extremely ascidiate (utriculate), the entire marginal region being involved in stigma differentiation. The single ovule is median, pendent, orthotropous, bitegmic and crassinucellar. Three scales atop the ovary in *Hedyosmum* have been interpreted as a reduced perianth (Swamy, 1953) or androecium (Burger, 1977). The ovary appears inferior in bisexual flowers, but since the oligomerous androecium is a highly zygomorphic structure, the situation is not clear.

The fossil record of old Chloranthaceae-like plants is unusually rich. *Clavatipollenites* from the Lower Cretaceous resembles *Ascarina; Asteropollis* and *Stephanocolpites* match *Hedyosmum* and *Chloranthus* (Couper, 1960; Walker and Walker, 1985; Chapman, 1987; Chlonova and Surova, 1988). Chloranthoid Early Cretaceous leaves have been found (Upchurch, 1985); and especially well-preserved androecia from the Early and Late Cretaceous of North America and Europe resemble *Chloranthus* (Friis *et al.*, 1986; Crane *et al.*, 1989; cf. Section III.A,C).

III. FOSSIL FLOWERS

A. EARLY CRETACEOUS FLOWERS

The earliest undisputed angiosperm fossils are monocolpate pollen grains described from the Hauterivian and Barremian (Hughes *et al.*, 1979; Doyle *et al.*, 1982; Brenner, 1984; Hughes and McDougall, 1987). There is no floral evidence of these earliest members and all floral structures currently available from the Early Cretaceous are slightly younger, being recorded from rocks of Albian age. A number of pre-Albian fossils superficially resembling angiosperm fruits or inflorescences have been recorded from Europe, Asia and North America (e.g. Chandler, 1958; Chandler and Axelrod, 1961; Samylina, 1961; Krassilov, 1982), but they are all problematic and their affinity with the angiosperms remains uncertain (Friis and Crepet, 1987). Some of the fossils described from the Lower Cretaceous of Mongolia as angiosperms or

angiosperm-like including *Gurvanella*, *Erenia*, *Potamogeton*-like fossil and *Sparganium*-like fossil (Krassilov, 1982, 1985) also show some characters of the gnetopsids. All are, however, poorly preserved and more characters are needed for a firm establishment of their affinity.

Floral structures from the Albian are known from Asia and North America. They comprise poorly preserved structures fossilized in the fruiting stage, leaving little information on the original floral organization, as well as slightly compressed or three-dimensionally preserved flowers with a wealth of details present. They show a considerable variation in organization and indicate that diversification of the Magnoliidae, Lower Hamamelididae and possibly also the lower Rosidae was already advanced by the Albian. More advanced groups of the Hamamelididae and Rosidae do not appear until later in the Cretaceous.

The most diverse assemblages of angiosperm flowers and fruits from the Albian has been discovered from the Potomac Group sediments of Virginia and Maryland in eastern North America. Several floral organs have already been described from this area (Dilcher, 1979; Crane *et al.*, 1986, 1989; Friis *et al.*, 1986, 1988), but most of the material was discovered recently and is currently under investigation (by P. R. Crane, A. Drinnan, E. M. Friis and K. R. Pedersen). Two larger fruiting structures (1.5–5 cm) were recorded from Albian strata of this area, but most of the flowers are minute (0.5–2 mm). The two larger structures comprise *Carpolithus conjugatus* and an unnamed fossil (Fontaine, 1889; Dilcher, 1979). Both apparently have apocarpous gynoecia with relatively few carpels (three in *C. conjugatus* and six to eight in the unnamed fossil) and the floral base is not elaborate. None of these larger fossils afford any information on other floral organs and it is unknown whether they were bisexual or unisexual. *Williamsonia recentior* described from the Albian Blairmore Group of Alberta, Canada (Dawson, 1886) also comprises larger structures, interpreted by Crane and Dilcher (1985) as apocarpous fruits resembling the Cenomanian *Archaeanthus* and *Lesqueria* (cf. Section III.B). The Canadian fossils are, however, poorly preserved and their angiospermous affinity not established with certainty.

The smaller angiosperm fossils from the Potomac Group are of mid- to Late Albian age and comprise a variety of well-preserved flowers, fruits, seeds and dispersed anthers. The flowers investigated thus far are unisexual and female flowers apocarpous, but a few dispersed syncarpous fruits have also been recovered. Information from this material is extensive and has provided detailed knowledge of the relationship of some Albian angiosperms. The most completely understood of the mid-Albian fossils are pistillate and staminate inflorescences of platanaceous affinity described from the West Brothers clay pit in Maryland (Fig. 3c–g; Friis *et al.*, 1988). Pistillate as well as staminate flowers are crowded in unisexual balls that are borne on elongated axes (Fig. 3d). The pistillate flowers, *Platanocarpus marylandensis*, consist of five free carpels surrounded by a number of perianth parts of which the

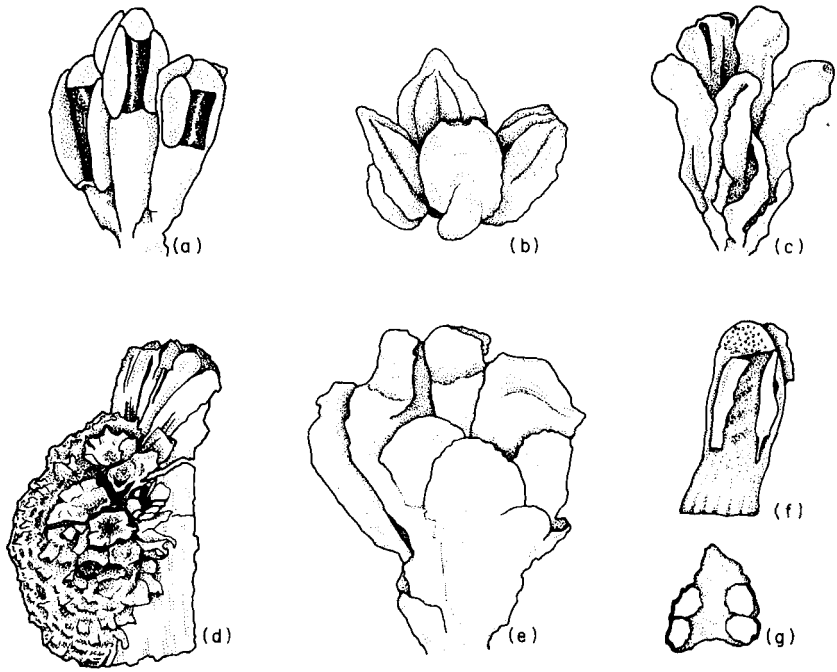

Fig. 3. Early Cretaceous (mid-Albian) flowers from Maryland, USA. (a) Chloranthoid androecium; (b) tetramerous staminate flower; (c, d) *Platanocarpus marylandensis*: (c) dispersed pistillate flower and (d) fragment of inflorescence; (e–g) *Platananthus potomacensis*: (e) staminate flower, (f) dispersed stamen and (g) section through stamen. Redrawn from Friis *et al.*, 1986 (a); Friis and Crane, 1989 (b, f, g); Friis *et al.*, 1988 (c–e).

outer are short and broad and the inner longer, linear to spathulate. The carpels are narrowly obtriangular in outline with an expanded "peltate" apical region and without a distinct style (Fig. 3c). The staminate flowers, *Platananthus potomacensis* consist of five stamens surrounded by prominent perianth parts apparently all of the same length. The anthers are elongated and borne on short filaments. The connective between the four pollen sacs is prominent with an apical shield-like expansion (Fig. 3e–g). Dehiscence was apparently valvate (Fig. 3f). Pollen grains found *in situ* in the anthers are tricolpate and reticulate, similar to the dispersed pollen species *Tricolpites minutus*. A pentamerous arrangement of androecium and gynoecium is also characteristic of Late Cretaceous platanoids (Friis *et al.*, 1988) and has been described from Early Tertiary forms as well (Manchester, 1986) while flowers of modern *Platanus* are irregular. Pistillate and staminate inflorescences and flowers, morphologically very similar to those of *Platanocarpus* and *Platananthus* and also with a pentamerous arrangement of carpels and stamens, have been recovered from two mid-Albian Potomac Group localities. The pistillate and staminate flowers are linked together based on common occurrence and the presence of pollen of the same kind on the surfaces of the carpels and *in situ*

in the anthers. These fossils differ mainly from the platanaceous flowers in epidermal characters and in having tricolpate pollen. The stamens also differ in having a hook-shaped apical expansion of the connective. They occur together with leaves of *Sapindopsis* and may represent early rosids (Crane *et al.*, 1986; Friis and Crane, 1989). Another staminate flower from the mid-Albian has a tetramerous androecium. The stamens are dorsifixed on short filaments and have a short triangular apical extension of the connective (Fig. 3b). Pollen grains are tricolpate and coarsely reticulate. Follicles associated with the staminate flowers have a decurrent stigmatic surface that extends for almost the full length of the ventral margin. The systematic position is uncertain although the flower shows some conformity with members of the Magnoliidae as well as the Hamamelididae (Friis and Crane, 1989; Drinnan *et al.*, 1990a).

A small tri-parted androecium from the West Brothers clay pit was tentatively referred to the Chloranthaceae (Friis *et al.*, 1986; Crane *et al.*, 1989). It consists of three stamens that are fused at their bases (Fig. 3a). Each stamen has a fleshy filament that grades into the connective which is apically expanded into a small triangular extension. Anthers of all three stamens of the androecium have four pollen sacs unlike modern *Chloranthus* (cf. Section II.E) and the Late Cretaceous *Chloranthistemon* (cf. Section III.C) that have four pollen sacs on the central stamen, but only two on the lateral.

Several different angiospermous fruiting structures have been described from mid-Albian strata of Kazakhstan and North-east Siberia. Most are preserved as impression fossils, but a few compressions have also been reported. *Caspiocarpus paniculiger* from western Kazakhstan comprises small elongated infructescences of densely crowded follicles (Vachrameev and Krassilov, 1979; Krassilov, 1985). The follicles are very small, about 1 mm long, attached to the axis by a short stalk, and usually opened. Each carpel contains two to four seeds described as bitegmic and anatropous. The organization of the inflorescences is obscure, but Krassilov (1985) referred to them as panicles. No remains of perianth and stamens were preserved and the systematic position of the fossils is uncertain. Krassilov (1985) suggested a ranunculidean affinity, but characters of the attached leaves, *Cissites* cf. *parvifolius*, indicate that *Caspiocarpus* may be an early member of the Trochodendrales (Friis and Crane, 1989). *Hyrcantha karatcheensis* is another fruiting structure from western Kazakhstan (Vachrameev, 1952; Krassilov *et al.*, 1983). The fruits are apocarpous with three to five elliptical follicles about 7 mm long and borne on a flattened receptacle. The calyx is persistent and comprises free and scaly sepals. Remnants of filaments suggest that the flower was bisexual and the small size of the sepals, pedicel and receptacle suggest that *Hyrcantha* was minute in the flowering stage. The fossils were compared to members of the Ranunculaceae and Paeoniaceae, but their affinity could not be established with certainty (Krassilov *et al.*, 1983). *Ranunculaecarpus quinquecarpellatus* is another possible ranunculidean fruiting structure. It

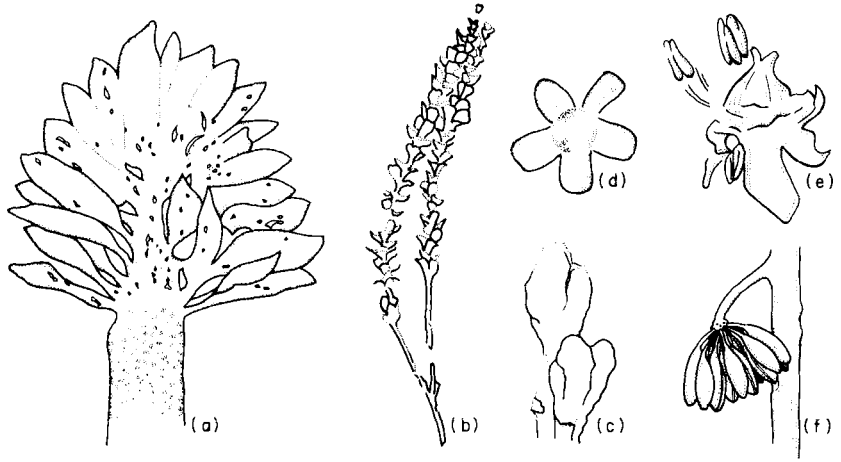

Fig. 4. Late Cretaceous (early Cenomanian) reproductive organs from Kansas (a–c, f), Nebraska (e) and New Jersey (d), USA. (a) Unnamed apocarpous fruit; (b, c) *Prisca reynoldsii*: (b) inflorescence and (c) detail of inflorescence showing lobed units; (d) dispersed calyx of *Calycites parvus*; (e) bisexual flower; (f) reconstruction of *Caloda delevoryana*. Redrawn from Friis and Crepet, 1987, based on Dilcher, 1979 (a, d); Retallack and Dilcher, 1981a (b, c); Friis and Crepet, 1987, based on Basinger and Dilcher, 1984 (e); Dilcher and Kovach, 1986 (f).

was described from the mid-Albian Kolyma Basin (Samylina, 1960) and consists of five free carpels with several seeds in each carpel. From the same deposits Samylina (1960) reported a small two-lobed structure, *Araliaecarpum kolymensis*, that possibly represents a syncarpous angiospermous fruit, but the nature of this fossil is obscure. Other fossils from the same flora (*Caricopsis compacta*, *C. laxa* and *Kenella harrisiana*) were assigned to the angiosperms by Samylina (1960, 1968), but their affinity with the angiosperms has not been established with certainty.

B. MID-CRETACEOUS FLOWERS

The Lower Cenomanian Dakota Formation of Kansas and Nebraska has yielded a number of different angiosperm flowers and fruiting structures exhibiting a considerable diversity of forms (Dilcher, 1979; Retallack and Dilcher, 1981a; Basinger and Dilcher, 1984; Crane and Dilcher, 1985; Dilcher and Crane, 1985; Dilcher and Kovach, 1986). Small, unisexual or bisexual structures crowded into heads or elongated, simple or compound inflorescences are common. They include species assigned to *Platanus* (Dilcher, 1979), *Caloda* (Dilcher and Kovach, 1986), two unnamed inflorescences (Dilcher, 1979) and probably also *Prisca reynoldsii* (Fig. 4b,c). This species was originally described by Retallack and Dilcher (1981a) from the Hoisington and Linnenberger localities in Kansas as a multifollicular apocarpous fruit with 50–90 carpels spirally arranged along an elongated, slen-

der floral base. No scars were observed below the supposed carpels and the structure was interpreted as derived from a single unisexual and apetalous flower. This interpretation is, however, equivocal. Units described by Retallack and Dilcher (1981a) as carpels are lobed (Fig. 4c) and probably represent compressed lateral branch systems and the fossil is probably an inflorescence similar to the lauraceous fossils from Elk Neck (Drinnan *et al.*, 1990b; see also p. 113). Also occurring with *Prisca* at the Hoisington locality is another elongated reproductive structure of small unisexual units described by Dilcher and Kovach (1986) as *Caloda delevoryana*. It is a dense infructescence consisting of an elongated main axis bearing spirally arranged secondary axes. Each secondary axis terminates in a spherical to ovate receptacle with numerous small and stalked follicles about 2 mm long (Fig. 4f). The fossils are strongly compressed and the original floral structure cannot be established with certainty, but Dilcher and Kovach (1986) suggested that the flowers were originally apetalous and unisexual. The characters available on the fossil do not afford sufficient evidence of the systematic affinity and the fossil was compared to a number of different modern groups within the dicots as well as the monocots. Another possible pistillate infructescence from the Dakota Formation in Kansas was figured by Dilcher (1979, fig. 49), and from the same deposits Dilcher (1979) also reported a staminate inflorescence consisting of closely packed and spirally arranged flowers. The flowers were apparently unisexual with a tetramerous androecium. The stamens have elongated pollen sacs, a prominent apical extension of the connective and contain zonocolpate pollen (Dilcher, 1979).

Larger, multiparted floral structures with an apocarpous gynoecium are also common in the Dakota Formation of Kansas (Fig. 4a) and include *Archaeanthus linnenbergeri*, *Lesqueria elocata*, and two different *Archaeanthus*-like fossils (Crane and Dilcher, 1985; Dilcher and Crane, 1985). Of these structures *Archaeanthus linnenbergeri* is probably the best understood, but all of the fossils are preserved in the fruiting stage and do not provide full information on the original floral structure. The apocarpous fruit of *Archaeanthus* is borne terminally on a stout stalk. It consists of an elongated floral base which in mature specimens is more than 10 cm long. Follicles are stalked and borne spirally in a loose arrangement along the floral base. A number of different scars below the gynoecium may indicate the position of stamens and perianth. Associated laminar organs assigned to two different species of *Archaepetala* were interpreted as inner and outer perianth parts of *Archaeanthus*, and Dilcher and Crane (1985) reconstructed the flower as a bisexual showy flower very similar to modern members of the Magnoliaceae. An unnamed fruiting structure also from the Dakota Formation of Kansas (Fig. 4a) has a similar elongated floral base and stalked follicles, while *Lesqueria* has a shorter, conical floral base (Crane and Dilcher, 1985). *Palaeanthus problematicus* described from the Amboy Clays of New Jersey (Newberry, 1895;

Crane and Dilcher, 1985) and several unnamed structures from the Dakota Formation (Crane and Dilcher, 1985) have a flat base. The structure of these fossils is, however, unclear and their angiospermous affinity has not been established with certainty.

A whorled arrangement of all floral parts has also been observed in Cenomanian angiosperms (Fig. 4d,e). Basinger and Dilcher (1984) described a medium-sized bisexual flower from the Dakota Formation of Nebraska, about 3–4 cm in diameter and with a pentamerous condition of all organs. The perianth parts are clearly differentiated into calyx and corolla and stamens are short, placed opposite the petals (Fig. 9b). The carpels are fused to form a syncarpous, five-loculed ovary and styles are free (Fig. 4e).

A number of small well-preserved fossil flowers are extracted from Lower Cenomanian Potomac Group sediments collected at the Elk Neck Peninsula, Maryland, USA. Flowers studied so far show a relationship to members of the Magnoliidae and Lower Hamamelididae and include the earliest record of the Lauraceae. The lauraceous flowers are trimerous with 2 perianth whorls and three whorls of fertile stamens; dehiscence of anthers are by two valves indicating bisporangiate condition. Flowers are crowded on elongated axes similar to those of *Prisca* (Drinnan *et al.*, 1990b).

A variety of floral structures have also been described from the Cenomanian of Czechoslovakia (Velenovský, 1889; Bayer, 1914; Velenovský and Viniklář, 1926, 1927, 1929, 1931). These are slightly younger than the North American fossils and exhibit a wide range of forms from large apocarpous structures such as *Triplicarpus purkynei* (Velenovsky and Viniklář, 1926) to small unisexual platanoids and possible myricaceous inflorescences as well as a range of cyclic flowers (Velenovský and Viniklář, 1926, 1929). Several flowers with inferior ovaries have also been reported from the Czechoslovakian mid-Cenomanian floras (Velenovský, 1889; Bayer, 1914; Velenovský and Viniklář, 1926) and represent the earliest occurrence of this gynoecium type. The structure of all these fossils is, however, not fully understood and there is no modern account of the material. From the same mid-Cenomanian strata, Knobloch and Mai (1986) reported a number of minute angiospermous fruits and seeds, among these *Caryanthus triasseris*. Related species from the Santonian/Campanian have Normapolles pollen *in situ* and have been assigned to the Juglandales/Myricales (cf. Section III.C).

C. LATE CRETACEOUS FLOWERS

A large number of angiosperm fruits, seeds and flowers have been recovered from Upper Cretaceous strata of Europe, Asia and North America. From Central Europe more than 100 different horizons containing angiosperm reproductive organs were reported by Knobloch and Mai (1986). The post-Cenomanian Late Cretaceous floras from Europe and North America show

considerable similarity in composition. With very few exceptions the angiosperm reproductive organs are all minute, generally ranging from about 0.5–5 mm. Although most of the fossils are fruits and seeds that provide little information on the original floral structure, well-preserved flowers with a wealth of details intact are also present and afford evidence of an appreciable diversity. The Santonian/Campanian flora of Scania, southern Sweden, includes a great number of different floral structures, preserved as three-dimensional charcoalifications or slightly compressed lignite fossils and is among the most informative of Late Cretaceous floras (Friis and Skarby, 1981, 1982; Friis, 1983, 1985a,b,c, 1990; Friis *et al.*, 1986, 1988; Crane *et al.*, 1989). Other floras of similar age with well-preserved fruits, seeds and flowers are the Martha's Vineyard flora (Tiffney, 1977) and the Neuse River flora (Friis, 1988; Friis *et al.*, 1988), both from eastern North America. Most of the flowers in these floras are bisexual with floral parts in a whorled arrangement and with a syncarpous gynoecium. They include only a few representatives of the Magnoliidae while the Hamamelididae and the Rosidae are represented by a large variety of forms.

The only magnoliid fossils so far encountered in the Scanian flora are the androecia of *Chloranthistemon endressii* (Crane *et al.*, 1989). The androecium of this species is dorsiventrally flattened and three-lobed, probably representing three stamens that are laterally fused at their bases (Fig. 10b). The central lobe (stamen) is slightly longer than the lateral ones and has two pairs of pollen sacs while the laterals have only one pair. The connective is extensive and fleshy, apically expanded into a triangular appendage, and with large intercellular spaces seen in transsections. These features indicate a close relationship with modern *Chloranthus* of the Chloranthaceae (Friis *et al.*, 1986; Crane *et al.*, 1989). In the Neuse River flora the Magnoliidae is represented by small seeds resembling *Liriodendron* (Magnoliaceae) as well as trimerous flowers and fruits of lauraceous affinity (E. M. Friis, in preparation).

The Hamamelididae is represented in the flora from Scania by a variety of forms. Except for the platanoid fossils they are all bisexual. The Platanaceae include the pistillate *Platanocarpus* sp., the staminate *Platananthus scanicus* as well as an unnamed pistillate and staminate structure. Like the Early Cretaceous platanoids these have a pentamerous arrangement of carpels and stamens (Friis *et al.*, 1988). Similar pentamerous platanoids were also discovered in the Neuse River flora and described as *Platanocarpus carolinensis* and *Platananthus hueberi* (Friis *et al.*, 1988). The Hamamelidaceae are represented in the Scanian flora by two small flowers closely resembling modern *Hamamelis* (Figs 5a–c and 10f). They are probably hypogynous. Perianth and androecium are in whorls of six to seven, while the gynoecium has three carpels in one specimen and possibly two in the other. The introrse stamens are bisporangiate with valvate dehiscence (P. K. Endress and E. M. Friis, in preparation).

Small bisexual flowers of juglandalean/myricalean affinity are among the

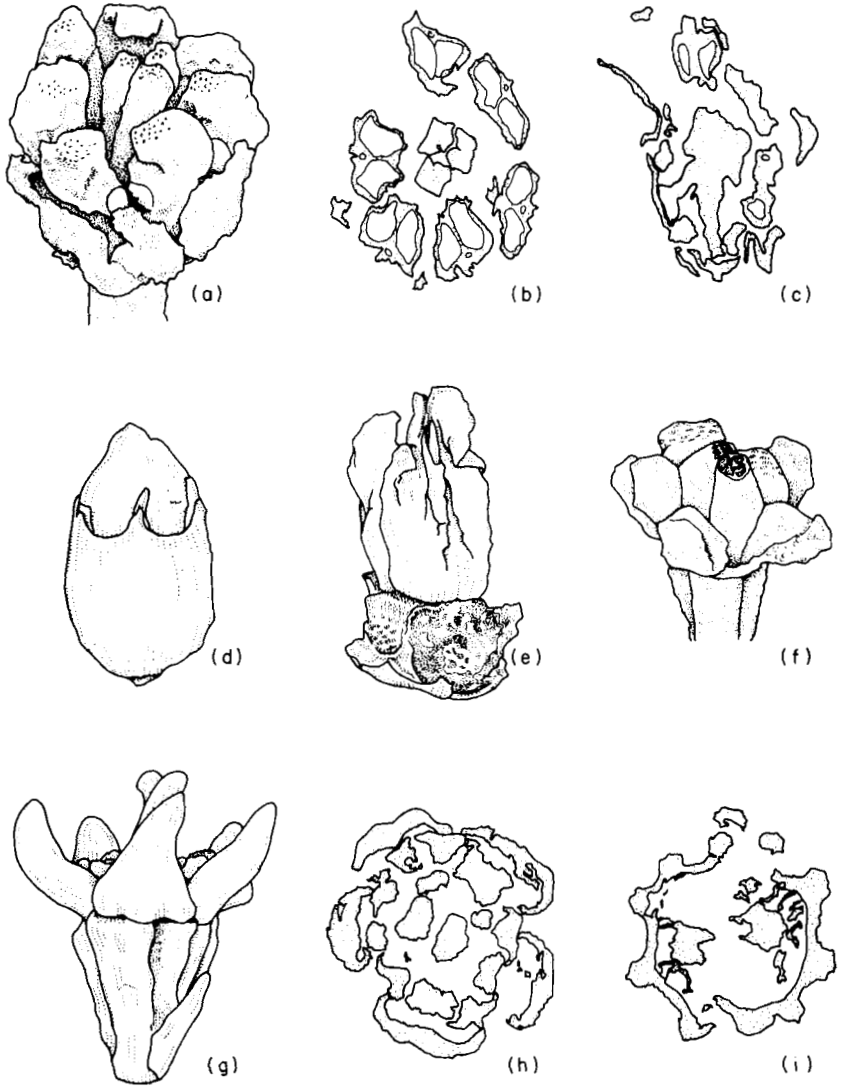

Fig. 5. Late Cretaceous (Santonian/Campanian) flowers from Scania, southern Sweden. (a–c) Hamamelidaceous flower: (a) specimen with seven stamens, section of the same flower (one stamen removed) (b) near apex and (c) near floral base; (d) *Antiquocarya verruculosa*; (e) *Actinocalyx bohrii*; (f) unnamed saxifragalean flower with five-lobed nectary disc; (g–i) *Scandianthus costatus*: (g) flower with calyx preserved, and sections of flower bud through (h) perianth region and (i) ovary. Redrawn from Friis and Crepet, 1987 (d–f); Friis and Skarby, 1982 (g–i).

most abundant in the fossil flora of Scania being represented by several thousand specimens. They may be grouped into six species in three genera, *Antiquocarya*, *Caryanthus* and *Manningia* (Friis, 1983). They are all epigynous, with a single whorl of perianth parts (Fig. 5d) and with a unilocular ovary containing a single orthotropous ovule. Triporate pollen referable to the extinct Normapolles complex has been found inside the flowers. *Antiquocarya* and *Manningia* both have radially symmetrical flowers with three carpels and five (*Manningia*) or six (*Antiquocarya*) tepals and stamens. *Caryanthus* are disymmetrical with two carpels, six stamens and four tepals. They are distinguished from modern members of the Juglandaceae and Myricaceae mainly in their bisexual flowers. However, bisexual flowers may occur sporadically in modern members of these families and modern *Rhoiptelea* (Juglandales) has bisexual as well as unisexual flowers on the same plant. A large number of fossils closely resembling the Scanian Normapolles flowers have also been reported from Central Europe (Knobloch and Mai, 1986), and more than 30 Late Cretaceous species can with some confidence be included in this ancestral Juglandales/Myricales complex (Friis and Crane, 1989).

Floral structures of rosidean affinity constitute another important element in the fossil flora of Scania. They are represented by a large variety of forms, most of which are probably related to the Saxifragales (Fig. 6). This group includes *Scandianthus costatus* and *S. major* (Friis and Skarby, 1982) *Silvianthemum suecicum* (Friis, 1990) as well as a number of unnamed forms (Figs 5f–i and 6c,d). The fossil saxifragalean flowers are characterized by being bisexual and epigynous with free perianth parts differentiated into calyx and corrolla. The androecium is in one or two whorls and stamens have a distinct filament and small tetrasporangiate anthers that open by longitudinal slits. The gynoecium is syncarpous with stout, usually free styles, and the ovary septate or unilocular (Fig. 5i) with numerous anatropous ovules. Sepals, petals and stamens are commonly in whorls of five (Fig. 5g,h), more rarely in whorls of four, and the gynoecium is di- or trimerous. In several of these saxifragalean flowers a distinct lobed nectary is present between the androecium and the gynoecium (Fig. 5f).

The Dilleniidae is the only other major group of angiosperms recognized thus far among the fossil flowers from Scania. It includes *Actinocalyx bohrii* (Friis, 1985a) and an unnamed flower probably of thealean affinity. The flowers of *Actinocalyx* are minute, hypogynous, radially symmetrical with five free sepals and five petals that are fused to form a short corolla tube with five ligulate lobes (Fig. 5e). There are five stamens alternating with the corolla lobes and the gynoecium is syncarpous and formed by three carpels. The characters of the fossils indicate a close relationship with modern members of the Ericales although they cannot be assigned to any modern family.

Few angiosperm floral structures are currently available from Asia. In the permineralized flora from the Turonian-Santonian of Hokkaido (Japan), Stopes and Fujii (1910) described small three-loculed fruits which they tentatively assigned to the Liliaceae. Two other reproductive organs from the

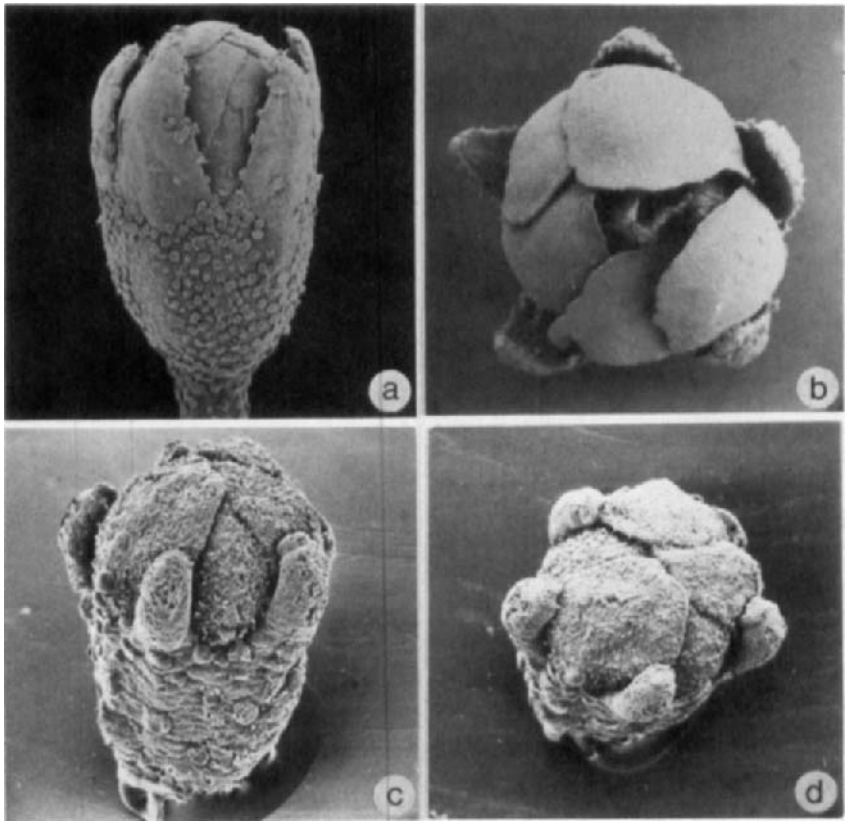

Fig. 6. Extant (a, b) and fossil (c, d) saxifragalean flowers. (a) *Quintinia* cf. *sieberi* (Escalloniaceae), × 17; (b) *Quintinia verdonii*, × 30; (c, d) *Silvianthemum suecicum* from the Late Cretaceous (Santonian/Campanian) of southern Sweden, (c) × 33, (d) × 30.

same flora were assigned to the angiosperms. They represent closely related multicarpellate gynoecia borne terminally on a thick peduncle (Nishida, 1985; Ohana and Kimura, 1987). Both fossils have a broad flattened or slightly concave floral base and numerous apocarpous follicles in a spiral arrangement (Fig. 7a,b). Carpel number is about 50 in one of the fossils (Nishida, 1985), but exceeds 400 in the other (Ohana and Kimura, 1987). Nishida (1985) assigned the fossil to the Magnoliidae and compared it with members of the Austrobaileyaceae and Monimiaceae. However, in a later publication, Nishida and Nishida (1988) were more cautious and did not assign the fossil (now named *Protomonimia*) to any particular family of the Magnoliidae.

From the Santonian/Campanian of Kazakhstan two different floral structures were decribed (Krassilov *et al.*, 1983). *Sarysua pomana* is probably preserved in the fruiting stage and comprises an elongate syncarpous fruit, about 7 mm long, with five free styles, and a persistent pentamerous calyx. It was

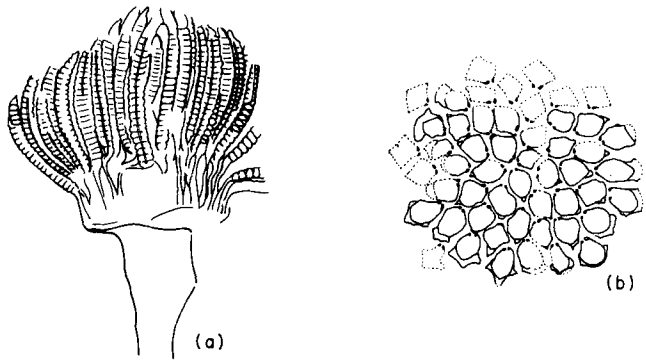

Fig. 7. Multicarpellate magnoliid gynoecia from the Late Cretaceous (Turonian-Santonian) of Japan. (a) Gynoecium with stalked follicles; (b) section through gynoecium. Redrawn from Ohana and Kimura, 1987 (a); Nishida, 1985 (b).

compared to modern members of the Geraniales. The other floral structure, *Taldysaja medusa*, is a compound inflorescence of minute simple flowers. It is, however, much compressed and no details of the floral structure were revealed (Krassilov *et al.*, 1983).

A number of well preserved permineralized flowers have been described from the Deccan Intertrappean beds of Mohgoan-kalan in India. The age of these beds has not been established with certainty. While they were previously regarded as of Early Tertiary age based on their content of fossil plants (e.g. Sahni, 1934) they are now regarded by some authors as of Maastrichtian age (e.g. Chitaley, 1977). This is supported by palaeozoological and palaeomagnetic data (Sahni *et al.*, 1984; Acton and Gordon, 1989). Flowers described from these strata are all small and show a large variety of forms also including several monocotyledons. The Arecidae is represented by trimerous flowers and fruits of *Deccananthus*, *Nipa*, *Palmocarpon*, *Tricoccites* and *Viracarpon* (Rode, 1933; Sahni, 1934; Prakash, 1954; Chitaley and Nambudiri, 1969; Chitaley and Kate, 1974).

D. EARLY TERTIARY FLOWERS

The early Tertiary record of angiosperm reproductive organs is vast, including mainly fruits and seeds. Floral structures from two areas have been especially important in elucidating organizational levels of Early Tertiary angiosperms. These are the compression fossils from the Palaeocene and Eocene of Tennessee (Crepet and Dilcher, 1977; Crepet, 1978, 1985; Crepet and Daghlian, 1980, 1981a,b; Zavada and Crepet, 1981; Crepet and Taylor, 1985, 1986; Taylor and Crepet, 1987), and the Late Eocene to Oligocene flowers of the Baltic Amber (Göppert, 1836; Göppert and Berendt, 1845; Conwentz, 1886). From this time interval the range of floral diversity has increased considerably and a number of advanced floral types associated with

bee and butterfly pollination was present already in the Palaeocene (Crepet and Friis, 1987). These Palaeocene forms include *Protomimosoidea buchanensis* (Crepet and Taylor, 1986) and several different unnamed papilionoid flowers (Crepet and Taylor, 1985). The latter is distinctly zygomorphic with the calyx unevenly lobed and the corolla differentiated into standard, wings and keel. The mimosoid flower has a deeply lobed calyx and five valvate petals. The stamens have long thread-like filaments and the anthers are versatile and sagittate (Crepet and Taylor, 1985). From the same Palaeocene beds are also two different types of euphorbiaceous inflorescences composed of pseudanthial units borne along an elongated axis. In one of these, assigned to the tribe Euphorbieae, the pseudanthium consists of a whorl of bracts subtending an apparently tricarpellate pistillate flower and at least three staminate flowers (Friis and Crepet, 1987). The other is related to the tribe Hippomaneae and the Eocene *Hippomaneoidea warmanensis* which has a staminate inflorescence (Crepet and Daghlian, 1981a). Advanced flowers from the Eocene of Tennessee include a number of unnamed forms with deep, narrow corolla tubes as well as more open, funnel-shaped forms such as the gentianaceous *Pistillipollenites*-producing flower (Crepet and Daghlian, 1981b). The flower is sympetalous with seven corolla lobes. Pollen grains found in the flower are triporate with a distinct gemmate ornamentation. Similar pollen has also been found dispersed in strata of Palaeocene age suggesting that this floral type was also present at this time.

Unisexual flowers of hamamelididean affinity are diverse in the Early Tertiary floras and include representatives of the Platanaceae, Urticaceae, Casuarinaceae, Fagaceae, Betulaceae, Myricaceae and Juglandaceae. The Platanaceae is represented in the Eocene of North America by pistillate and staminate inflorescences described as *Macginicarpa glabra* and *Platananthus synandrus* (Manchester, 1986). They are similar to the Cretaceous platanoids in the pentamerous arrangement of carpels and stamens and represent the youngest of these ancestral platanoids. The Fagaceae are represented by a large variety of forms referable to modern genera such as *Quercus*, *Castanea* and *Fagus* (e.g. Conwentz, 1886) or to extinct forms such as *Castaneoidea* (Crepet and Daghlian, 1980). They are mostly represented by staminate flowers while pistillate flowers are more rare.

IV. EVOLUTION OF FLORAL ORGANS AND FLORAL ORGANIZATION

A. PHYLLOTAXIS

The floral organs arise in a certain pattern of arrangement, like the arrangement pattern of leaves, which is called the phyllotaxis. For flowers we use "floral phyllotaxis". Leppik (1961) used the term "anthotaxis" to distinguish the floral from the vegetative regions. This would logically refer to the

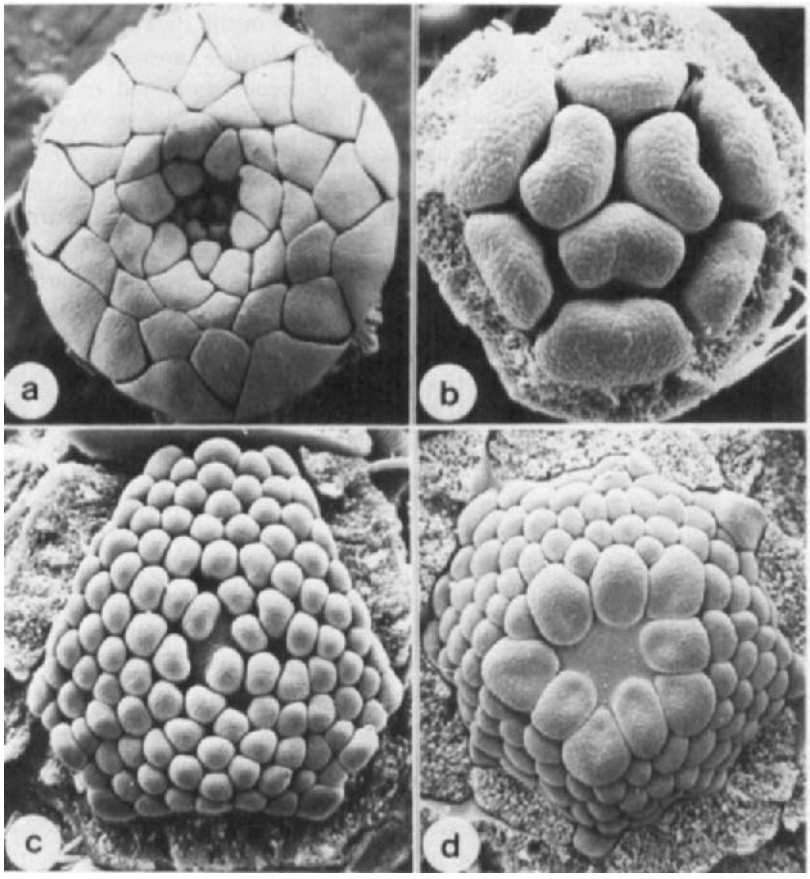

Fig. 8. Phyllotactic patterns of floral organs. (a) Spiral phyllotaxis in *Galbulimima baccata* (Himantandraceae), × 25; (b) whorled phyllotaxis in *Cinnamomum camphora* (Lauraceae), × 90; (c) complex symmetrical phyllotaxis in *Artabotrys uncinatus* (Annonaceae), × 60; (d) chaotic phyllotaxis in *Exospermum stipitatum* (Winteraceae), × 60. From Endress (1986a) with permission.

arrangement of flowers in an inflorescence rather than the arrangement of floral organs in a flower.

The two most common patterns are spiral and whorled phyllotaxis. In the *spiral* pattern subsequent organs arise in equal time intervals (plastochrons) and in equal distances from each other (divergence angles) (Fig. 8a). As seen in a projection subsequent organs are situated in a spiral, the ontogenetic spiral. In the *whorled* pattern there are rhythmic changes in the plastochrons and divergence angles. Here, entire groups of organs arise in coordination so that each group forms a whorl of an equal number of organs (Fig. 8b). Subsequent whorls arise in equal plastochrons and divergence angles. Within a whorl the organs do not arise exactly simultaneously, but with very short plastochrons in the same sequence as in the spiral pattern. Within a whorl the

distance of immediate neighbours (not subsequent organs) is more or less equal in contrast to the spiral arrangement where there are two distance classes between corresponding members.

In extant angiosperms the whorled pattern by far prevails. The spiral pattern is abundant only in the subclass Magnoliidae, while it is much rarer in the other subclasses (cf. Endress, 1987a). In Magnoliidae it coexists with the whorled pattern, often even at a low level of the systematic hierarchy (e.g. within Ranunculaceae, Monimiaceae). In other cases there are transitions between the two types: in Magnoliaceae flower development starts with whorls and changes to a spiral (Erbar and Leins, 1982). More complicated (e.g. Annonaceae) or irregular (chaotic) patterns (Fig. 8d; e.g. Winteraceae) also exist (cf. Endress, 1986a, 1987a).

The phyllotaxis of the Early Cretaceous floral structures is difficult to establish, partly because of the preservation. Spiral arrangement of parts appear to be the most common organization, but the spirals are generally compressed and there are no indications of very elongated floral axes among the earliest forms. *Williamsonia recentior* has a flat or slightly conical floral base. Several mid-Cretaceous forms figured by Crane and Dilcher (1985) have similar flat receptacles apparently with spirally arranged parts, while others have elongated receptacles with distinct spiral arrangement. In *Archaeanthus* the floral base becomes extremely long, up to 137 mm in mature specimens (Dilcher and Crane, 1985). The perianth is apparently in whorls while stamens and carpels are in spiral arrangement. A distinct spiral arrangement of carpels was documented for the two magnoliid fossils from the Upper Cretaceous of Japan (Nishida, 1985; Ohana and Kimura, 1987).

The presence of a small syncarpous fruit from the mid-Albian West Brothers locality indicates that whorled phyllotaxis was also developed in Early Cretaceous. This is also indicated by the structure of the small tetramerous male flower from the same locality. By the mid-Cretaceous whorled organization was common indicated by several calyces (illustrated by Dilcher, 1979), the Nebraskan flower (Basinger and Dilcher, 1984), the Lauraceae flower from the Potomac Group (Drinnan *et al.*, 1990b) and a number of compressed flowers from Czechoslovakia (Velenovský, 1889; Velenovský and Viniklář, 1926, 1931). In the Upper Cretaceous floras of Scania and North America whorled phyllotaxis prevails as in modern flowers.

B. NUMBER OF PARTS

The floral organs (sepals, petals, stamens, carpels) occur in most cases in a fixed, relatively low number, somewhere between 10 and 30. This is mainly true for those flowers with a whorled phyllotaxis. Here the number of organs per whorl as well as the number of whorls per flower is more or less limited and fixed. Whorls often contain five (dicots) or three organs (monocots). In

flowers with spiral phyllotaxis there is much more variation in organ number since there is no limitation by the completion of cycles. The range in organ number may be substantial within families, genera, species and even individuals in several groups of the Magnoliidae (e.g. Ranunculaceae, Schöffel, 1932; Winteraceae, Vink, 1970; Monimiaceae, Lorence, 1985; see also Endress, 1986a). In the genus *Tambourissa* (Monimiaceae) organ number per flower may vary between about 40 (*T. purpurea*) and 2000 (*T. ficus*); in the species *Tasmannia piperita* (Winteraceae) in male flowers between about 10 and over 100.

In flowers with whorled phyllotaxis there is also a possibility to increase the number of floral parts either by increasing the number of parts per whorl (e.g. Crassulaceae) or by increasing the number of whorls (e.g. Rosaceae). However, these instances are rare. More frequent is an increase of stamen number by subdivision of five or three primary primordia into numerous secondary primordia that differentiate into perfect stamens (e.g. many Dilleniales, Theales, Malvales).

Extremely low numbers of organs per flower may occur in both spiral and whorled patterns. *Ascarina* (Chloranthaceae), *Cercidiphyllum* (Cercidiphyllaceae) and some Araceae include flowers consisting of a single organ.

Within a flower the number of stamens tends to exceed that of the carpels. In the dicots dimerous or trimerous gynoecia within otherwise pentamerous flowers are very common in many families (Hamamelididae, Rosidae, Dilleniidae, Asteridae). In the monocots trimerous gynoecia are common in flowers with six stamens (in two trimerous whorls).

Among the Early Cretaceous fossils, the number of floral organs is generally low and floral phyllotaxis patterns are difficult to establish. Perianth parts are only rarely preserved. In *Platananthus/Platanocarpus* the number of tepals has not been conclusively demonstrated, but it does not exceed 15. In the mid-Albian *Hyrcantha* the number of perianth parts was apparently also low, but none of the specimens has a completely preserved perianth. No multiparted androecia are recorded from this time interval. In the four androecial structures from the West Brothers locality one is three-parted, one has four and two have five stamens. Carpel number in the Early Cretaceous forms generally ranges from three to eight. Only in *Williamsonia recentior* is the number considerably higher and is estimated to be about 150–200 (Crane and Dilcher, 1985). In the mid- and Late Cretaceous several multiparted forms were described and in *Archaeanthus* and *Lesqueria* carpel number was estimated to be about 100 and 200, respectively, and in one of the petrified reproductive organs from Japan, Ohana and Kimura (1987) reported more than 400 carpels.

The mid-Cretaceous flowers with whorled phyllotaxis and fixed number of parts are typically isomerous with an equal number of floral parts in all whorls. Trimerous, tetramerous, pentamerous and a single hexamerous form have been reported. In the Late Cretaceous the flowers are generally hetero-

merous with pentamerous (or more rarely tetramerous) perianth and androecium and trimerous or dimerous gynoecium.

C. FLORAL SIZE

Flower size is partly correlated with floral organ number, but may also vary considerably among groups with fixed floral organ number.

The largest flowers of the angiosperms occur among highly specialized groups of the Magnoliidae in Aristolochiaceae and Rafflesiaceae. There, the perianth may reach 1 m in length or diameter. Stamens and carpels, however, remain relatively small, even in such giant flowers. Especially the size of anthers (thecae) and ovules is constrained. Thecae are never longer than a few centimetres. Ovules are never longer than a few millimetres. The length of styles and filaments is more variable. Among the Magnoliales, Magnoliaceae contain the largest flowers (up to about 30 cm in diameter); the smallest are in the Myristicaceae and Canellaceae (about 1 cm long) and some Winteraceae (about 0.5 cm in diameter). Many Magnoliales have flowers ranging within 2–5 cm in diameter (e.g. Annonaceae, Eupomatiaceae, Himantandraceae, Degeneriaceae, Austrobaileyaceae, Illiciaceae, Calycanthaceae, some Winteraceae).

Among the Laurales most groups contain relatively small flowers of 1 cm or less in diameter (Chloranthaceae, Trimeniaceae, Amborellaceae, Lauraceae, Monimiaceae, Gomortegaceae). The largest flowers occur within the highly plastic monimiaceous genus *Tambourissa* and reach 8 cm in diameter (cf. Endress and Lorence, 1983; Lorence, 1985).

The vast majority of flowers recovered from the Cretaceous are minute, generally only a few millimetres in diameter. Evidence from fruiting structures and dispersed perianth parts indicates, however, that larger flowers were also present.

In the mid-Albian *Caspiocarpus paniculiger* the small flowers, which are about 2 mm long, are densely crowded in elongated inflorescences. Elongated inflorescences with densely crowded small flowers have also been recorded from the mid-Cretaceous and include *Prisca reynoldsii*, *Caloda delevoryana*, the Lauraceae flower and an unnamed inflorescence (Dilcher, 1979, fig. 45). Minute flowers crowded in globose inflorescences from the mid-Albian include *Platananthus potomacensis*, *Platanocarpus marylandensis* and an undescribed possibly rosidean form from the West Brothers locality, and several undescribed fossils from other Potomac Group sediments.

D. FLORAL SYMMETRY

The most common type of floral symmetry is *radial* or actinomorphic (with three or more planes of symmetry), and occurs in spiral as well as whorled

flowers. *Disymmetric* patterns (with two planes of symmetry) occur only in flowers with dimerous whorls (e.g. some Berberidaceae, Papaveraceae, Cruciferae, Oleaceae).

Monosymmetric or *zygomorphic* flowers (with only one plane of symmetry) occur in many different groups. Most of them have whorled phyllotaxis and a fixed moderate number of organs. Here zygomorphy is intimately correlated with pollination biology (mostly Hymenoptera; e.g. Scrophulariales, Leguminosae). Zygomorphy may also occur in flowers with spiral phyllotaxis (e.g. some Ranunculaceae). Another type of zygomorphy occurs in flowers with extreme low numbers of floral organs (e.g. Chloranthaceae, Piperaceae, Hippuridaceae) and may have been attained by reduction (Endress, 1987c; Leins and Erbar, 1988). Zygomorphy may also occur as a transient stage in the early development of flowers that become actinomorphic or disymmetric (e.g. Trochodendrales; cf. Pervukhina and Yoffe, 1962; Endress, 1986b). The symmetry of zygomorphic flowers may be established in different stages of development (cf. Tucker, 1984a,b, 1987).

Asymmetric flowers (without a plane of symmetry) are rare. They occur in some groups with reduction in floral organ number (e.g. Valerianaceae, Marantaceae, Cannaceae).

Floral symmetry may be difficult to establish in compressed fossil material, but apparently most Early Cretaceous forms show radial symmetry. This is distinct in the mid-Albian platanoid and rosidean flowers as well as the tetramerous male flower from the West Brothers locality. The possible chloranthoid androecium with the three stamens apparently in a linear arrangement indicate that the type of zygomorphy associated with extreme low numbers of floral parts existed in the Early Cretaceous. This type is further developed in the Late Cretaceous *Chloranthistemon endressii*. The first floral evidence of true zygomorphic flowers is in the Maastrichtian *Raoanthus* (Chitaley and Patel, 1975). In this flower the zygomorphy is only weakly developed. Strongly zygomorphic flowers, however, are recorded from the Early Tertiary and include among others papilionoid flowers with distinct keel, wing petals and a standard (Crepet and Taylor, 1985). The presence of zingiberaceous seeds in the Upper Cretaceous of North America and Europe (Friis, 1988) may indicate that true zygomorphy was established as early as the Santonian/Campanian.

E. DISTRIBUTION OF SEX

Bisexual angiosperm flowers always have a central gynoecium surrounded by the androecium. This pattern is constant and suggests a basic condition.

Unisexual flowers may show sterile parts of the opposite sex or not. If these organs are present they have the same position as in bisexual flowers.

Both bisexual and unisexual flowers occur in all subclasses and in most

smaller groups of the angiosperms. This distribution suggests an easy transition between both conditions. Within the Magnoliidae bisexual flowers are predominant, for example in Magnoliaceae, Annonaceae, Degeneriaceae, Austrobaileyaceae, Himantandraceae, Illiciaceae, Ranunculaceae and Nymphaeaceae, while unisexual flowers prevail in Monimiaceae, Myristicaceae, Ceratophyllaceae, Menispermaceae and Lardizabalaceae. In the Winteraceae and Chloranthaceae genera with unisexual flowers as well as genera with bisexual flowers occur. Among the floral biological classes there is a high amount of unisexual flowers in the wind-pollinated groups, often correlated with small floral size. Since among the angiosperm subclasses the Hamamelididae are especially characterized by small, wind-pollinated flowers, they also have (besides the Arecidae) the highest percentage of unisexual flowers.

The distribution of sex may be difficult to establish with certainty for fossil material. Stamens in many cases are shed at an early stage without leaving distinct scars. From well-preserved material it has, however, been possible to show the presence of both unisexual and bisexual forms in the Cretaceous. Among the relatively few floral structures known from the Early and mid-Cretaceous a remarkably high proportion have unisexual flowers. This has been firmly established for *Platananthus* and *Platanocarpus*, the tetramerous West Brothers flower, *Platanus* and two undescribed flowers from the Dakota Formation (Dilcher, 1979). It is probably also the case for *Caspiocarpus* and *Caloda*. The only well-established bisexual flowers from this time interval are the Cenomanian flower from Nebraska and the Lauraceae flower from the Potomac Group, but bisexuality is also suggested for *Archaeanthus* based on a number of distinct scars below the gynoecium, and for *Hyrcantha* based on possible remains of filaments.

In the Late Cretaceous, bisexual flowers are by far the most dominant and also prevail in flowers that otherwise show adaptation for wind-pollination such as the Normapolles flowers.

The pistillate flowers of *Platanocarpus* have several layers of membranous floral parts surrounding the gynoecium. The elongated inner parts have clusters of pollen grains on their adaxial surfaces and they may well be staminodes suggesting a derivation from a bisexual ancestor (Friis *et al.*, 1988).

F. PERIANTH

The perianth in angiopsperms is often differentiated into an outer part (calyx) and an inner part (corolla) with different structure, development and function (Fig. 9a,b). The *calyx* is a complex of organs (sepals) protecting the young inner floral organs in bud. The *corolla* is a complex of optically attractive organs (petals) displayed at anthesis. Typical sepals are green, robust, early differentiated, with a broad base that is supplied with three

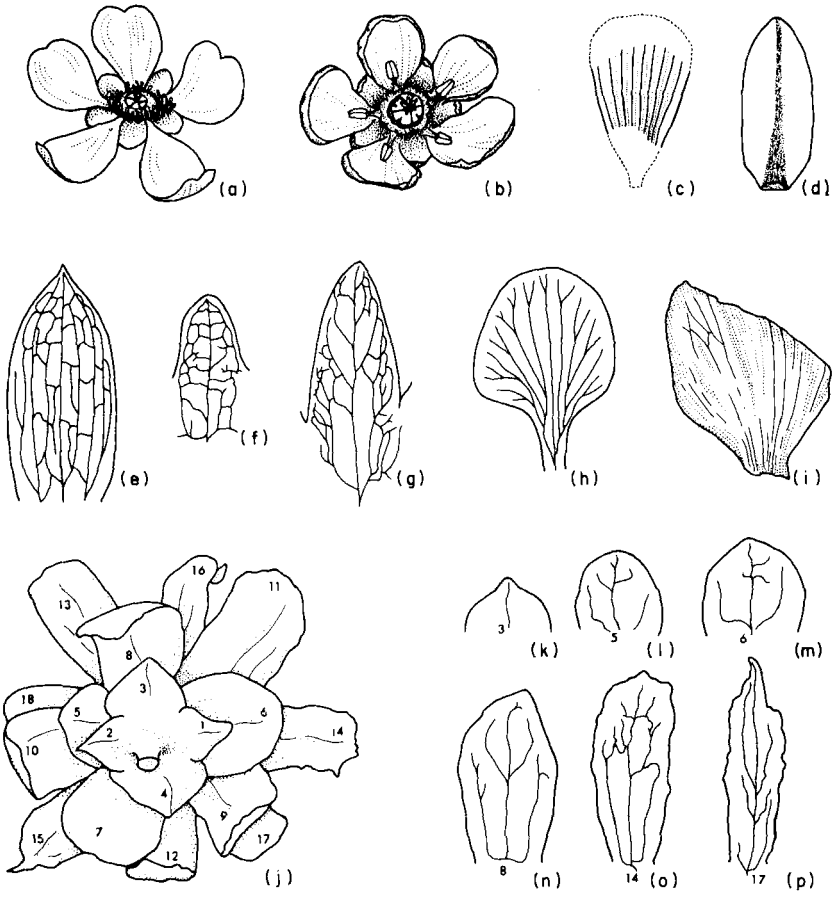

Fig. 9. Perianth morphology in extant (a, e–h, j–p) and Late Cretaceous (early Cenomanian) (b–d, i) flowers. (a) *Exochorda serratifolia*; (b) reconstruction of the fossil flower from Nebraska; (c, d) possible perianth parts of *Archaeanthus linnenbergeri*: (c) *Archaepetala obscura*, inner perianth part and (d) *Archaepetala beekeri*, outer perianth part; (e) sepal of *Pelargonium radula*; (f) sepal and (g) petal of *Solanum luteum*; (h) petal of *Aneilema beniniense*; (i) fossil *Magnolia palaeopetala*; (j–p) transition of tepal morphology in a spiral flower of *Hortonia angustifolia*. Redrawn from Hooker, 1877–79 (a); Basinger and Dilcher, 1984 (b); Dilcher and Crane, 1985 (c, d); Rohweder and Endress, 1982 (e–h); Hollick, 1903 (i); Endress, 1980b (j–p).

vascular bundles and with an acute tip (Fig. 9e). They are initiated with relatively long plastochrons. Typical petals are coloured or white, thin, ephemeral, differentiated late (although initiated in the normal acropetal sequence of the floral organs), with a small base that is supplied with one vascular bundle, and with a broad, rounded, sometimes emarginated or even bilobed apical region (Fig. 9h). They are initiated almost simultaneously (cf. Hiepko, 1965; Erbar and Leins, 1985; Tucker, 1987). From this difference the hypothesis has been derived that sepals are modified bracts while petals are modified staminodes (Hiepko, 1965). This clear-cut differentiation occurs mainly

in the middle evolutionary level of the dicots (Rosidae, Dilleniidae, Caryophyllidae) but is less common in the more primitive (Magnoliidae) and more advanced (Asteridae) groups or in monocots.

In the more highly evolved Asteridae the petals are often congenitally fused and often the stamens are congenitally fused with the petals as well (see also p. 152). Under this condition the petals are more robust and differentiated earlier and therefore less different from the calyx in behaviour (Fig. 9f,g). The only distinction that remains is that they are the second perianth whorl.

In the Magnoliales and Laurales there are no typical petals. Although the inner perianth members are often somewhat different from the outer ones in having more attractive than protective functions, in shape, anatomy, and development they resemble more the typical sepals than petals (Fig. 9j–p). Among Magnoliidae only some Ranunculales have differentiated typical petals inside the sepals.

In many monocots outer and inner perianth parts do not differ much from each other and it is more difficult to interpret them as being derived from either bracts or staminodes (cf. Weber, 1980; Vogel, 1981).

In some families of the Magnoliidae and Lower Hamamelididae a conventional perianth is absent altogether. The protective function is then performed by the subtending floral bract (e.g. Chloranthaceae, Piperales) or by organs still lower on the inflorescence axis (Trochodendrales) or by single bracts closed around the floral axis (Eupomatiaceae). Attractive organs are stamens or staminodes, sometimes inner staminodes (between androecium and gynoecium) that are highly differentiated (Eupomatiaceae, Himantandraceae, Austrobaileyaceae, Degeneriaceae; Endress, 1984b).

The only Early Cretaceous floral structure with well-preserved perianth parts are the two platanoid forms, *Platanocarpus* and *Platananthus*. The latter apparently has an undifferentiated perianth or membranous laminar parts, all of equal length, while in *Platanocarpus* the perianth consists of two different sets of organs. The outer set has smaller, bract like parts while the inner has longer, spathulate to linear parts. These inner perianth parts are not typically petal-like in appearance and may be modified staminodes. In *Caspiocarpus* only small bract-like parts seem to be present. In *Archaeanthus* a number of larger scars at the base of the floral axis suggest the presence of a well-developed perianth. Associated with *Archaeanthus* Dilcher and Crane (1985) described two different laminar structures, *Archaepetala beekeri* (Fig. 9d) and *Archaepetala obscura* (Fig. 9c) thought to represent, respectively, an outer calyx-like and an inner corolla-like perianth of *Archaeanthus*. *Magnolia palaeopetala* is another petal-like fossil from the Early Cenomanian (Hollick, 1903). It has a broad, short base and a broad rounded apex (Fig. 9i).

The earliest evidence of differentiation into a distinct calyx and corolla is in the flower from Nebraska, which has relatively thick, persistent sepals and more delicate petals (Fig. 9b) that are otherwise rarely preserved. Among the

Late Cretaceous floral structures well-differentiated perianth types are predominant and show some variability with free or more rarely fused parts.

G. ANDROECIUM

The structural units of the androecium are the *stamens*. The fertile part of a stamen, the *anther*, has two lateral *thecae*, each composed of two *pollen sacs*; each theca opens by a longitudinal slit between the two pollen sacs to release the pollen. The sterile part below the anther, often developed as a thread-like *filament*, raises the anther from the floral base at anthesis. More rarely a sterile part is also developed above the anther as a *connective protrusion*, the connective being the central part of the anther between the two thecae.

Mainly in the Magnoliidae and Lower Hamamelididae connective and connective protrusion are often well developed (Fig. 10a–f). In some Magnoliidae the stamens even appear laminar without a distinct anther but with two thecae on the ventral or dorsal side or on the margins of a more or less flattened organ (Fig. 10a, e.g. Degeneriaceae, Himantandraceae, Austrobaileyaceae, Chloranthaceae, Schisandraceae; cf. Canright, 1952).

In such stamens with well-developed connectives and connective appendages, often the dehiscence pattern is more complicated. The longitudinal slits between the two pollen sacs may bifurcate at both ends, which results in two valves (Fig. 10c,d; e.g. Degeneriaceae, Himantandraceae, Annonaceae, Chloranthaceae, Trochodendrales, Hamamelidales; cf. Endress, 1986b, 1989a; Endress and Hufford, 1989; Hufford and Endress, 1989). In some families the valves have become more elaborated (Berberidaceae and Laurales), consequently the two pollen sacs of a theca have become more independent of each other and eventually one of them has disappeared (Fig. 10e,f; some Lauraceae, Monimiaceae, Hernandiaceae, Gomortegaceae, some Hamamelidaceae). Valvate anther dehiscence is not known so far from subclasses other than Magnoliidae and Hamamelididae.

Another type of anther dehiscence is by a pore at the base or top of each theca. This is often correlated with dry pollen and buzz-pollination by bees (e.g. some Dilleniales, Ericales, many smaller groups of higher advanced dicots and monocots; cf., e.g. Buchmann, 1983) or with sticky pollen and pollination by flies (e.g. Rafflesiaceae, Araceae).

Congenital fusion of the stamens occurs in several groups and sometimes is characteristic for families (e.g. Myristicaceae, Canellaceae, Schisandraceae, Meliaceae, Malvaceae, Bombacaceae, Nepenthaceae; e.g. van Heel, 1966). In taxa where primary primordia are secondarily subdivided in early floral ontogeny, the stamen groups sometimes form bundles of stamens that are united at the base (e.g. Theales, Erbar, 1987; Myrtales, Juncosa and Tomlinson, 1987).

Postgenital fusion of anthers occurs in some highly advanced dicots (Com-

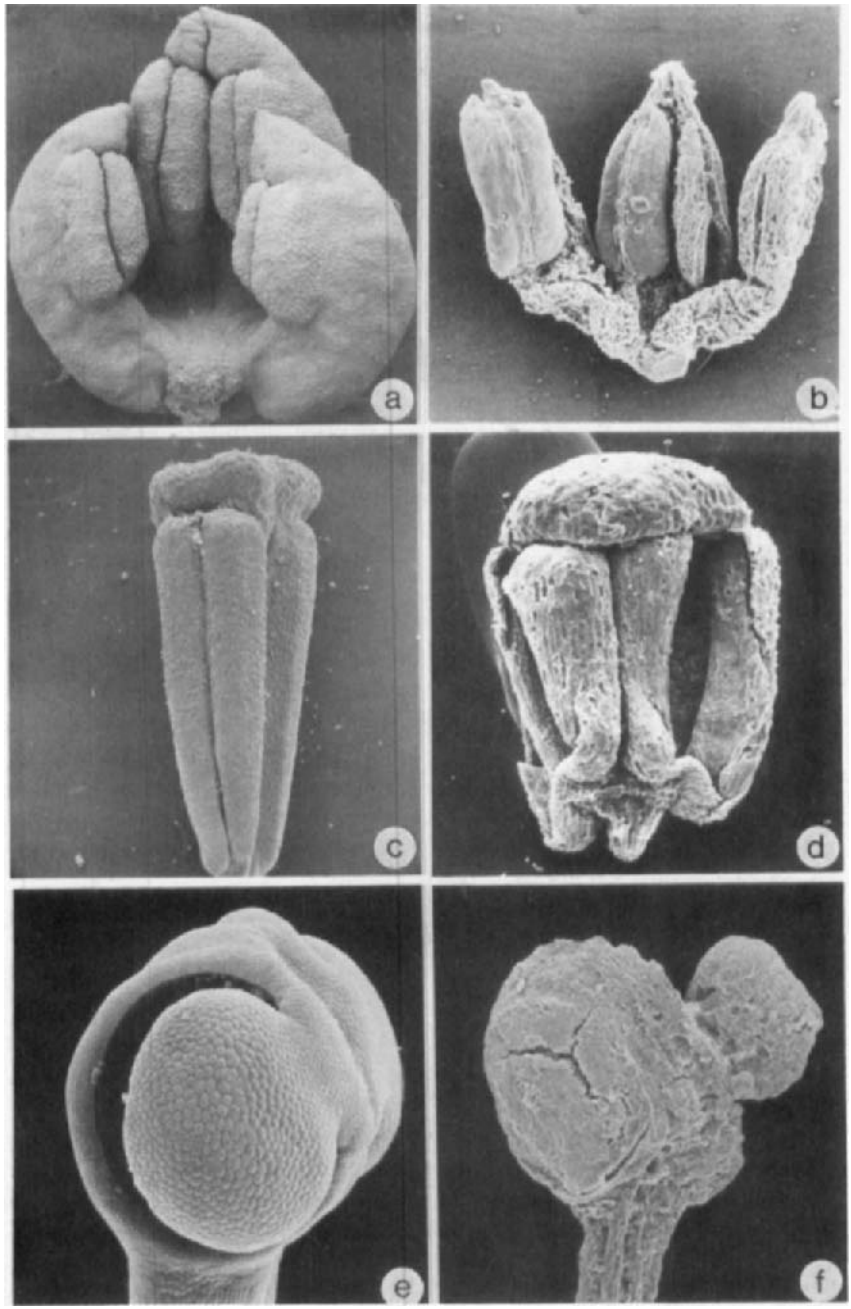

Fig. 10. Stamen morphology in extant (a, c, e) and fossil (b, d, f) Chloranthaceae (a, b), Platanaceae (c, d) and Hamamelidaceae (e, f); fossil stamens all from the Late Cretaceous (Santonian/Campanian) of southern Sweden. (a) *Chloranthus spicatus*, × 16; (b) *Chloranthistemon endressii*, × 50; (c) *Platanus orientalis*, × 25; (d) dispersed stamen, × 60; (e) *Hamamelis japonica*, × 66; (f) Dispersed stamen, × 90.

positae and some other Campanulales, Gesneriaceae; e.g. Trapp, 1956, Thiele, 1988).

Fossil stamens recovered so far from Lower Cretaceous strata are rather uniform in structure. They are all tetrasporangiate with four pollen sacs in two pairs. Anthers are generally borne on a short, fleshy filament and the pollen sacs are relatively long. In the possible chloranthoid stamens the sterile part below the pollen sacs is longer and merges gradually into the connective. The connective is well developed in most species and all have a more or less distinct apical connective appendage. The dehiscence is by longitudinal slits or valvate with longitudinal slits that bifurcate at both ends. Laminar stamens without a distinct anther have not been recorded from the Early Cretaceous.

Stamens recovered from Upper Cretaceous strata exhibit a greater variety in structure. Most of the species studied so far are tetrasporangiate, but bisporangiate forms were apparently also developed by the Late Cretaceous as represented by the lauraceous flower from the Potomac Group and by the hamamelidaceous flower from southern Sweden. Among the Late Cretaceous stamens forms with long thread-like or band-like filaments, inconspicuous connectives and without connective appendages are most abundant, while forms with short filaments and expanded connectives that characterize the early forms are less common. Dehiscence in the Late Cretaceous stamens is usually by simple longitudinal slits, more rarely valvate (Fig. 10b,d,f; in *Chloranthistemon*, the lauraceous flowers, *Platananthus* and the hamamelidaceous flowers). Anther dehiscence by pores has not been observed in any of the Cretaceous stamens. This feature is, however, fully developed in the Early Tertiary observed in the Late Palaeocene flower *Sezanella*, from France (Viguier, 1908).

H. GYNOECIUM

The angiosperm gynoecium is the most complicated organ of the flower, mainly because parts of it are not freely exposed but enclosed and form a system of canals and chambers.

Although in the majority of the angiosperms the gynoecium is a unified (*syncarpous*) structure the structural units, the *carpels*, are almost always easy to recognize. In some more primitive groups the carpels remain completely separated, the gynoecium is *apocarpous* (=choricarpous; e.g. many Magnoliidae and Alismatidae, some Rosidae) or, quite often, there is only one carpel. Some larger families are entirely monocarpellate, such as Lauraceae, Hernandiaceae, Myristicaceae, Berberidaceae (Magnoliidae) or Leguminosae and Proteaceae (Rosidae). The syncarpous gynoecium or the free carpels are differentiated into an *ovary* containing the ovules, a *stigma* receiving the pollen grains, and often a *style* raising the stigma for better exposition.

The carpel is a conduplicate (involute) or ascidiate structure (cf. van Heel, 1981). Mostly the adaxial side is lower and the flanks of the upper parts are pressed together and form a closed (occluded) *ventral slit*. The ventral slit forms the *pollen tube transmitting tract*. The region with the slit is the *plicate* zone, the region below the *ascidiate* zone (Leinfellner, 1950).

A carpel contains from one up to many *ovules* (Bouman, 1984; Boesewinkel and Bouman, 1984). Usually the ovules are positioned along the carpel margins; they are curved (anatropous) away from the ventral slit so that their apex, the micropyle, again faces the ventral slit. Therefore, pollen tubes growing down through the ventral slit can reach the ovules directly via the micropyle (Tilton and Lersten, 1981).

If several ovules occur in a carpel, they are often arranged in two rows along both carpellary flanks. If a single ovule occurs, it is often located at the lower end of the ventral slit either on one side (lateral) or in the middle (median position). This condition may be constant in a larger group. Magnoliales, for example, mostly have several or one *lateral* ovules, while Laurales mostly have one *median* ovule.

Anatropous, *bitegmic*, *crassinucellar* ovules are by far the most common in the angiosperms. *Orthotropous* (uncurved) ovules are rare and occur either in simple gynoecia or carpels, where the micropyle of the single basal ovule is in direct contact with the stylar canal (e.g. Piperaceae, Polygonaceae, Juglandaceae) or where a mucilaginous secretion mediates the pollen tubes to the micropyles (e.g. Chloranthaceae some Nymphaeaceae, Lardizabalaceae, Rafflesiaceae, Arales; cf. Endress, 1989b). *Tenuinucellar*, *unitegmic* ovules are highly constant in the highest evolved dicots (Asteridae) and monocots (Orchidaceae) but much rarer in the lower groups.

In more than 80% of the extant angiosperm species the gynoecium is syncarpous (Endress, 1982). The carpels are congenitally fused in such a way that also the inner morphological surfaces of the carpels merge. The syncarpous zone with continuous inner space is the "symplicate" region, the zone below where internal continuity ceases and each ovarial cavity is completely secluded from the other ones, is the *synascidiate* region (Leinfellner, 1950). Consequently the pollen tube transmitting tracts of all carpels are united for some distance. The united portion is the *compitum* (Carr and Carr, 1961). The compitum allows crossing of pollen tubes from one carpel to the other. Functional aspects of the compitum are the regular distribution and, still more important, the centralized selection of pollen tubes (Endress, 1982).

In some groups a compitum is formed by postgenital fusion of free carpels, such as Sterculiaceae, Rutaceae, Apocynaceae, Asclepiadaceae, Staphyleaceae, and some Bromeliales and Liliales (Endress *et al.*, 1983; see also Hartl and Severin, 1980; Ramp, 1987). This construction is, at least in some cases, derived from a syncarpous condition.

On the other hand, in many syncarpous groups the carpels are free in the upper or entire stylar region. A compitum is then formed in the upper part of

the ovary. This is mainly the case at the middle evolutionary level of the dicots (Hamamelididae, Rosidae, Dilleniidae, Caryophyllidae), while in the Asteridae the fusion is more often up to (or almost up to) the apex of the carpels.

There is a large variety of stigma and ovary differentiation. Stigmas may be dry or wet (with heavy secretion), smooth or papillate, extended or restricted to a point (see e.g. Heslop-Harrison and Shivanna, 1977; Heslop-Harrison, 1981; Schill *et al.*, 1985; Heslop-Harrison and Heslop-Harrison, 1985; Knox *et al.*, 1986). The stigma is an important site for compatibility/incompatibility reactions. Ovaries or syncarpous gynoecia are unilocular or divided into as many locules as there are carpels. Often they are subdivided only at the base but unilocular at the top. The ovules are borne at the inner angles of the locules in a subdivided ovary (*axile* placentation) or at the periphery of the locule in an unilocular ovary (*parietal* placentation), in any event mostly at or near the carpellary margins (see e.g. Rohweder, 1982).

Only a few complete gynoecium structures are known from Lower Cretaceous strata, all from the Albian. The majority of these are apocarpous, but a few syncarpous forms have also been recorded. Several small detached fruits or fruitlets may represent monocarpellate gynoecia or may be dispersed units derived from apocarpous gynoecia. Among the Early Cretaceous apocarpous forms *Platanocarpus* and the related flower of possible rosidean affinity have elongated and narrow fruitlets. The apical part is expanded and "peltate" with a distinct ventral slit that appears incompletely closed. In other structures, such as the Albian *Williamsonia recentior* and, the Cenomanian *Archaeanthus linnenbergeri*, the carpels appear conduplicate.

In the Cenomanian, apocarpous forms still constitute a significant part of the floral structures, but flowers with syncarpous gynoecia diversified strongly during the Late Cretaceous and dominate the floras at least by the Santonian-Campanian.

In none of the apocarpous gynoecia from the Cretaceous has a distinct style been observed. The Early and mid-Cretaceous forms seem to have a decurrent stigmatic area, while a well-defined rounded stigma has been observed in an apocarpous (possibly saxifragalean) gynoecium from the Late Cretaceous. The syncarpous gynoecia often have well-developed styles. In most species the styles are free, but forms with a single style were also established in several different groups in the Late Cretaceous.

The number of ovules per carpel is low in the Early Cretaceous floral structures and varies from one (in *Platanocarpus*) up to possibly ten (impressions of seeds in *Hyrcantha*). A larger number of ovules (about 100) has been reported for the Cenomanian *Archaeanthus* and in the Santonian-Campanian flora of southern Sweden the number of ovules varies considerably.

While organization of the ovules could be established for many Late Cretaceous forms it is less clear for most Early Cretaceous forms. Anatropous ovules were reported for *Caspiocarpus* and ovules of *Platanocarpus marylan-*

densis were most likely orthotropous as observed in the Late Cretaceous species of *Platanocarpus*. In the Santonian/Campanian floras anatropous ovules predominate, but orthotropous as well as campylotropous types are also present and diverse.

The number of integuments may be difficult to establish for fossil seeds. This is especially true for the angiosperms where the integuments usually are not separated by cutinized membranes, and no unequivocal evidence on seed-coat structure has thus far been reported from the Early Cretaceous.

I. POSITION OF PARTS

Apart from phyllotaxis, which shows the *arrangement* of parts in a vertical projection of the flower, the *position* of parts is understood as their relationship in a horizontal projection. This is correlated with the formation and elaboration of the floral base.

The floral base can be elongated, for example as in Magnoliaceae and some Ranunculaceae, or it can be dilatated (thickened). If it is dilatated outside the gynoecium, it becomes bowl- or cup-like, for example as in Calycanthaceae, some Monimiaceae, Rosaceae, Chrysobalanaceae and Leguminosae. If it is dilatated in the region of the gynoecium, the ovary becomes *inferior* (e.g. Myrtaceae, Rubiaceae, Campanulales, Orchidales).

These processes may also occur in combination, e.g. inferior ovaries and floral cups occur in Combretaceae, or elongated and dilatated floral bases occur in *Potentilla* and *Geum*.

The floral base is often referred to as the floral "axis" (e.g. Weberling, 1981). However, it is more practical to consider this zone as a transition region between the floral organs (phyllomes) and the floral axis. It is influenced by both regions (Endress, 1977a; Leins and Erbar, 1985).

Early Cretaceous angiosperm flowers all have superior ovaries and an unelaborated floral base. *Williamsonia recentior* represents an exception with its elongated and conical base, but its angiospermous affinity is equivocal (cf. Section III.A). The early Cenomanian flowers apparently also all had superior ovaries. In some cases, such as in *Archaeanthus*, floral bases were very elongated. Inferior ovaries apparently became established by the mid-Cenomanian and were extremely common by the Santonian-Campanian where epigyny is present in about two-thirds of all recorded floral structures (Friis and Crepet, 1987). Bowl- or cup-like floral bases have not been observed in any of the Cretaceous flowers but are present in a variety of forms by the Early Tertiary, e.g. *Paleorosa similkameensis* (Basinger, 1976).

J. NECTARY

Reproductive nectaries in the floral region are distinguished from non-

reproductive nectaries outside of the floral region with no direct relationship on pollination or fruit dispersal (Schmid, 1988; cf. also Vogel, 1977).

Nectaries in flowers are located on floral organs (sepals, petals, stamens, carpels) or on a disc in the centre of the flower that cannot be ascribed to one of the floral organs but rather to the floral base. The disc usually surrounds the gynoecium or extends to the region of the androecium (Fahn, 1952).

If nectaries are located on the outer floral organs (sepals, petals, stamens) they often fall off with the organ after anthesis (cf. Smets, 1986), if on a disc or on the gynoecium they may persist up to fruit dispersal and remain active in some instances (e.g. *Ajuga*; Lüönd and Lüönd, 1981).

In the Magnoliidae and in monocots nectaries are usually located on floral organs, such as petals (tepals; e.g. Ranunculales, Liliaceae p.p.), stamens (Laurales), or carpels. Carpellary nectaries may apear as septal nectaries (many monocots; e.g. Daumann, 1970; Schmid, 1985) or as stigmatic surfaces with sweet exudates (e.g. Lardizabalaceae, Aristolochiaceae; cf. Baker, 1973).

Disc nectaries occur in the middle and higher evolutionary levels of the dicots, such as Rosidae, Dilleniidae, and Asteridae. Nectary discs are characterized by late ontogenetic initiation (cf. Gut, 1966; Endress, 1967). They may be simple, as in Rhamnaceae, Celastraceae, Sapindaceae, Rutaceae, Umbelliferae, Labiatae (e.g. Rudall, 1981; Magin, 1983; Kumari, 1986) or lobed, as in Hamamelidaceae, Cunoniaceae (e.g. Endress, 1967; Dickison, 1975) or one-sided, as in some Capparales (e.g. Brown, 1938; Weberling and Uhlarz, 1983). There are also cases where disc lobes might represent specialized staminodes. This is difficult to interpret (e.g. Crassulaceae; e.g. Wassmer, 1955).

Histologically, nectaries are smaller or larger secretory regions where nectar is secreted either by the epidermis or by subepidermal layers through stomata ("water pores") (cf. Vogel, 1977; Fahn, 1979). More rarely nectar is secreted by multicellular hairs (Malvales, Dipsacales; cf. Wagenitz and Laing, 1984).

Nectar is a watery solution of sugars and other substances (cf. Bentley and Elias, 1983; Baker and Baker, 1983). If oil predominates in the secretion, the secretory region is called an *elaiophore* rather than a nectary (e.g. in neotropical Malpighiaceae in contrast to the paleotropical representatives of the family; cf. Vogel, 1974, 1986; Buchmann, 1987). Oil is collected by some specialized bees.

Nectaries are present in a variety of Late Cretaceous flowers. Although the Early Cenomanian Nebraskan flower (Fig. 9b) was reconstructed as having a lobed nectary disc (Basinger and Dilcher, 1984) the earliest unequivocal evidence of nectaries are afforded by the Santonian/Campanian flowers from Scania. The nectaries are diverse in morphology, but most are disc-shaped, directly related to the floral base and inserted between the androecium and gynoecium. All of these nectary discs are lobed with five, eight or ten prominent lobes. Their surfaces are rugulate or smooth with stomata-like openings.

They occur in flowers of possible Rosidean affinity such as *Scandianthus*. More rarely the secretory regions are located on the carpels either as a pronounced ring at the base of the carpels or diffuse with scattered stomata on the surface of the carpels. Nectaries located at the base of stamens have been observed in a small flower from the early Campanian of Aachen. They are developed as prominent two-lobed structures and they also have prominent stomata-like openings on their surfaces. All Santonian/Campanian nectaries are arranged radially symmetrical. In later forms, such as *Sahnianthus* from the Deccan Intertrappean Beds, one-sided nectaries have also been observed (Shukla, 1944, 1958). In *Sahnianthus* nectaries are present as a single scale or two stalked bodies.

Nectaries located on sepals and petals have not been observed in any Cretaceous flower so far available, but they are known from the Early Tertiary malpighiaceous flower, *Eoglandulosa warmanensis* (Taylor and Crepet, 1987). In this flower each sepal has a pair of glands very similar to elaiophores of modern neotropical Malpighiaceae and they probably had the same function as oil secretory structures.

V. RELATED GYMNOSPERMS

In the cladistic analysis of seed plant phylogeny and angiosperm origin by Crane (1985a, 1986) and Doyle and Donoghue (1986a,b, 1987a,b) the angiosperms form a monophyletic group with the Bennettitales, Pentoxylales and Gnetopsida. To emphasize that a dense aggregation of sporophylls into flower-like structures occurs in all of these taxa Doyle and Donoghue referred to them as the anthophyte clade. In addition to having flowers, basic characters of the anthophytes are once-pinnate leaves, syndetocheilic stomata, apical meristem with differentiation of tunica and corpus, scalariform pitting in secondary xylem, uni-ovulate cupule, bitegmic ovules, reduced megaspore wall and granular monocolpate pollen (Doyle and Donoghue, 1986b).

A short summary of the anthophytic gymnosperm is given below with emphasis on the floral organs. Reproductive organs of other related gymnosperms that are important for understanding the phylogeny of the flower are discussed at the end of the section.

A. BENNETTITALES

The Bennettitales (or Cycadeoidales) is an extinct group of gymnosperms with a worldwide distribution. It arose in the Late Triassic and persisted into the Late Cretaceous with maximum diversification and distribution in the Jurassic. The bennettitalean plants were probably shrubs or small trees that might have colonized open habitats (Harris, 1973; Crane, 1987).

The bennettitalean flowers exhibit considerable variation in structure and size with the largest flower reaching up to about 10 cm in diameter. With a few exceptions the flowers are unisexual (Fig. 11a,b), a condition considered basic within the group (e.g. Crane, 1985a, 1986). They are naked or more commonly surrounded by perianth-like bracts. The microsporophylls are apparently mostly whorled while other floral organs are helically arranged. In bisexual flowers the ovulate structure is central surrounded by the pollen producing organs (Fig. 11c).

The ovulate reproductive structure is conical or spherical consisting of an expanded receptacle with densely packed ovules and intermixed interseminal scales. Ovules are numerous in most taxa and *Vardekloeftia* with five, rarely more, ovules forms an exception. The ovules are orthotropous and erect, arising directly from the receptacle without subtending bracts. Reinvestigation of the early bennettitalean form, *Vardekloeftia*, from the Upper Triassic of East Greenland, indicates that the ovule is basically bitegmic with the nucellus protected by an inner integument and an outer cupule (outer integument; Fig. 11h; Pedersen *et al.*, 1989). A resistant megaspore membrane was apparently not developed. Based on structural and morphological similarities the interseminal scales have been considered as homologous with the ovule (Seward, 1913; Delevoryas, 1968). Based on well preserved petrified material of *Cycadeoidea* from North America, Crepet (1974) demonstrated that linear tetrads of megaspores also had developed in the Bennettitales.

The microsporophylls are generally of complex structure, pinnate and bearing numerous microsporangia that are mostly clustered in synangia (Fig. 11d–g; e.g. Wieland, 1906; Nathorst, 1912; Harris, 1944). They are free or partly fused and may form a cup-shaped structure as in *Weltrichia* (Fig. 11a; Nathorst, 1911). Pollen grains are monocolpate and non-saccate.

B. PENTOXYLALES

The Pentoxylales is a small extinct order restricted to Jurassic and Lower Cretaceous sediments from the Gondwanan province (India, Australia and New Zealand). It includes five genera of dispersed organs (stems, *Pentoxylon*, *Nipanioxylon;* leaves, *Nipaniophyllum*; male flowers, *Sahnia*; female flowers, *Carnoconites*). It was first recognized as a separate group by Sahni (1948) and a detailed reinvestigation of the various organs of the *Pentoxylon* plant was presented by Bose *et al.* (1985). The *Pentoxylon* plant was probably shrubby and may have grown at the edge of open waters (Bose *et al.*, 1985).

The flowers are unisexual and naked, borne on woody short shoots. Both microsporophylls and ovules are apparently helically arranged.

The ovulate reproductive structure is ovoid or elongated consisting of a central axis with densely packed ovules (Fig. 11g; e.g. Srivastava, 1946; Harris, 1962; Bose *et al.*, 1985). The number of ovules varies from about 20

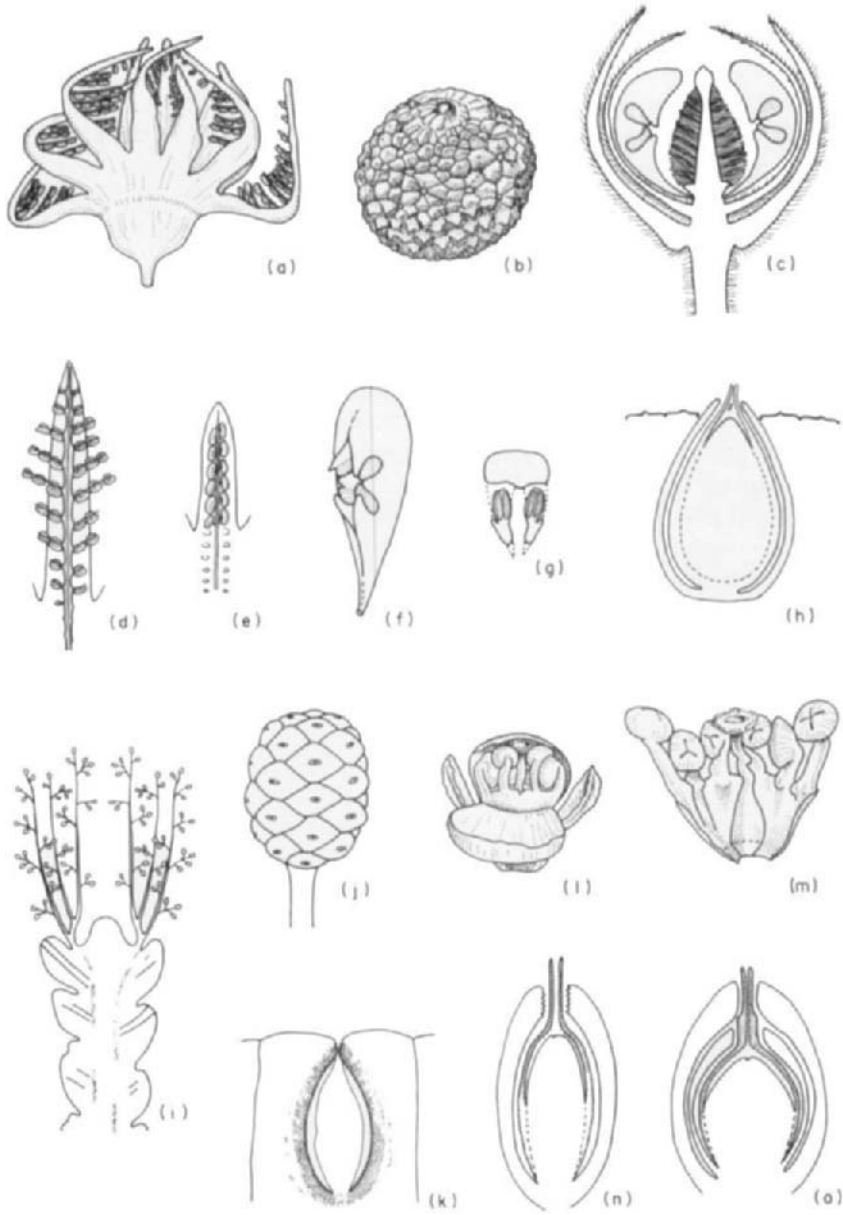

Fig. 11. Morphology of Bennettitales (a–h), Pentoxylales (i–k) and Gnetopsida (l–o). (a) Reconstruction of the microsporangiate fossil *Weltrichia spectabilis*; (b) reconstruction of the ovulate fossil *Williamsonia netzahualcoyotlii*; (c) reconstruction of the bisexual fossil *Williamsoniella coronata*; (d–g) Microsporangia-bearing units of (d) *Weltrichia spectabilis*, (e) *Weltrichia whitbiensis* and (f, g) *Williamsoniella coronata*; (h) schematical section of ovule and inter-seminal scales of *Vardekloeftia sulcata*; (i) reconstruction of a male flower of *Sahnia*; (j) reconstruction of the ovulate fossil *Carnoconites cranwelliae*; (k) schematical section of a *Carnoconites* ovule; (l, m) male flower of *Welwitschia bainesii*; (l) complete and (m) opened flower with bracts removed; (n, o) schematical section through ovule of (n) *Ephedra* and (o) *Gnetum*. Redrawn from Thomas, 1913 (a); Delevoryas and Gould, 1973 (b); Harris, 1944 (c, f, g); Nathorst, 1912 (d, e); Pedersen *et al.*, 1989 (h); Bose *et al.*, 1985 (i); Harris, 1962 (j); Crane, 1985a (k); Hooker, 1863 (l, m).

to more than 100. The ovules are orthotropous and erect (Fig. 11k) arising directly from the central axis without subtending bracts as in Bennettitales. The nucellus is free and surrounded by an inner sclerotesta and an outer fleshy sarcotesta. The sclerotesta was interpreted by Crane (1985a) as an inner integument and the sarcotesta as an outer integument homologous with the cupule of mesozoic pteridosperms.

The arrangement of the microsporophylls has not been fully established but they are either in a true spiral or irregular. They are borne on a broad flat receptacle. They are branched, but the arrangement of branches is uncertain. Each microsporophyll bears numerous sporangia that are not clustered in synangia (Fig. 11i; Vishnu-Mittre, 1953; Bose *et al.*, 1985).

Pollen grains are poorly known. They are non-saccate and apparently monocolpate. In the various cladograms presented by Crane (1985a, 1986) and Doyle and Donoghue (1986a,b, 1987a,b) the Pentoxylales generally associates with the Bennettitales. There is no unequivocal indication in the analyses which of the two groups is basal. Crane (1985a) suggested a derivation of the Bennettitales from the Pentoxylales by a reduction of fertile ovules to form sterile interseminal scales. The Bennettitales, however, is known in older strata and for stratigraphic reasons Doyle and Donoghue (1986b) suggested a reverse position.

C. GNETOPSIDA

The class Gnetopsida comprises three distinct related extant genera (*Ephedra*, *Gnetum* and *Welwitschia*) usually placed in separate families and separate orders. *Ephedra* is mainly distributed in warm temperate to subtropical dry areas of North America, in the Mediterranean and Asia. *Gnetum* is restricted to tropical areas in Asia, Africa and South America, and *Welwitschia* to the desert of southwestern Africa. Growth form is different for all of the three extant genera. *Ephedra* comprises small shrubs with strongly reduced xerophytic opposite or whorled leaves. *Welwitschia* has a unique conical stem of which only a small part is above ground. It has a broad flat apex with two persistent leaves that grow throughout the life of the plant. *Gnetum* comprises lianas, small trees and shrubs with opposite dicotyledonous reticulate leaves (Sporne, 1965). The gnetopsids possess a number of features otherwise restricted to the angiosperms such as vessels in the wood, siphonogamy, cellular embryogeny and reticulate leaves with several orders of venation (Pearson, 1929; Bierhorst, 1971; Martens, 1971; Crane, 1985a; Doyle and Donoghue, 1986b).

Ephedra, *Gnetum* and *Welwitschia* all have minute flowers borne in spike-like inflorescences or solitary on short shoots in some species of *Ephedra*. The flowers are unisexual or male flowers may have a central, sterile ovulate

organ. Floral parts are arranged in whorls or decussate pairs (e.g. Martens, 1971).

The female flowers are simple consisting of a single erect, orthotropous ovule subtended by one to several bracts. A resistant megaspore membrane is absent in all three genera and the nucellus is surrounded by an inner integument with an elongated micropylar canal that extends beyond the outer protecting envelopes. In *Ephedra* the inner integument is surrounded by an outer envelope and subtended by a bract (Fig. 11n). The wall structure of the outer envelope is identical to that of the subtending bract and it has generally been interpreted as two fused bracts rather than a second integument. The ovule of *Welwitschia* is apparently also unitegmic and is protected by two sets of bracts. In *Gnetum*, however, the inner integument is surrounded by two distinct envelopes (Fig. 11o). The outer envelope has generally been interpreted as modified bracts while the second envelope has been variously interpreted as a second integument, aril, fruit wall or modified bracts (cf. Wettstein, 1935; Pearson, 1929; Martens, 1971; Crane, 1985a).

The male flowers are simple, consisting of a single or few sporangiophores subtended by bracts. The sporangiophores have two to eight fused sporangia in an apical position (Fig. 11m) resembling the stamens in angiosperms. In *Welwitschia* the sporangiophores are fused at the base and surround a sterile ovulate structure (Fig. 11l,m), which attracts insects to the flower by producing a sweet secretion. Also some male flowers of *Gnetum* have a central sterile ovulate structures with similar function occur in groups of male flowers (e.g. Martens, 1971). Pollen grains of *Ephedra* and *Welwitschia* are monocolpate with characteristic longitudinal ribs while those of *Gnetum* are inaperturate and spiny (Erdtman, 1957, 1965).

The earliest record of possible gnetopsid affinity is striate ephedroid pollen found in the male cones of *Masculostrobus clathratus* from the Late Triassic (Ash, 1972). Associated vegetative shoots and ovulate organs described as *Dechellyia gormanii* (Ash, 1972) were tentatively assigned to the conifers but may also be of gnetopsid affinity (see below). Other reproductive organs have recently been discovered from Jurassic and Lower Cretaceous sediments (Krassilov, 1986; Wu *et al.*, 1986; Crane and Upchurch, 1987; Krassilov and Bugdaeva, 1988) indicating that the extant genera represent only a small part of the total diversity of the group. This is also suggested by the fossil record of pollen, which indicates maximum diversification and distribution of the group in the Early and mid-Cretaceous (Brenner, 1976; Doyle *et al.*, 1977; Herngreen and Chlonova, 1981). They are especially conspicuous within the African–South American microfloral province (Herngreen and Chlonova, 1981), which may be the evolutionary centre of the group.

A fossil reproductive organ of considerable interest is *Eoantha zherikhinii* described by Krassilov (1986) from the Lower Cretaceous of the Lake Baikal area. It is minute and consists of four wedge-shaped ribbed appendages, each

containing a single orthotropous ovule. They are arranged in a whorl and subtended by a whorl of linear bracts. Bracts are also observed above the ovulate organs suggesting that the structure was possibly borne in an inflorescence. The ovules contain ribbed *Ephedripites*-pollen in the pollen chamber. Krassilov (1986) compared the ovulate appendages with opened angiosperm carpels, but also noted some similarity with the bracts of *Welwitschia*.

The structure of *Masculostrobus clathratus* from the Upper Triassic which contains the earliest ephedroid pollen, is unclear (Ash, 1972). It is a small spike-like structure apparently with whorled or decussate bracts and sporangiophores. Associated with these male cones are foliar shoots with ovulate organs described as *Dechellyia* (Ash, 1972). The branches and the needle-like leaves are borne opposite and decussate and the leafy shoots are shed with the foliage. The ovulate organs consist of an ovule attached to a lanceolate lamina with two prominent veins. They are very similar to the leaves of the Early Cretaceous *Drewria potomacensis* described by Crane and Upchurch (1987) and assigned to the gnetopsids. A gnetopsid affinity for the *Dechellyia* plant is thus probable and it may represent the vegetative and female parts of the *Masculostrobus clathratus* plant.

D. MESOZOIC PTERIDOSPERMS

The Caytoniales, Corystospermales, Glossopteridales and the Peltaspermales constitute a heterogenous group of extinct seed plants often referred to as the Mesozoic pteridosperms. In the cladistic analyses by Doyle and Donoghue (1986b, 1987a), the Caytoniales and Glossopteridales come out as the closest relatives of the anthophytes by having reticulate leaf venation and a thick nucellus cuticle. The Corystospermales and the Peltaspermales also come out close to these groups, but their position in the cladogram is less fixed. They are, however, strongly linked to Caytoniales and Glossopteridales by having quasisaccate pollen with sacci filled with exinal elements (Pedersen and Friis, 1986; Meyen, 1987), a character that was not included in the analyses by Doyle and Donoghue. The reproductive organs of the Mesozoic pteridosperms are generally interpreted as modified pinnate leaves but some authors (e.g. Stebbins, 1974) have interpreted them as pluriaxial systems. They have often been considered in various theories regarding the derivation of the angiosperm carpel and to some extent also the angiosperm stamen (cf. Section VI.A).

The Caytoniales is a small extinct order distributed in Upper Triassic to Upper Cretaceous strata of Europe, eastern Asia and Greenland. It comprises three different genera (leaves, *Sagenopteris*; seed-bearing organs, *Caytonia*; pollen-bearing organs, *Caytonanthus*) linked together by common association, conformity in epidermal structure and the presence of *Caytonan-*

thus pollen in the micropyles of the *Caytonia* seeds (Thomas, 1925; Harris, 1933, 1940a,b, 1941, 1951). A small fragment of a stem has been associated with *Caytonia* (Harris, 1971) indicating that it was a woody plant, but nothing is known of its stature. The *Sagenopteris* leaves are pinnate and reticulate.

The seeds and microsporangia are borne on unisexual structures that have been interpreted as pinnate mega- and microsporophylls (Thomas, 1925). This was supported by epidermal studies that indicated a dorsiventral organization of the axes (Harris, 1940a).

Caytonia is a long slender structure with small fruit-like organs borne laterally on short unbranched stalks in subopposite pairs along the main axis (Fig. 12a). The fruit-like organs, usually referred to as cupules, are almost spherical in outline and reflexed towards the axis. They are open only along a narrow mouth between the stalk and the swollen lip. Each cupule encloses 8–30 orthotropous, unitegmic seeds with micropyles facing the mouth of the cupule (Fig. 12b,c).

In *Caytonanthus* the microsporangia are borne in clusters on lateral appendages that are subdivided into a few short branches, each terminated by a synangium of four elongated microsporangia (Fig. 12d). Pollen grains extracted from the synangia are small and quasisaccate (Pedersen and Friis, 1986; Zavada and Crepet, 1986).

The Corystospermaceae (Corystospermales) was instituted by Thomas (1933) for pteridosperms with forking leaves and reproductive organs on branched structures. Plants included in this group are widely distributed in Triassic sediments from the Gondwanan province (South Africa, Australia, Argentina and India). The group includes several different genera of dispersed organs (stems: *Rhexoxylon*; leaves: *Dicroidium*; seed-bearing organ: *Umkomasia*, *Pilophorosperma*; pollen-bearing organs: *Pteruchus*). They were linked together based on common association and the presence of *Pteruchus* pollen in the micropyles of the seeds (Thomas, 1933). The corystosperms were small woody plants, but little is known about their growth habit.

Two northern hemisphere genera, *Pachypteris* (leaves) and *Pteroma* (pollen-bearing organs) from the Jurassic of Yorkshire have also been included in the corystosperms by some authors (Townrow, 1965; Stewart, 1983; Crane, 1985a). The leaves of *Pachypteris* are, however, unforked and no seed-bearing organs have been associated with these fossils (Harris, 1964); thus their relationship with the corystosperms remains uncertain.

The seed- and microsporangia-bearing organs are compound structures. Based on the presence of bract-like organs along the seedbearing axis, Thomas (1933) and Stebbins (1974) interpreted the structures as pluriaxial systems. This interpretation was questioned by Townrow (1962) who regarded these organs as sterile pinnules on a pinnate sporophyll.

The two seed-bearing organs, *Umkomasia* and *Pilophorosperma* are very similar in external morphology. They have small helmet-shaped cupules

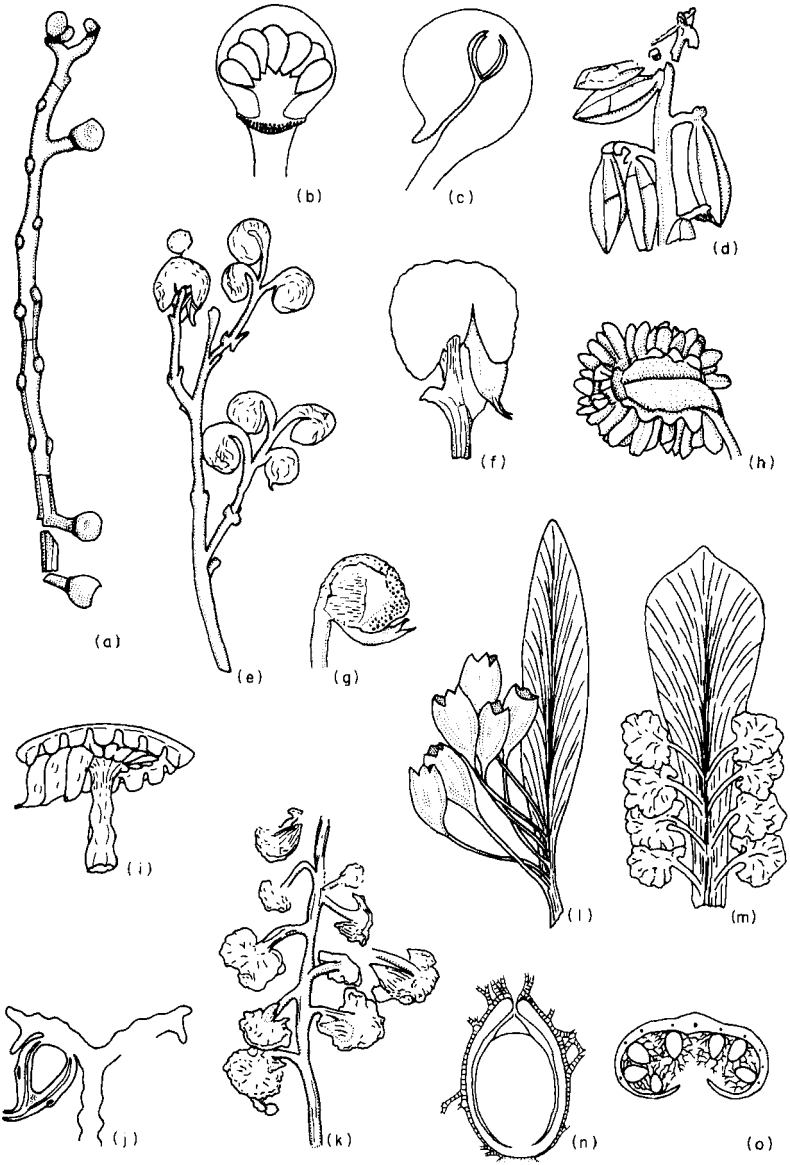

Fig. 12. Morphology of Caytoniales (a d), Corystospermales (e h), Peltaspermales (i k) and Glossopteridales (l o). (a) Cupule bearing axis of *Caytonia nathorstii*; (b,c) schematical sections through a *Caytonia* cupule with seeds; (d) axis of *Caytonanthus arberi* bearing synangia; (e) cupule bearing axis of *Umkomasia macleani*; (f) cupule and seed of *Umkomasia macleani*; (g) cupule of *Pilophorosperma granulatum*; (h) microsporangiate heads of *Pteruchus africanus*; (i,j) reconstructions of cupulate disc and ovules of *Peltaspermum ottonis*; (k) seed-bearing axis of *Lepidopteris natalensis*; (l) *Denkania indica* with uniovulate cupules; (m) *Lidgettonia mucronata* with multiovulate heads; (n, o) schematical sections through (n) seed and (o) seed-bearing head of a petrified glossopterid fossil. Redrawn from Harris, 1940a (a); Crane, 1985a (b,c); Harris, 1964 (d); Thomas, 1933 (e–g,k); Townrow, 1962 (h); Harris, 1932 (i,j); Surange and Chandra, 1976 (l,m); Stewart, 1983, based on Gould and Delevoryas, 1977 (n, o).

borne solitary or in pairs along the branches of the reproductive structure (Fig. 12e). The cupules are stalked and reflexed towards the stalk. They are bivalved and each contains a single seed with an elongated, curved, bifid micropyle that projects beyond the cupule (Fig. 12f,g). There are no details on the seed wall and the nature of the cupule plus seed is poorly understood.

In *Pteruchus* the microsporangia are borne in clusters on small rounded or elongated heads arranged along the branches of the organ. The heads are stalked and laterally attached and composed of an expanded lamina with numerous microsporangia on one side (Fig. 12h). The sporangia are apparently free (Townrow, 1962) and not synangial as stated by Thomas (1933). Pollen grains are quasisaccate with the two sacci filled as in the pollen of *Caytonanthus* (Taylor *et al.*, 1984).

The Peltaspermales comprises a number of different genera widely distributed in Permian and Triassic floras in the northern and southern hemisphere. They form a conspicuous constituent of many Late Permian and Early Triassic floras of the Subangaran and Angaran area (e.g. Meyen, 1982, 1987). The peltasperms are also characteristic of many Late Triassic floras with closely related forms having been reported from East Greenland, Europe and South Africa (Nathorst, 1908; Antevs, 1914; Harris, 1932; Thomas, 1933). The three genera, *Lepidopteris* (leaves), *Peltaspermum* (seed-bearing organs) and *Antevsia* (pollen-bearing organs), were linked together based on epidermal features and characteristic swellings on the surfaces of the organs (Antevs, 1914; Harris, 1932). They are probably the best understood peltasperms currently available, but leaves of *Callipteris*, *Compsopteris* and *Tatarina* and the seed-bearing organ *Autunia* are also known in some detail. No stems have so far been attributed to the peltasperms and nothing is known about their habit.

The seed-bearing organ, *Peltaspermum*, consists of a long main axis bearing stalked peltate heads in a subalternate arrangement. The heads have a flattened disc-shaped lamina attached to the stalk at the centre and with 10–20 seeds arranged along the margin of the lamina. The seeds are orthotropous and unitegmic with slightly curved micropyles (Fig. 12i,j). There are no cupules, but Harris (1932) referred to the seed-bearing lamina as a "cupulate disc". The heads of *Autunia* differ in having lamina laterally attached to the stalk and in having a smaller number of seeds (Fig. 12k).

The pollen-bearing organ, *Antevsia*, consists of a main axis bearing appendages that are subdivided into two or three branches, each with a number of free microsporangia. Harris (1932) interpreted the organ as a pinnate microsporophyll, but he also pointed out that branches arose in different planes, which may suggest that *Antevsia* was a pluriaxial system. Pollen grains of *Antevsia* are monocolpate and non-saccate (Pedersen, 1981), whereas some older members of the group produced quasisaccate pollen (Meyen, 1987).

The Glossopteridales is a diverse group of extinct plants distributed mainly in the Gondwanan Province where it constituted a conspicuous element in

Permian and Triassic floras. The group is represented by a wide variety of fossils including roots, stems, leaves, female and male reproductive organs as well as dispersed seeds and pollen. The glossopteridalean plants were large trees with trunks of typical gymnospermous wood (Pant, 1977; Gould and Delevoryas, 1977). The leaves are simple and reticulate with a distinct midrib (*Glossopteris*) or lacking the midrib (*Gangamopteris*).

The organization of the glossopteridalean reproductive organs is diverse and for most genera poorly understood. Female as well as male structures were borne on leaves. The ovulate structure *Lidgettonia* consists of a disc-shaped and stalked lamina bearing many seeds on one side (Fig. 12m). The seed-bearing laminae were arranged in two subalternate rows on the surface of the leaf (Thomas, 1958; Surange and Chandra, 1974). Other ovulate structures such as *Ottokaria*, *Dictyopteridium* and *Venustostrobus* have a single seed-bearing lamina attached to the subtending leaf (Pant and Nautiyal, 1965; Chandra and Surange, 1976, 1977). A petrified seed-bearing structure similar to *Dictyopteridium* shows seeds attached to the surface of the lamina and enclosed by the incurved margins (Fig. 12o). The seeds are unitegmic (Fig. 12n) and contain *Arberiella* pollen in the pollen chamber (Gould and Delevoryas, 1977). In *Denkania* (Fig. 12l) the seed-bearing unit is cupulate rather than laminate and contains a single seed (Surange and Chandra, 1973).

Microsporangia are free and clustered on branched or simple stalks in a subalternate arrangement along the middle of the subtending leaf. Pollen grains are quasisaccate or asaccate and striate (Meyen, 1987).

VI. PREVIOUS IDEAS ON DERIVATION OF FLORAL ORGANS

A number of hypotheses about the primitive nature of the angiosperms have been developed. Opposing Euanthium and Pseudanthium theories developed at the beginning of this century have received special attention and have greatly influenced phylogenetic classification of angiosperms.

A. EUANTHIUM OR ANTHOSTROBILUS THEORY

According to this theory the angiosperm flower is homologous with a gymnosperm strobilus as present in Cycadales and Bennettitales (Hallier, 1901; Arber and Parkin, 1907). The flower is basically a uniaxial system, consisting of an axis with lateral appendages (bracts and sporophylls). The strobiloid flowers of the Magnoliaceae and related families were considered most primitive among living angiosperms (Bessey, 1897, 1915). The theory has received substantial support from most modern systematists (e.g Cronquist, 1968,

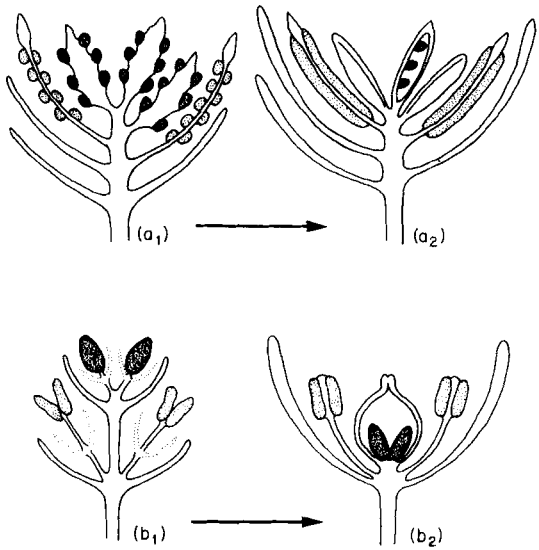

Fig. 13. (a) Derivation of the angiosperm flower (a_2) from a uniaxial stobilus (a_1) according to the Euanthium Theory; (b) derivation of the angiosperm flower (b_2) from a pluriaxial cone (b_1) according to the Pseudanthium Theory. Redrawn from Firbas (1947).

1981, 1988; Thorne, 1968; Hutchinson, 1969; Takhtajan, 1958, 1959, 1969, 1980, 1987) and a description of the hypothetical basic angiosperm flower was given by Takhtajan (1969). According to this floral parts were numerous, spirally arranged and free. The flower was large, insect-pollinated, with a perianth of modified bracts. The stamens were broad and laminar without differentiation into filament and anther. The carpels were large and leaf-like, and the large seeds developed from anatropous and bitegmic ovules.

The Euanthium Theory was formulated after the discovery of bisexual bennettitalean flowers that superficially resembled the *Magnolia* flower. A direct derivation of the angiosperms from the Bennettitales was, however, impeded by difficulties in deriving the simple tetrasporangiate anthers and the multiovulate follicles of *Magnolia* from the bennettitalean organs.

A hypothetical link between the two groups, the Hemiangiospermeae, was introduced to overcome the problem (Arber and Parkin, 1907). It was provided with *Cycas*-like megasporophylls bearing seeds along the margins. A simple folding and fusion of the margin would lead to the angiosperm follicle (Fig. 13a), and a strong reduction of the megasporophyll to the naked seed of the Bennettitales. At the time when the Euanthium Theory was developed, the Bennettitales and the Cycadales were considered as closely related and the mixture of characters plausible. Later studies have, however, concluded that similarities between the groups are superficial and that they are only remotely related (e.g. Harris, 1932). This has also been strongly supported by recent cladistic analyses of seed plant phylogeny (Crane, 1985a, 1986; Doyle

and Donoghue, 1986a,b, 1987a,b) and is a serious objection against the Euanthium Theory in its original form.

B. PSEUDANTHIUM THEORY

According to this theory the angiosperm flower is homologous with a gymnosperm conus as it occurs in Coniferales or Gnetopsida (e.g. Wettstein, 1907; Neumayer, 1924; Janchen, 1950). The flower is basically a pluriaxial structure (system), consisting of a primary axis and more or less numerous secondary axes, both with lateral appendages. The name "Pseudanthium Theory" implies that the angiosperm flower is basically derived from a gymnospermous group of reproductive organs (an "inflorescence"), which has been condensed to a simple flower-like structure (Fig. 13b). Under this view the sporangia (pollen sacs, nucelli) are terminal on axes (stachyosporous; Lam, 1950). Carpels basically represent subtending bracts of the ovular axes. Thus the functional unit of carpel and ovules represents a construction of elements of axes of two orders. According to the Pseudanthium Theory small, simple and unisexual flowers of anemophilous Hamamelididae such as Casuarinales, Fagales, Myricales and Juglandales are the most primitive among modern angiosperms. These plants commonly have small flowers with a single whorl of perianth parts. The gynoecium consists of a simple ovary with a single or few ovules. They were grouped by Wettstein (1907) in the Monochlamydeae together with a number of other families with small unisexual flowers (e.g. Piperaceae and Chloranthaceae). The discovery of small bisexual flowers of juglandalean and possibly fagalean affinity in the Upper Cretaceous indicates that the unisexual condition is derived at least for some of these groups (Friis, 1983). This has also been suggested based on the occasional presence of bisexual flowers in living representatives of these groups.

C. MIXED THEORIES

There are other theories where only part of the angiosperm flowers are interpreted according to the Euanthium or Pseudanthium Theory. Some authors surmise that angiosperm flowers are basically of two kinds. Some groups have flowers that are homologous to entire clusters of flowers of other groups. The two types may go back to different gymnosperm ancestors or they may stem from the same gymnosperm group or they may even have been developed within the angiosperms.

In the view of Karsten (1918) the first group is represented by the big, multiparted flowers of, for example, Magnoliaceae, the second by the small, few-parted flowers of, for example, Casuarinaceae. He derives both from the

gnetopsids, the first from a *Gnetum*-like, the second from an *Ephedra*-like model.

Similarly, Lam (1950), sees the main gap within angiosperms between a phyllosporous and a stachyosporous group but he does not explicitly speculate on gymnospermous ancestors of the two clades.

Hagerup (1934) hypothesizes the existence of at least two basically different angiosperm groups, the one with simple gynoecia with "false" carpels derived from Gnetopsida, the other with true carpels derived from Caytoniales.

Like Karsten, Fagerlind (1947) derives the angiosperms from Gnetopsida, but interpreted the flower as partly euanthial, partly pseudanthial.

In Meeuse's "anthocorm" theory (e.g. Meeuse, 1986) conventional flowers ("FRU" = "functional reproductive unit") are of basically two different kinds: modified "holanthocorms" (pluriaxial systems) in some groups (e.g. Magnoliaceae), and modified "gonoclads (anthoids)" (uniaxial systems) in others (e.g. Chloranthaceae). The basic system is the "anthocorm", a branched reproductive system as it occurs in gymnosperms such as Gnetopsida. The terminology used by Meeuse is to some extent based on Neumayer (1924).

Melville (1983) in a revised version of his "gonophyll" theory (Melville, 1962, 1963) interpreted the angiosperm flower as composed of a number of reproductive shoot systems with their subtending bracts, derived from reproductive shoot systems of the Glossopteridales. The number of such shoot systems (gonophylls) is reflected by the number of floral sectors. There are a few simple angiosperm flowers that represent a single gonophyll (e.g. *Chloranthus*).

Burger (1977) considers the monocot (and dicot) flower as derived from a cluster of simple flowers such as those of the Chloranthaceae. Hence, most flowers are pseudanthia, except very few relictual forms that represent true flowers (uniaxial structures).

D. PARTIAL THEORIES

In this section we consider partial theories that do not refer to all angiosperms or not to all floral organs. Most of them are modifications of the classical Euanthium Theory in that they view the flower as an uniaxial structure. They have mainly been presented by palaeobotanists.

Thomas (1931) suggested a derivation of the angiosperm carpel from the *Caytonia* ovulate axis by a reduction of cupule number to two and a fusion of the two multiovulate cupules to form an ovary. This theory affords no explanation of the origin of the second integument in angiosperm ovules. Stebbins (1974), Doyle (1978), Crane (1985a, 1986) and Doyle and Donoghue (1986b, 1987a) also suggested a derivation of the carpel from *Caytonia* (Fig. 14). In contrast to Thomas they derived the carpel from the cupule bearing "rachis" by broadening and involution of the rachis. The anatropous and

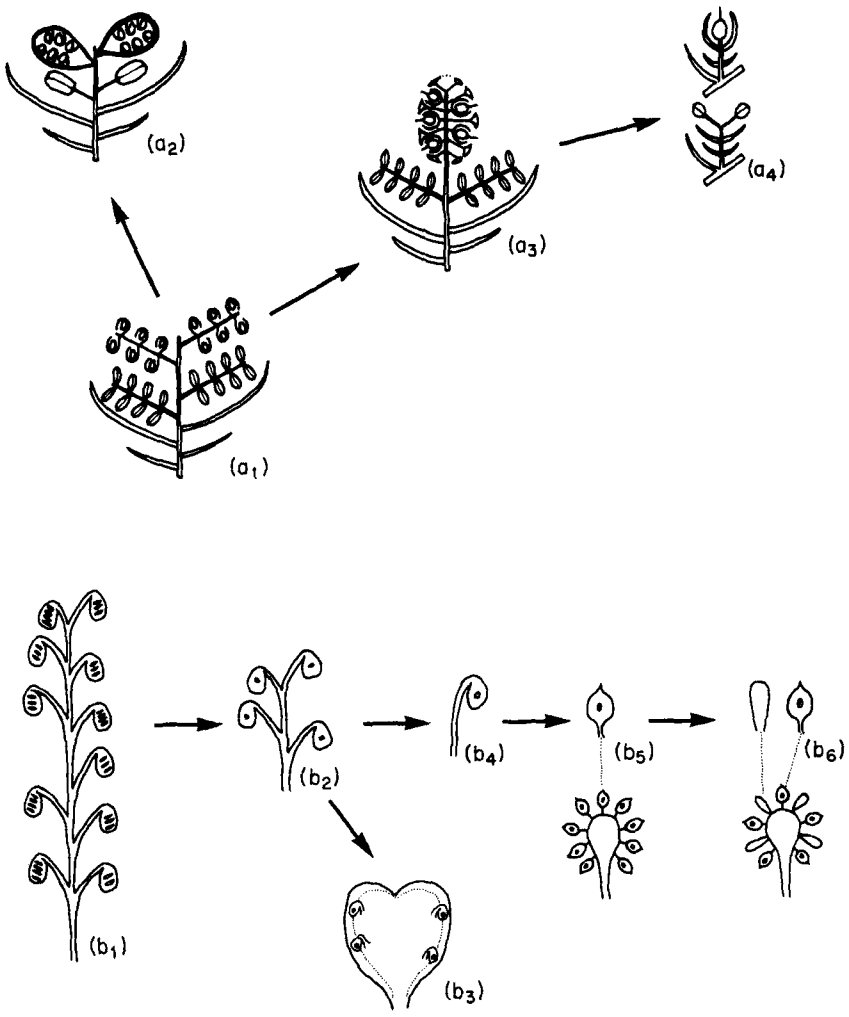

Fig. 14. (a) Derivation of the angiosperm flower (a_2) from a hypothetical *Caytonia*-like ancestor (a_1), (a_3), bisexual bennettitalean flower, (a_4) unisexual gnetopsid flowers; (b) derivation of the angiosperm carpel (b_3) from *Caytonia* (b_1) via a hypothetical uniovulate structure (b_2), (b_4) hypothetical "uniculpulate" structure, (b_5) Pentoxylales, (b_6) Bennettitales. Redrawn from Doyle and Donoghue, 1987a (a); Crane, 1985a (b).

bitegmic angiosperm ovule was homologized with a reduced cupule containing a single orthotropous seed. The outer integument of the angiosperm ovule is then derived from the cupule wall. A similar view had already been raised by Gaussen (1946). A close relationship between the angiosperms and the Caytoniales has been supported by recent cladistic analyses (Crane, 1985a; Doyle and Donoghue, 1986b, 1987a) and in the analyses by Doyle and Donoghue the Caytoniales always come out as a sister group to the

anthophytes. A direct derivation of the angiosperms from the Caytoniales, however, affords several problems and Doyle and Donoghue (1986b, 1987a) also introduced a hypothetical ancestral form (Fig. 14a).

Long (1977) derived the angiosperm carpel from a pteridosperm cupule, the outer integument from an outgrowth of the inner cupule surface.

Other authors have hypothesized a derivation of the angiosperm carpel from the glossopterids. Retallack and Dilcher (1981b) suggested that the basic angiosperm flower had multiovulate carpels with orthotropous and bitegmic ovules. They homologized these ovules with the uniovulate cupules of *Denkania* and the carpel with the subtending leaf-like bract.

Another explanation of the angiosperm carpel was presented by Meyen (1988), who derived the angiosperms from the Bennettitales. The gynoecium provides the greatest difficulty in the derivation of the bisexual angiosperm flower from that of the Bennettitales (see also p. 145). Meyen elegantly overcame this problem by hypothesizing "gamoheterotopy" in that a bennettitalean microsporophyll became female bearing ovules at the place of microsporangia and subsequently became involute. Crane (1986) provided additional support to this theory with the carpel-like microsporophyll of *Leguminanthus*.

Partial theories on the angiosperm androecium have been less elaborated. Stebbins (1974) noticed the possible homology of a stamen bundle to groups of microsporangiophores in Caytoniales. Hagemann (1984) interpreted the stamen as a stalked synangium and homologized a stamen with a microsporophyll (without specifying a particular gymnospermous group).

At present, all these hypotheses are still open to discussion. None can be regarded as compatible with all the available evidence.

VII. DISCUSSION

A. EVOLUTIONARY ORIGIN OF THE ANGIOSPERM FLOWERS

Darwin's "abominable mystery" has not yet been solved. However, it is no longer hidden in deepest darkness, since the facts that are necessary for an eventual resolution have become astonishingly diverse.

Extended neobotanical research on the floral structure and development of many groups of angiosperms has corroborated the hypothesis of the homogeneous and euanthial nature of the flower (e.g. Sattler, 1974; Eyde, 1982; Endress, 1977a; Rohweder and Endress, 1982; Leins *et al.*, 1988). These studies, however, leave the question of the angiosperm ancestors open. Also the palaeobotanical point of view now tends to the euanthial (i.e. uniaxial) nature of the flower (e.g. Friis *et al.*, 1987; Doyle and Donoghue, 1986b, 1987b) but arguments for a mixed or pseudanthial nature have also been raised (Krassilov, 1977; Dilcher, 1979).

As possible ancestors of angiosperms various groups have been taken into account (cf. Section VI.A). The most important ones that have to be seriously further considered are Gnetopsida, Bennettitales, Caytoniales, Glossopteridales, and other pteridosperms.

The Gnetopsida especially have come into focus again as the putatively closest relatives in the last few years (e.g. Crane, 1985a; Doyle and Donoghue, 1986a,b, 1987a,b). Only now are more complete fossil Gnetopsida beginning to be found (Krassilov, 1986; Wu *et al.*, 1986; Crane and Upchurch, 1987; Krassilov and Bugdaeva, 1988; Krassilov and Ash, 1988). Conversely, chloranthoid fossils are among the oldest angiosperm remains that are attributed to a special modern group with some certainty (see Section III and review in Endress, 1987c). Since the two groups, Gnetopsida and Chloranthaceae, share a number of special traits, one is inclined to ask whether there could be a phylogenetic bridge from the gymnosperms to the angiosperms via Gnetopsida and Chloranthaceae. They share: opposite leaves, partly with fused bases sheathing the axis; small, simple flowers, arranged in spikes; male flowers partly with a single stamen, occurrence of filamentous stamens in Gnetopsida as in many angiosperms, bisporangiate anthers in *Ephedra* and *Gnetum*, synandry in *Ephedra* and *Chloranthus*; female flowers with a single ascidiate "carpel" and a single orthotropous, bitegmic ovule, secretion at the "stigma", tetrasporic embryo sac in *Welwitschia* and *Gnetum* as in some Piperaceae (possibly related to Choranthaceae), double fertilization in *Ephedra* as in angiosperms (detected by Herzfeld, 1922, confirmed by Khan, 1943 and other authors, but criticized as being not comparable with the condition in angiosperms by Martens, 1971 and Moussel, 1979); fleshy organs surrounding ripe gynoecia/seeds (in *Ephedra* and *Hedyosmum*).

However, the two groups differ in basic characteristics. The ovules in Gnetopsida have another kind of development than those of angiosperms. The integument and envelope in *Ephedra* (Takaso, 1985) and *Gnetum* (Takaso and Bouman, 1986) are initiated centripetally, not centrifugally as in bitegmic angiosperm ovules. The inner integument functions as a pollen repository organ (but not as a style, since pollen does not germinate there) like the single integument in other gymnosperms; its tube-like extension is shared with Bennettitales (cf. Martens, 1971); both characters do not occur in angiosperms. A pollen chamber is formed in the ovular apex where pollen eventually germinates. A primary haploid endosperm is formed in Gnetopsida, in contrast to the secondary mostly triploid angiosperm endosperm. Archegonia are still differentiated in *Ephedra*, although they are lacking in *Gnetum* and *Welwitschia* (cf. Martens, 1971). The bilobed anthers do not show thecal differentiation as is so characteristic throughout the angiosperms. The anther wall differentiates an exothecium as in other gymnosperms, and not an endothecium as in angiosperms. Pollen is spindle-shaped, inaperturate (but monocolpate in *Ephedra* and *Welwitschia*), striate, and has another exine structure than angiosperms (incl. Chloranthaceae)

(Kedves, 1987). Pollen kitt is lacking in Gnetopsida (Hesse, 1984), while it is essentially present in angiosperms, even in those with dry, powdery pollen.

All these many differences make a direct evolutionary relationship between Gnetopsida and Chloranthaceae very unlikely. Probably many of the (mainly superficial) similarities have to be interpreted as results of convergent evolution. The Triassic *Sanmiguelia* claimed to have special similarities with Gnetopsida and Chloranthaceae (Cornet, 1986), is still very problematical. Yet, of all gymnosperms the Gnetopsida could still be the closest relatives of the angiosperms. This has also been discussed by Arber and Parkin (1908) and by Doyle and Donoghue (1986a). The Chloranthaceae are then not necessarily close to the common ancestors of Gnetopsida and angiosperms.

This general problem continually arises: the more reduced, simplified certain structures are, the more it becomes probable that they become similar to some other structures. The most reduced female flowers in the angiosperms, those of the parasitic Balanophoraceae, resemble moss archegonia. This is an extreme case, and nobody would seriously consider close evolutionary relationships between mosses and angiosperms via the Balanophoraceae.

Probably both Gnetopsida and Chloranthaceae are alike by reduction. Gnetopsida may be the closest relatives of the angiosperms, but they are not their ancestors (cf. Arber and Parkin, 1908; Ehrendorfer, 1976; Crane, 1985a, 1986; Doyle and Donoghue, 1986b, 1987a). Certainly the identity of their common ancestor is still in question, but Caytoniales and Corystospermales are, at present, favoured, as expressed by the detailed discussions by Crane (1985a) and by Doyle and Donoghue (1986b).

B. PROPERTIES OF THE FIRST ANGIOSPERM FLOWERS

The classical form of the Euanthium Theory (cf. e.g. Hallier, 1901; Arber and Parkin, 1907; Eames, 1961) regarded a large strobilus with many spirally arranged floral organs, as it occurs in the Magnoliaceae, as the primitive flower type. Based on structural and biological studies of Annonaceae and Winteraceae (Gottsberger, 1974, 1988; Gottsberger *et al.*, 1980) this view has later been modified to that of the primitive flower being of medium size, unspecialized, open (bowl-shaped, unprotected), protogynous, acyclic, with relatively few stamens and carpels loosely arranged on a short floral axis.

The results of the last decade have further modified our expectations of how the first angiosperm flowers were organized. The new sources are studies on numerous Cretaceous fossil flowers that have been discovered recently and studies on several archaic extant Magnoliidae families. Early flowers were characterized by a loose general organization. Synorganization of parts was largely lacking in many respects. This was expressed by a great plasticity in floral organ number, floral phyllotaxis, and floral size. A perianth was especially unelaborated. Tepals were bract-like and could easily be reduced

and lost, since they were not closely integrated in the floral architecture. Attractiveness of the mainly fly and beetle (and wasp; cf. Crepet and Friis, 1987 and Willemstein, 1987) pollinated flowers was mainly exhibited by the androecium and not the perianth. Smaller flowers with relative few organs probably prevailed. Bisexuality with a central (apocarpous) gynoecium was basically established but unisexual flowers often evolved (see also Crepet and Friis, 1987; Friis and Crepet, 1987; Endress, 1987c).

C. MAJOR EVOLUTIONARY STEPS IN ANGIOSPERM FLOWERS

The flower is a highly plastic system in many respects. Therefore, evolutionary modifications are immensely numerous but major evolutionary steps are comparatively few.

Within the angiosperms, there is a general tendency of elaboration of the *perianth*. Differentiation of a calyx with mainly protective functions and a corolla with mainly attractive functions dominates at the middle and higher evolutionary levels of the dicotyledons. The calycine organs have more properties of bracts, the corolline organs more of staminodes (cf. Hiepko, 1965; Vogel, 1981; and Section IV.F). It can be surmised that these structural and functional relationships also reflect their evolutionary history. A further step is congenital fusion of the corolla (and calyx) by basal intercalary meristems, often together with stamens. This has opened new avenues for pollination biological elaborations. It is a characteristic feature in the Asteridae.

The *androecium* has become fixed in the number of its constituents. The stamens have evolved well-defined anthers and more or less long filaments and lost attractive functions in parallel with the elaboration of a corolla.

One of the most general evolutionary advancements is the event of syncarpy in the *gynoecium* by congenital fusion of the carpels and the differentiation of a compitum, a common pollen tube transmitting tissue for all carpels (cf. Section IV.H). Another step in the gynoecium, however less general, is the change from a superior to an inferior ovary by an intercalary meristem in the floral base and, therefore, increased protection of the developing fruit and seeds. Progression from crassinucellar to tenuinucellar ovules opened the way for an increased seed production per fruit, which was explored by several parasitic groups and other specialists (cf. Endress, 1989b).

It has been hypothesized that the pentamerous and trimerous flowers have arisen from spiral ancestors (Kubitzki, 1987) or that pentamerous flowers have arisen from trimerous ones (Dahlgren, 1983). However, phyllotaxis is considerably more flexible as can be seen from studies on floral development in various groups, and all evolutionary directions seem possible (Endress, 1987a). Families like Ranunculaceae (Schöffel, 1932), Monimiaceae (Endress, 1980a,b), or Palmae (Uhl, 1988) are especially diverse in this respect. As a general trend, though, a whorled floral phyllotaxis has been

greatly favoured by the increased synorganization of parts, because whorls are a precondition of precise interactions of different parts.

Likewise, most of the traits in the breeding systems fall into the category of plastic evolutionary modifications rather than major evolutionary steps. A balance of inbreeding and outbreeding can be reached in many ways. Features such as self-incompatibility or temporal and spatial separation of sexes (dichogamy, herkogamy, dioecy) were explored and were lost in many different groups, primitive and advanced (surveys, e.g. Bawa and Beach, 1981; Willson, 1983; Lloyd, 1986a,b; Richards, 1986; Cruden, 1988). One of the exceptions is perhaps the differentiation of heterostyly. Although it is present here and there in many different groups, it occurs only in the medium and higher evolutionary level of the dicots and monocots. Representatives of the Magnoliidae, Hamamelididae, Alismatidae, and Arecidae are not known to be heterostylous, while the highest number of heterostylous genera occurs in the Asteridae. Heterostyly requires a floral organization that is not too open. Apparently, flowers with a fixed and low number of organs are more prone to become heterostylous (cf. Ganders, 1980).

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Bacterial Leaf Nodule Symbiosis

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I. INTRODUCTION

Leaf nodules represent the most visible aspect of a group of symbiotic relationships which are arguably the most complex, and certainly the most intimate associations between bacteria and higher plants. This intimacy lies in the fact that the bacteria are constant and obligate companions of the host throughout the plant's entire life cycle, and hence these relationships have

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come to be described as "cyclic symbioses". Colonies of bacteria are maintained in a protein/carbohydrate based mucilage within every vegetative shoot tip of the host plant, acting as a source of inoculum for the infection of each new developing leaf. At the onset of flowering, the bacteria are transferred into the floral shoot tip and, during floral organogenesis, are placed within the embryo sac of the developing ovule. As the ovule develops into a seed, the bacteria are somehow positioned on the epicotyl of the embryo where, upon germination of the seed, they become enclosed in the shoot tip of the seedling where they infect the first leaves of the next host plant generation.

In these relationships the leaf nodules *per se*, although highly visible, are but a relatively small part of the story, there being a more complicated sequence of plant–microbe interactions occurring at a microscopic level within the host plant throughout its entire life cycle. It is the purpose of this chapter to describe in some detail the present state of our understanding of the interactions which occur in these fascinating and complex cyclic symbioses, and to describe at the ultrastructural level the interesting, if somewhat bizarre, ecosystem to which these leaf nodule microsymbionts have become adapted.

A. OCCURRENCE AND DISTRIBUTION OF HOST PLANTS

Leaf symbioses have been described in two dicotyledonous plant families, Myrsinaceae and Rubiaceae, and in one monocotyledonous family, Dioscoreaceae. As will be domonstrated later, the symbiosis in the latter is different in character and apparent function to that observed in the two dicotyledonous families. Historically, however, these dioscoreaceous associations have been included in discussions on leaf nodulation, and will therefore, for the sake of completeness, be included in this dissertation.

1. Myrsinaceae

This is a medium size family of tropical and subtropical trees and shrubs, consisting of some 1000 species in 32 genera (Mez, 1902). Three genera have been reported to possess bacterial leaf nodules, these being *Ardisia*, *Amblyanthus* and *Amblyanthopsis* (Table I). The latter two genera are very small, consisting of only five species between them and all of these are restricted in distribution to the Indian state of Assam. Mez reported 235 species of *Ardisia* arranging them in 14 subgenera. Additions by later authorities increased the genus to 250 species (Walker, 1940). The 30 nodulated *Ardisia* species are grouped together in the subgenus *Crispardisia*. The nodules of myrsinaceous host plants are ellipsoid structures located solely on the margins of the leaves, giving the leaves a crenate appearance (Fig. 1a). The most widely known member of the subgenus is *Ardisia crispa* Thunb. A.DC. an attractive evergreen shrub known as "coral berry" and grown in botanic gardens and conservatories throughout the world for its ornamental value. As a result

TABLE I

General information on leaf-nodulated host plants

Family	Genus	No. of nodulated species	Range	Remarks
Dioscoreaceae	*Dioscorea*	2–4	Tropical West Africa	Two species, *D. sansibarensis* and *D. macroura* have been definitely shown to harbour symbiotic bacteria. Possibly at least two other symbiotic species
Myrsinaceae	*Amblyanthus*	2	Restricted to Assam	Mez (1902) described these species as having protein glands along the leaf margins. The presence of bacteria, however, has not been unequivocally demonstrated
	Amblyanthopsis	3	Restricted to Assam	
	Ardisia	30	India, Himalayas, China, mainland S.E. Asia, Philippines, Indonesia	Bacterial symbiosis has been described in *A. crispa*, *A. crenata*, *A. crednulata* and *A. hortorum*
Rubiaceae	*Neorosea*	14	South and West Africa	Bacterial symbosis definitely established for *N. andongensis*
	Pavetta	353	Tropical Pacific, Asia, Australia, Africa	Bacterial presence demonstrated in numerous species
	Psychotria	33–67	East Africa, West Africa	Symbiosis shown in numerous species. Systematics of *Psychotria* is somewhat in turmoil. Number of nodulated species varies widely between authorities

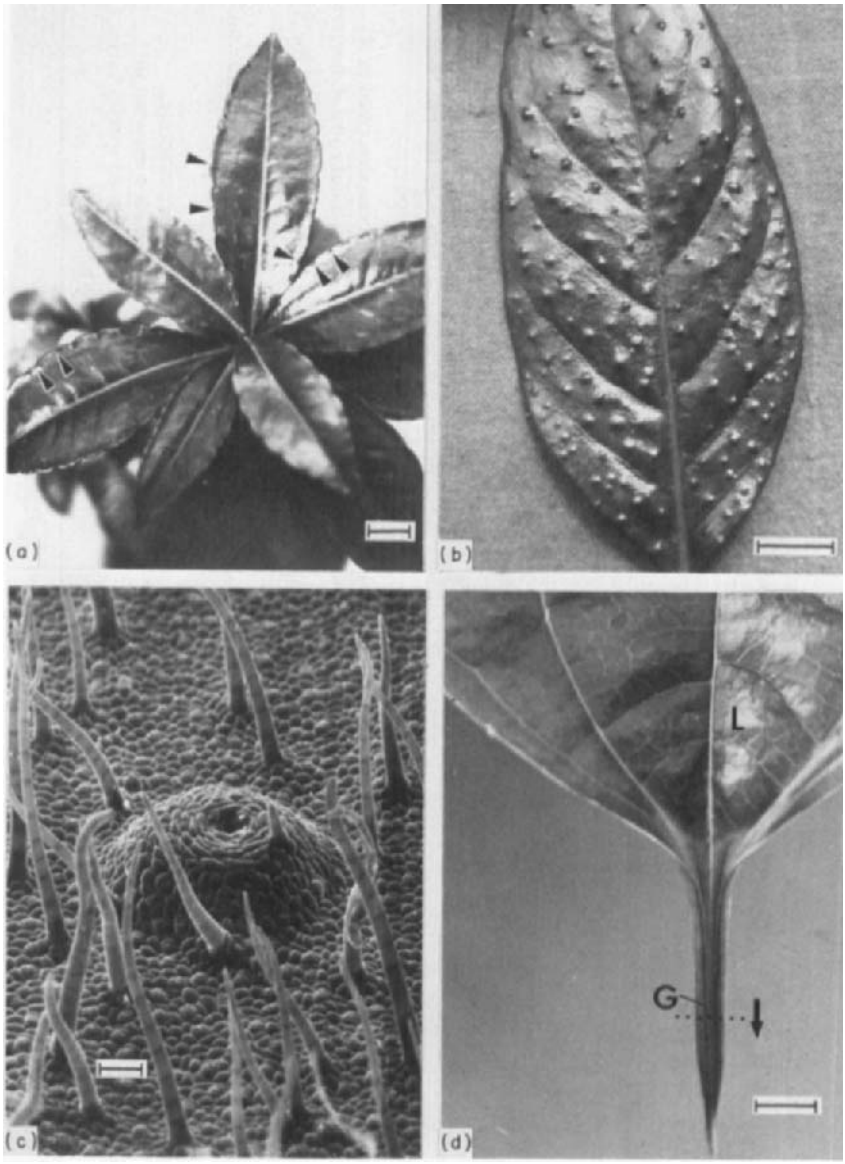

Fig. 1. (a) Leaves of a young *Ardisia crispa* specimen showing the small marginally located nodules (arrows). Bar = 18.0 mm. (b) A leaf of *Psychotria punctata* showing the random distribution of leaf nodules in the lamina. Bar = 10.0 mm. (c) SEM of a nodule on the lamina of *Psychotria kirkii* var. *kirkii*. The nodules appear as raised hemispherical swellings with a pronounced central depression or pit. This depression arises through cell division and movement (see Section III.A.2). A light micrograph of a transverse section through such a nodule is shown in Fig. 26. Bar = 50 μm. (d) The basal portion of a leaf from *Dioscorea sansibarensis* showing the long glandular acumen (G) or "drip-tip"; leaf lamina, L. The dotted line and arrow indicate the section and direction of view of the SEM in Fig. 30b. Bar = 10.0 mm.

A. crispa is readily available for study and consequently the majority of work on leaf nodulation in the family Myrsinaceae concerns this species. The natural geographic range of *A. crispa* is in hot humid regions of India, the Himalayas, China, mainland South East Asia and the offshore archipelagos of the Philippines and Indonesia.

2. Rubiaceae

This is a large family of herbs, shrubs and trees containing some 6000 species in about 500 genera, mostly tropical in distribution and adapted to moist conditions. Taxonomic revisions within Rubiaceae have been extensive in recent years leading to considerable confusion in the nomenclature of nodulated rubiaceous genera; presently nodulated species have been placed in three genera, *Psychotria*, *Pavetta* and *Neorosea*. The nodules of rubiaceaous hosts vary widely in shape, size and distribution. They can be spherical or rod-like, branched or unbranched and located variously at random on the leaf lamina, midrib or petiole (Fig. 1b,c).

Verdcourt (1958) divided the Rubiaceae into three subfamilies; one genus which contained nodulated species, *Psychotria*, was placed in the tribe Psychotrieae, subfamily Rubioideae. The other nodulated genera he placed in the tribe Ixoreae, subfamily Cinchonoideae. Bremekamp (1966) divided the family into eight subfamilies. In his classification the genus *Psychotria* was retained in the tribe Psychotrieae of the subfamily Rubioideae as in Verdcourt (1958). Bremekamp however placed the other nodulated genera in the tribe Ixoreae of the subfamily Ixoroideae. Bremekamp (1933, 1952, 1960) recognized a total of 49 nodulated *Psychotria* species.

Petit (1964, 1966) divided the genus *Psychotria* into two subgenera, *Psychotria* and *Tetramerae*, recognizing 67 nodulated species from the continent of Africa. The nodulated species, with two exceptions, he placed in the subgenus *Tetramerae*. Lersten (1974a) contended that these two species, placed by Petit (1964) in two different sections of the subgenus *Psychotria*, should in fact be transferred to the subgenus *Tetramerae*. Lersten based this contention on the fact that these two species possessed trichomes in their buds which were compatible with trichome morphology in the subgenus *Tetramerae* and incompatible with the trichome morphology found in the sections in which they were placed by Petit. In his recent taxonomic review of East African Rubiaceae, Verdcourt (1976) follows Petit and retains the two species under dispute in their respective sections. Steyermark (1972) corrected the anomalous nomenclature situation created by Petit by erecting a new subgenus, *Heteropsychotria*, to include all *Psychotria* species except those in the subgenus *Tetramerae*. Verdcourt (1976), following this subgeneric classification of Steyermark, now recognizes 27 nodulated species from tropical East Africa, having reduced many species to the level of infraspecific variants. Six West African nodulated species, outwith the defined geographic scope of Verdcourt's work brings this total to 33.

The genus *Psychotria* consists mainly of shrubs and trees and is distributed throughout the New and Old World tropics and subtropics. Nodulated *Psychotria* species, however, are found only in Africa and surrounding islands and most of these are further limited to the south-eastern portion of the continent.

The genus *Pavetta*, which consists mainly of trees and shrubs, has been described and classified thoroughly by Bremekamp (1929, 1934, 1939a,b, 1948, 1953, 1956). Out of 406 species, a total of 353 are nodulated and Pavetta therefore represents the largest single nodulated genus enjoying a wide distribution throughout the tropical Pacific, Australia, Africa and Asia. Unlike *Psychotria*, nodulated *Pavetta* species are found throughout the entire geographic range of the genus.

Neorosea is a small genus of trees and shrubs, created and subsequently expanded by Hallé (1970, 1972) and which now contains a total of 16 species, 14 of which are nodulated and all of which are found in southern and western Africa.

For a more detailed discussion on the systematics of nodulated rubiaceous and myrsinaceous plants see the review of Lersten and Horner (1976) and other systematic studies by Lersten (1974a,b, 1975, 1977).

3. Dioscoreaceae

This viny monocotyledonous family consists of over 600 species in 10 genera (Ayensu, 1972). Almost 90% of this family consists of the genus *Dioscorea*, the true yams. Although pan-tropical in distribution, the two *Dioscorea* species which have been shown to exhibit a leaf-located symbiosis, *D. macroura* and *D. sansibarensis*, occur naturally only in the rain forests of West Africa. In the strictest sense the structures which harbour the symbiotic bacteria are not "nodules"; rather, they are large glands or extrafloral nectaries on the leaf acumen which become beneficially infected by the microsymbiont (Fig. 1d). There may well be yet more *Dioscorea* species which are involved in symbiotic associations; the author has preliminary evidence which suggests that two other yams, *D. cochleari-apiculata* and *D. dodecaneura* also harbour symbiotic bacteria.

The number and range of nodulated species is summarized in Table I.

II. LEAF NODULE SYMBIOSIS IN ARDISIA (MYRSINACEAE)

A. INITIATION AND DEVELOPMENT OF LEAF NODULES

Nodules on the leaves of myrsinaceous species were first reported by von Höhnel (1881) who described "elongated swellings or callosities" on the margins of *Ardisia crenulata* Lodd (see Fig. 2a). He described the "nodule" as being encompassed by a sheath of flattened cells and that some of these

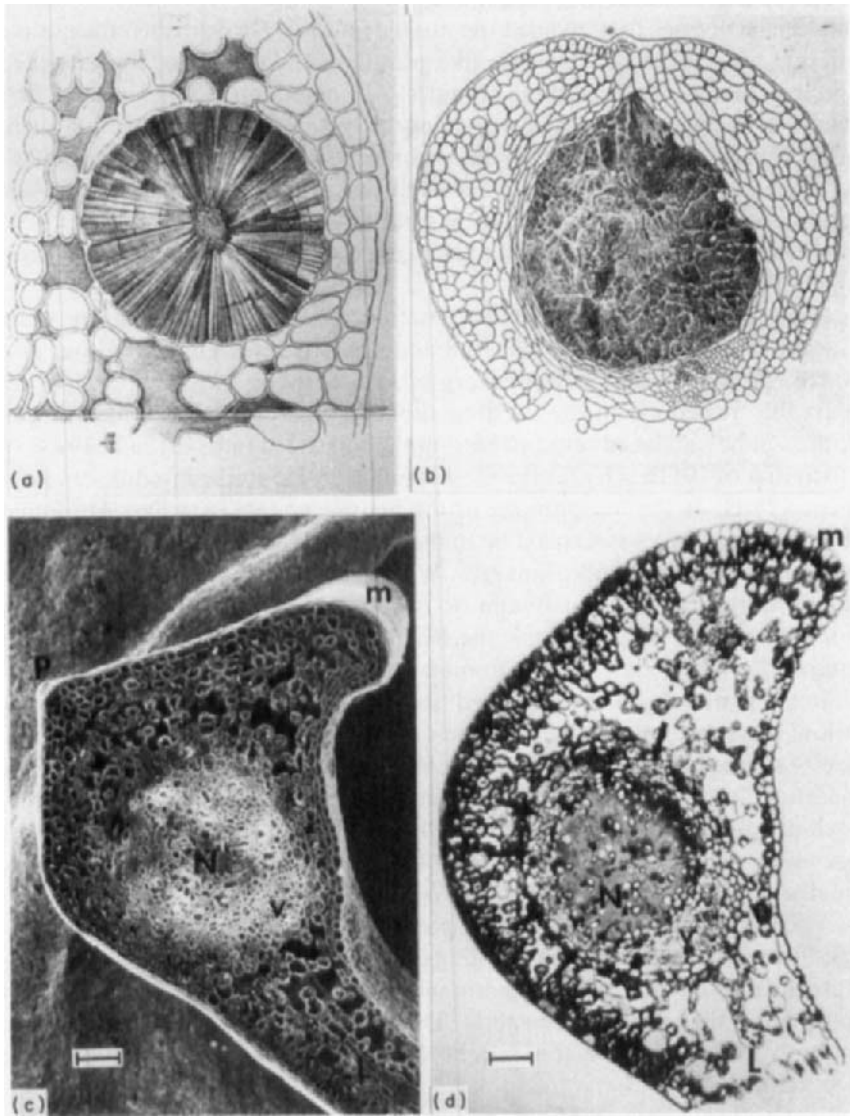

Fig. 2. Photographic reproduction of the *Ardisia* nodule (a) published in 1881 by von Höhnel; (b) published by Miehe in 1911. (c) SEM of an *Ardisia crispa* leaf transverse section showing a bacterial nodule (N) in the leaf margin. The now-sealed pore through which the bacteria entered this leaf is evident (P). Each nodule is served by a major vascular bundle (v) located basally on that side of the nodule proximal to the lamina (L) and distal to the nodule pore and leaf margin. The extreme leaf margin is indicated (m). Bar = 50 μm. (d) Light micrograph (LM) of an *Ardisia* nodule transverse section. Extreme leaf margin, m; leaf lamina, L. The demarcation between the bacterial nodule (N) and the surrounding vascularized sheath is very clear. The main bundle (v) consists of phloem, xylem, schlerenchymatous fibres and other supportive cells while the remainder of the nodule sheath is punctuated in places with small phloem bundles (arrows). Note that the bacterial region is traversed by an interconnected network of tubular, condensed and collapsed cells which are in places attached to cells of the sheath. Bar = 50 μm.

cells had processes that invaded the nodule interior. He described the nodule interior as consisting of "bacteria-like granules" and illustrated the branched and irregular structure of these granules. Although von Höhnel was the first observer of bacteria in *Ardisia* nodules, he did not interpret them as such; histochemical analysis of the nodule led him to believe that these "granules" were precipitated protein. He considered that the sheath cells and invasive cells secreted the protein and that the nodules themselves were some form of gland or protein storage organ and he made no attempt to study the development of these "glands".

Miehe (1911b) was the first to describe the swellings on the crenulate margin of *Ardisia crispa* as bacterial nodules. His research, carried out at a coffee research station at Buitenzorg in Java between 1909 and 1911, is remarkably revealing and interesting though largely devoid of illustration. Although he published seven papers on the topic (1911a,b, 1912a,b, 1913a,b, 1916) that of 1911b is by far the most significant. He studied nodule development as well as the morphology of the mature nodule (Fig. 2b). He found that in young leaves still rolled up in the bud, there were swellings at equidistant intervals along the leaf margin. Where these swellings occurred he also found marginally placed stomata or "water pores" which were larger than normal stomata developed on the leaf lamina. As leaf development progressed he found that the substomatal chamber became filled with a red-staining material which contained some bacteria. The cells immediately below the substomatal chamber then developed small "schizogenous lacunae" (intercellular spaces) which became partly filled with the red-staining material. At this point in development the stomatal pore became rigidly occluded by the fusion of adjacent cells which had the effect of pushing the bacterial "primary lacuna" deeper into the leaf tissue. This lacuna was very small and according to Miehe could very easily be mistaken for a plant cell.

As development proceeded the bacteria split the walls of adjacent tubular cells, forming new lacunae. According to Miehe (1911b) the micro-organisms were distributed intercellularly, although sometimes cells could be found that looked as if they contained bacteria. The endophyte continued to invade and multiply in the intercellular spaces between the tubular cells until the nodule was mature.

In the mature nodule, Miehe claimed that the tubular cells did not die but became very compressed, sometimes appearing at the light microscope level as only two lines in the bacterial mass. He illustrated the mature nodule as consisting of a central bacterial mass surrounded by a sheath or "covering" of flattened cells (Fig. 2b). The compressed tubular cells he showed were confluent with the cells of the flattened sheath. Miehe also described having seen "little bundles of cambiform cells which interrupt the covering places" but stated he knew nothing about them, and, surprisingly, left them out of his illustration of the mature nodule and discussed them no further! The significance of these "little bundles" is discussed in Section 2.A.3. As a comparison

to the early illustrations of von Höhnel and Miehe (Figs 2a and b) modern SEM and micrographs of transverse sections through an *Ardisia* leaf margin at a point where a nodule occurs are shown in Fig. 2c and d.

Miehe speculated on certain points arising from his investigations. The fact that the stomatal pores were marginal in location led him to suggest that *Ardisia* nodules were, in fact, modified hydathodes—a position which is reflected in his reference to the stomatal openings as water pores and the tissue inside the nodule as epithem. He thought that the water pore must become blocked and that secretions normally emitted via the pore were trapped within the nodule for use by the bacteria. Thus, he reasoned the bacteria are maintained by secretions from the cells which surround them.

All in all, Miehe's anatomical and developmental studies on *Ardisia* nodules were extensive and thorough and 20 years later De Jongh (1938), the next worker to study the *Ardisia* symbiosis in any depth, said of Miehe's work "without exaggeration it may be said that we possess one classic in the literature of foliar symbiosis, and this classic is the work of Hugo Miehe . . .". This all-encompassing statement perhaps explains why De Jongh unfortunately carried out only a cursory examination of the development and structure of the *Ardisia* nodule. He verified Miehe's observations almost completely, adding only that he found a few, isolated irregular tubular cells within the nodule.

Apart from the present author's work, the last comprehensive study of *Ardisia* symbiosis was that of Hanada (1954). The first part of Hanada's paper is concerned with the development and structure of *Ardisia* nodules and is a somewhat composite account derived from observations on both *A. crispa* and *A. hortorum*. Hanada's (1954) account of nodule development differs greatly from the descriptions put forward earlier by Miehe and De Jongh (1938). According to him, when the bacteria enter the leaf through a hydathode pore they enter the host plant cells and multiply therein. When the plant cell divides the bacteria are carried into each daughter cell. The cells then break open releasing the bacteria which move either by Brownian motion or swim with the help of cilia and flagella. He claimed that as development of the nodule progresses the walls of the mesophyll cells surrounding the nodule thicken, giving rise to the nodule sheath. These cells, which contain both chloroplasts and starch, surround the nodule almost completely except in the vicinity of what he called the "atemgewebe", a somewhat spongy, slimy bacteria-filled tissue connected the nodule to the hydathode pore. Hanada (1954) illustrated the mature nodule of *A. hortorum* showing a sheath of flattened cells surrounding the bacterial tissue which contained large round circular cell profiles. A large vascular bundle was shown connecting the nodule to the midrib which Hanada observed was composed mainly of phloem leading him to speculate that this phloem was involved in the translocation of substances produced within the nodule to other parts of the host plant.

The contributions of De Jongh (1938) and Hanada (1954) have not ad-

vanced our knowledge of the developmental morphology of the symbiosis much beyond the earlier work of Miehe. Although all these earlier workers agree on points such as entry of bacteria into the host via pores, formation of a nodule by some form of bacterial proliferation within the host tissue and the formation of a sheath to contain the nodule, they were unable to describe the specific mechanisms involved in nodule development. This was for the most part due to the limitations of light microscopy; with the introduction of the electron microscope a more searching examination of initiation and development of the nodule became possible (Gardner *et al.*, 1981; Miller *et al.*, 1983a, 1984a).

1. Morphology and Role of the Shoot Tip

To understand the process by which each and every *Ardisia* leaf becomes inoculated with its bacterial partner one must consider the structure and functional role of the vegetative shoot tips. In the shoot tips of *Ardisia*, leaves are initiated sequentially in a low order Fibonacci spiral arrangement. Although the main shoot of the plant produces leaves on a continuous basis, the lateral shoots produce foliage in spiral clusters of three to five leaves and then become dormant in preparation for flowering. In both the growing main and lateral shoot tips each young leaf is tightly convolute, the abaxial surface of the lamina from either side of the midrib being closely juxtaposed. This forms an enclosed apical chamber into which each successive leaf grows. A line drawing shows the overall gross morphology of the shoot tip and indicates the relationship between the constituent parts (Fig. 3). A low-power scanning electron micrograph showing the relative spatial arrangement of three young developing leaves is shown in Fig. 4a.

Arising from both the abaxial (outer) and adaxial (inner) epidermis of the young leaves are numerous multicellular trichomes. On the abaxial surface the trichomes are exclusively peltate scales (Fig. 4a,b). As yet no definite function with respect to the symbiosis has been attributed to these trichomes. On the adaxial surface, the predominant form of trichome is a stellate capitate form with swollen distal cells (Fig. 4c). These stellate trichomes are uniseriate, rotate structures consisting of up to eight multicellular arms radiating from a central stalk cell and terminating distally in a swollen, club-shaped tip containing two to four terminal cells. Figure 4 shows that a membrane-like film covers the stellate trichomes and lines the inner surface of the apical chamber. This represents the dehydrated remains of mucilage which almost completely fills the chamber in the fresh, living shoot tip. The source of this mucilage is the multicellular arms and tip cells of the stellate trichomes (Figs. 5 and 6). The trichome arm cells contain extensive sheets of rough endoplasmic reticulum, particularly around the nuclei and in the peripheral cytoplasm (Figs 5b and 6b). The endoplasmic reticulum is often found to be continuous with the plasmalemma. These trichome arm cells also contain considerable numbers of small dictyosomes and mitochondria. The cells of

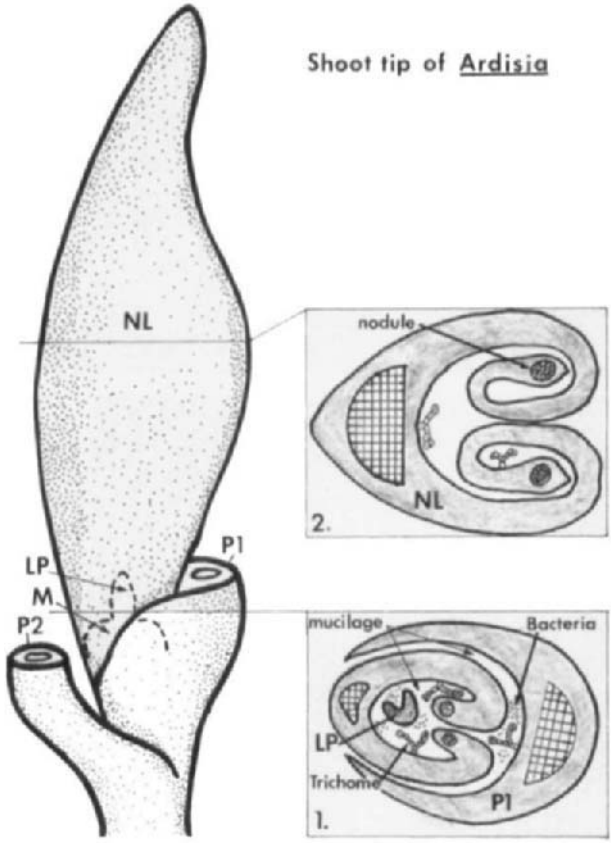

Fig. 3. Line drawing of the overall gross morphology of the shoot tip of *Ardisia crispa* showing the spatial relationships between the constituent parts. Arising from the apical meristem (M) is the leaf primordium (LP) of the most recently initiated leaf. It is totally enclosed by the basal part of the leaf which will be next to open out from the bud (NL). Often there may be one or two other leaves at intermediate developmental stages between LP and NL which add further to the protection both of the leaf primordium and bacterial colony from the external environment. For the sake of clarity in the diagram such additional leaves have been omitted. Toward the base of the shoot tip, just above the apical meristem (box 1) the secretory trichomes are very active and mucilage fills entirely the spaces between the constituent parts of the shoot tip. Inoculation of each leaf and the initiation of nodules occurs in this region. Further up the shoot tip, where only parts of the more mature leaves are found, trichomes, mucilage and bacteria are few or absent (box 2). At this stage in the leaf's development, the nodules are developing rapidly in concert with development of the procambial strands at the margins of the developing leaf lamina. P1 and P2 represent the petioles of the two preceding leaves.

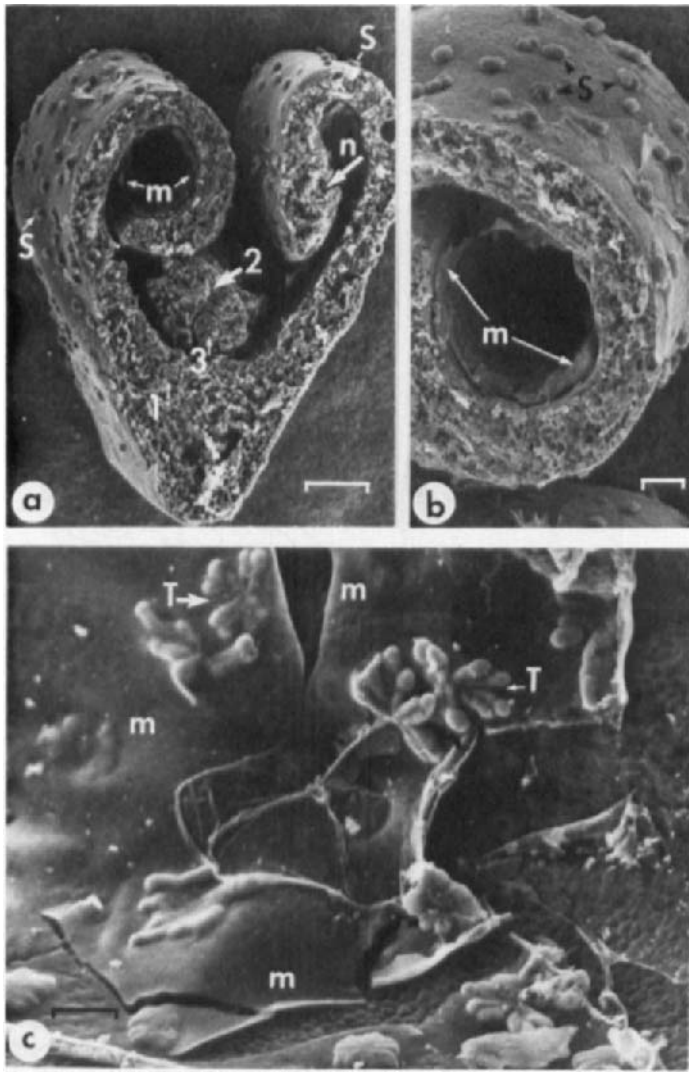

Fig. 4. (a) SEM of a transverse section through the shoot tip of *Ardisia crispa*. The oldest leaf (1) is rolled up forming a chamber into which the next leaf (2) develops. It in turn protects the primordium of yet the next leaf (3). Peltate scales (S) populate the abaxial surface but the stellate trichomes which populate the adaxial surface are obscured by the dehydrated remains of the mucilage (m). In comparison to the left hand leaf margin, the right hand margin is considerably more swollen, indicating the presence of a developing leaf nodule (n). Bar = 100 μm. (b) Rotated detail of the upper left portion of the oldest leaf (1) shown in (a). Young developing peltate scales (S) populate the abaxial leaf surface. Whether they have a role in the symbiosis is not yet known. Note the dehydrated layer of mucilage, which, in the fresh state, occupies much of the chamber delimited by the adaxial surface of the rolled up leaf. The stellate trichomes which secrete this mucilage are not visible at this scanning angle and magnification. Bar = 50 μm. (c) Detail of the adaxial surface mucilage layer in (b) showing the stellate trichomes (T). Note the uniseriate, rotate structure of these trichomes and the distal club-shaped tips. A layer of dehydrated mucilage (m) is superimposed on the trichomes. Bar = 25 μm.

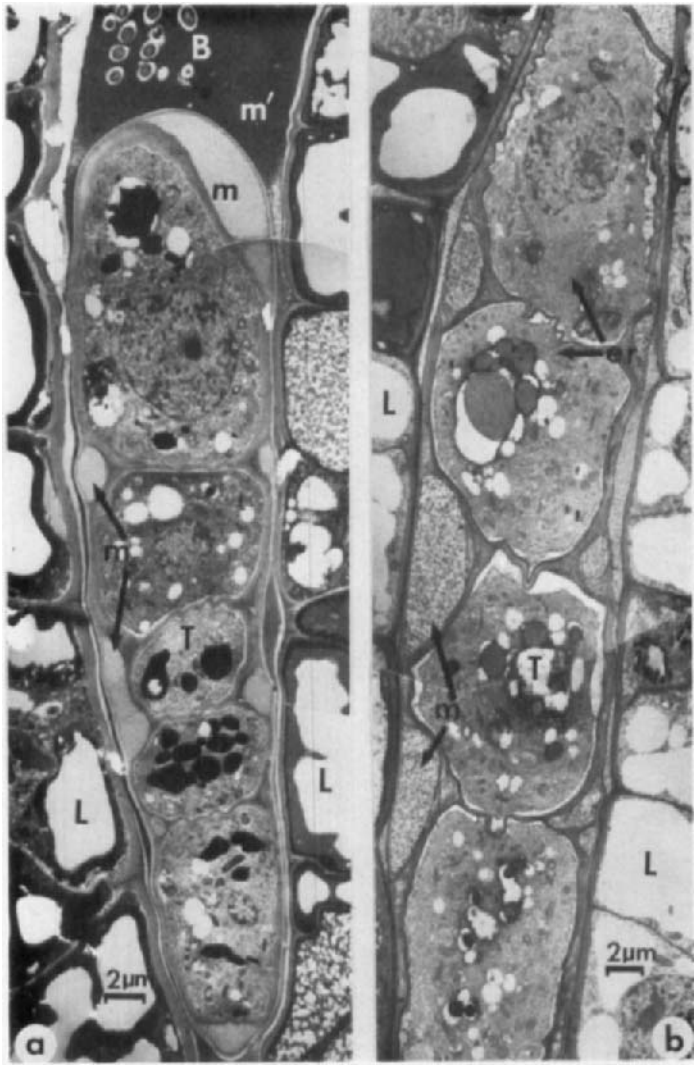

Fig. 5. (a) TEM photomontage of a multicellular arm of a stellate trichome sandwiched between the epidermes of two successive leaves (L). The mucilage (m) which has a fibrous to granular appearance, accumulates between the cuticle and the cell wall, particularly at the junctions between adjoining cells. The mucilage is presumably released by the rupture of the cuticle caused by the build up of pressure of the underlying mucilage. The mucilage is the medium in which the symbiotic bacteria (B) are maintained for use in the inoculation of each leaf. After the mucilage has been secreted from the trichome and fills the shoot tip chamber (m') it has a more electron dense appearance; this is possibly due to re-absorption of some of the water content of the mucilage by the host plant cells, effectively "concentrating" the mucilage. Tannin deposits (T) are frequently found in the trichome cells. (b) TEM photomontage of a stellate trichome are similar to (a) but stained using a carbohydrate specific PA-TSC-SP procedure. The mucilage (m) is well stained indicating a significant carbohydrate component. The trichome arm cells contain large amounts of rough endoplasmic reticulum (er) indicating that the mucilage probably also contains a significant protein content. L, successive host leaves; T, tannin droplets.

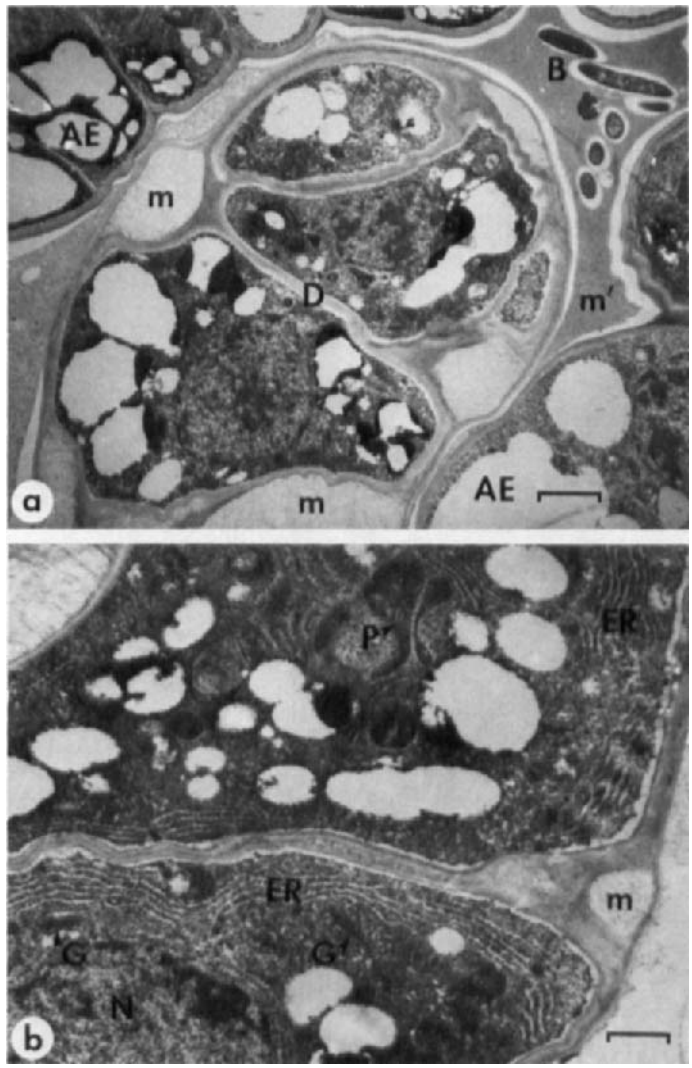

Fig. 6. (a) Detail of the three or four cell thick distal club-shaped tip (D) of a stellate trichome enclosed by the adaxial epidermis (AE) of the leaf from which it arises and surrounded by secreted mucilage (m'). Colonies of symbiotic bacteria (B) thrive in this mucilage throughout the entire basal part of the host plant shoot tip. The distal tip is also involved in the secretion of mucilage evidenced by the large sub-cuticular accumulations of material (m). Bar = 2 μm. (b) Detail of a stallate trichome cell showing typical distribution of cellular components. Large quantites of endoplasmic reticulum (ER) are typically found in the peripheral cytoplasm and around the nucleus (N). Often the ER is contiguous with the plasmalemma. These cells also contain Golgi bodies (G) and bizarre-shaped electron dense plastids as well as numbers of mitochondria and condensation vacuoles. Bar = 1 μm.

the club-shaped tips are similar to the arm cells but, however, contain less endoplasmic reticulum. The most pronounced features of these trichome cells, and the feature most crucial to the symbiosis, is the subcuticular accumulation of mucilage (Figs 5a,b and 6a). This mucilage, which is granular to fibrous in appearance, eventually exerts so much pressure on the cuticle that it bursts, releasing the contents into the apical chamber. A specific cytochemical staining procedure (Thiéry, 1967) shows that this mucilage contains a significant carbohydrate component (Fig. 5b). Given this, in addition to the large amounts of rough endoplasmic reticulum in the trichome cells, it can be concluded that the secreted material is a protein/carbohydrate type mucilage; it is in this mucilage that the bacteria are maintained as a source of inoculum for the initiation of leaf nodules.

In their studies on *Ardisia* both Miehe and De Jongh (1938) described the presence of trichomes in the shoot tips and speculated on their possible secretory role; however, they did not link this role with the symbiosis, considering that the mucilage in the apical chamber was of bacterial origin. The fact that the trichomes may be secretory and involved in the symbiosis was first put forward by Lersten (1977) and, indeed, this is the case. Not only does the mucilage secreted from these trichomes maintain the symbiotic bacteria, but it also acts as a vehicle for the successful intitiation of nodules in each young, developing leaf.

2. Infection of Young Leaves

Each new leaf is initiated from the apical meristem in the form of a crescent-shaped primordium, and is completely surrounded by mucilage secreted from the stellate trichomes (Fig. 7a). As the young leaf begins to develop, the distal arms of the crescent-shaped primordium, which are destined to become the leaf lamina, elongate and grow in towards each other (Fig. 7b). This inward growth results in the formation of a small chamber in which a small amount of mucilage containing the symbiotic bacteria becomes trapped. In essence, the very young developing leaf has "captured" its bacterial partner, and it will maintain its own colony from this point onward; dependence on the preceding leaf, therefore, ceases at a very early stage in the development of each new leaf. The fact that the young leaf has taken charge of its prospective bacterial partner is reflected in the appearance of the secretory trichomes on its adaxial surface (Figs 7b–e and 8a).

As development continues, the lamina-forming "arms" of the primordial leaf become closely appressed and begin to grow inwards into the bacteria-filled chamber, towards the leaf's adaxial surface (Figs 7e and 8a). At this stage in development the chamber is filled entirely with the cells of the stellate trichomes, their secretion product and the bacteria; the bacterial population in this secreted mucilage continues to thrive (Fig. 8b). At this early ontogenic stage, procambial strands have arisen both in that part of the primordium which will become the midrib vascular bundle, and near the ends of the

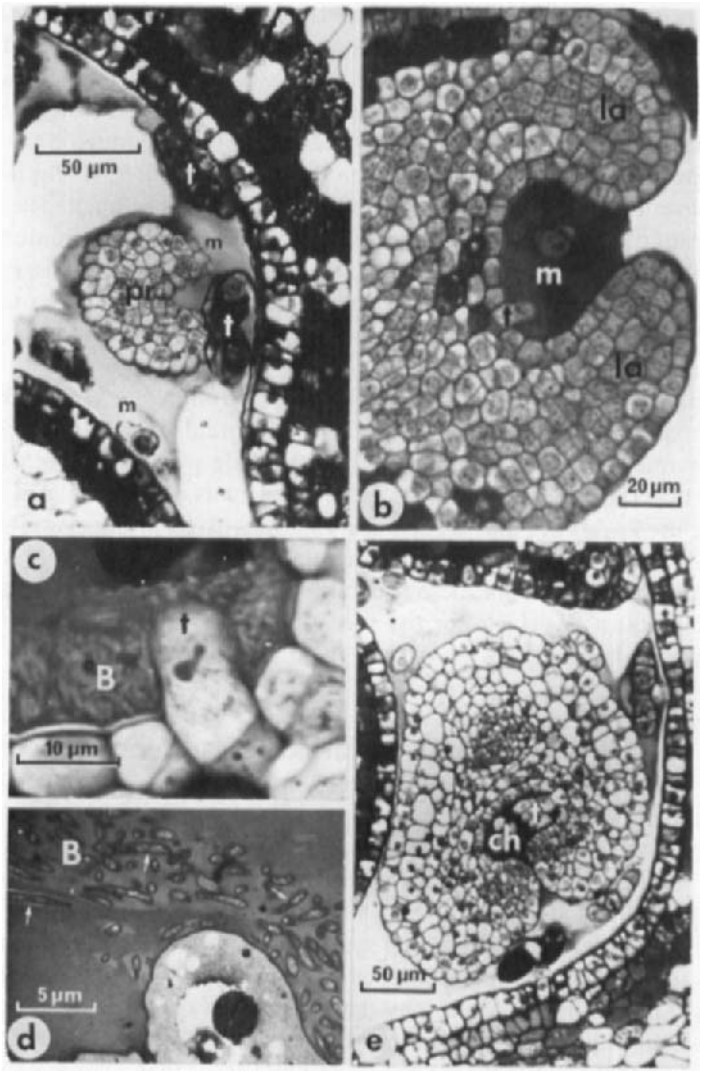

Fig. 7. (a) Light micrograph of a transverse section through an *Ardisia* shoot tip showing the crescent-shaped primordium (pr) bathed in mucilage (m) secreted from the stellate trichomes (t) which arise from the adaxial surface of the preceding leaf. The primordium and surrounding leaf are at approximately the same developmental stage as leaves 3 and 2, respectively shown in Fig. 4a. (b) As the primordium develops, the distal arms (1a) which are to become the leaf lamina, begin to grow in towards each other. Some of the mucilage (m) containing some of the symbiotic bacteria, becomes trapped between these arms. At this early stage the new leaf takes control of the maintenance of its bacterial partner, evidenced by the young trichome (t) arising from the adaxial protoderm. (c) Oil immersion detail of that young trichome and surrounding bacteria (B). (d) TEM detail of the same trichome. Many of the bacteria (B) are dividing (arrows). (e) As the primordium develops further, the lamina-forming arms have come together completely forming a small enclosed chamber (ch) which is filled with mucilage, bacteria and the now well-developed young secretory stellate trichomes (t).

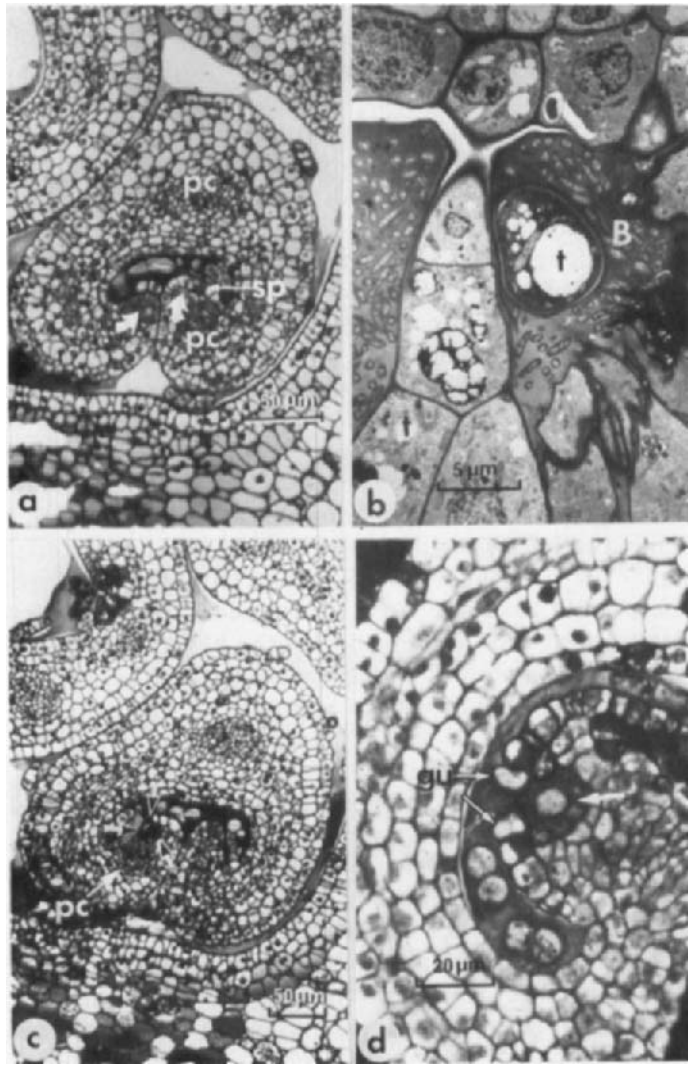

Fig. 8. (a) As the leaf primordium continues to develop the lamina-forming arms begin to grow inwards into the chamber towards the adaxial protoderm (curved arrows). Note that the chamber is filled with trichome cells and densely staining mucilage. At this stage, early-forming stomatal-like pores (sp) begin to develop in the protoderm at the tips of the lamina-forming arms. Procambial tissue (pc) has developed at the midrib and at the tips of the lamina-forming arms which will become the main and marginal vascular bundles, respectively. (b) TEM detail of part of the chamber showing the trichome cells (t) and the now rapidly increasing bacterial population (B). (c) A sub-stomatal chamber has developed beneath the stoma-like pore (delimited by arrows) and has become filled with bacteria-laden mucilage from the chamber. A leaf nodule has thus been initiated. This sub-stomatal chamber lies in direct contact with the procambial strand (pc). (d) Oil immersion detail of a nodule initiation event. Guard cells (gu) sit above a mucilage filled sub-stomatal chamber (arrow) at the tip of the inward-grown lamina-forming arm of the young leaf.

lamina-forming arms which will become marginal vascular bundles (Fig. 8a,c). An important phenomenon now occurs which is central to the initiation of leaf nodules. In the epidermis, at the tips of the lamina-forming arms, early-forming or "precocious" stomatal-like pores arise which are ontogenetically "out of step" with the surrounding tissue. When the pore opens, some mucilage containing the symbiotic bacteria fills the substomatal cavity, which in turn lies immediately adjacent to the procambial strand (Fig. 8c,d). A leaf nodule is thus initiated.

There is some disagreement among previous authors as to what this pore represents in terms of the true ontogeny of the leaf. That is, is the pore a regular stomatal pore or is it a hydathode? Both Miehe (1911b) and De Jongh (1938) have favoured regarding it as a premature hydathode while Hanada (1954) regarded it as a stoma. To this author, this seems rather an academic question. Procambial tissue certainly lies immediately adjacent to the cavity underlying the pore, suggestive of a developing hydathode (Fig. 8c,d). However at a later stage in leaf development rows of hydathodes develop normally in *Ardisia*, quite independently of nodules (Miller, 1982). It would seem more appropriate to consider these pores not as hydathodes or stomatal pores but quite simply as "nodule pores" unique to the somewhat specialized circumstances under which they arise. Given the role of the bacteria in the growth, development and differentiation of the host (discussed in Section V.B.2) it would not be untoward to suggest that these nodule pores may well be induced by the presence of the microsymbiont.

3. Development of Leaf Nodules

Now that the leaf has become infected with its bacterial partner, development of the nodule occurs in close concert with leaf development. A period of rather rapid growth and development of the young leaf ensues, producing the final convolute form it assumes before opening out (Fig. 9a). During this period of growth, differentiation of the leaf tissues, particularly the vascular tissue, is underway. The main vascular bundle is prominent and the growing marginal vascular bundles have caused the margins of the developing leaf to swell. This swelling is most prominent at the points where bacterial infection has occurred. Also during this growth period the nodule pore has become occluded by the transverse division and growth of the cells surrounding the pore; this essentially isolates the bacterial mass from the shoot tip chamber by positioning the mass and its associated procambial bundle deeper within the developing leaf tissue (Fig. 9b). The important point here is that the bacterial mass has remained in close contact with the procambial strand. The exact mechanism by which this occurs has not been studied in great detail. However, proliferation of some of the cells surrounding the pore causing a subsequent subduction of the epidermis is one possibility.

Considerable longitudinal growth also occurs during this period of occlusion of the nodule pore. This results in the bacterial mass, which at the time

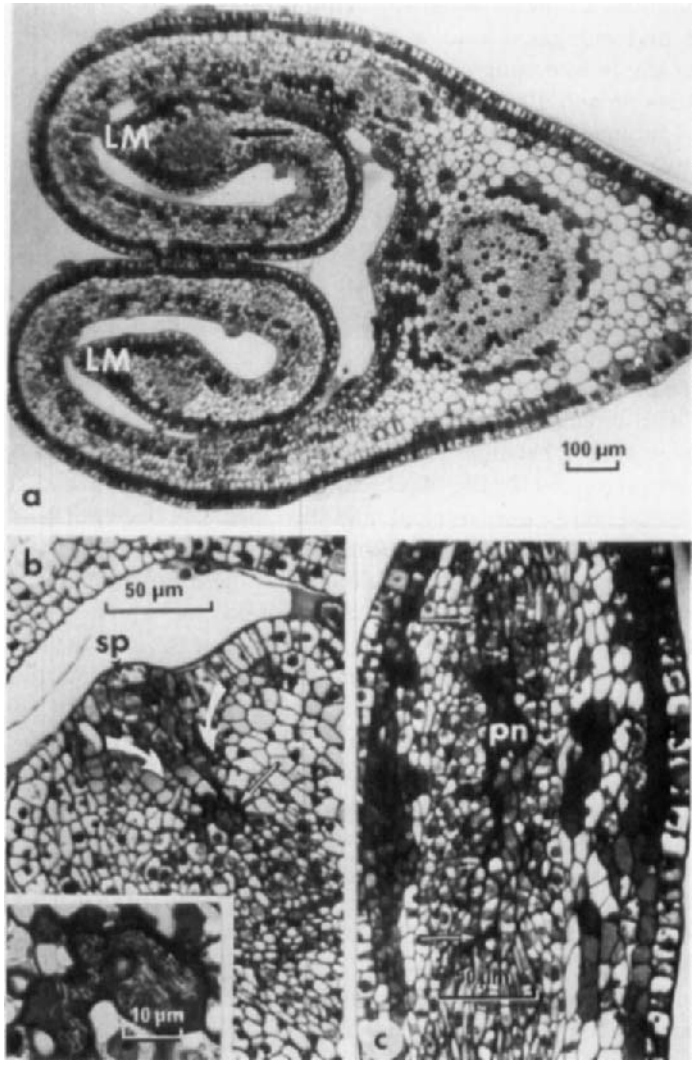

Fig. 9. (a) Transverse section through a young leaf showing its final convolute shape just before opening out (cf. box 2, Fig. 3). The developing procambial strands give the leaf margins (LM) a swollen appearance—the swelling is more pronounced at those points in the strand where a nodule has been initiated (arrow). (b) Horizontal transverse section of a leaf margin at a similar developmental stage to the leaf in Fig. 9a. The stomatal-like pore (sp) has become occluded by proliferation of the cells subtending it. This cellular proliferation has pushed the sub-stomatal cavity (arrow) along with its bacterial population (see inset) deeper into the leaf margin. The general inward movement of the substomatal cavity caused by the cellular proliferation is indicated by the curved arrows. During this growth, the bacterial mass and the procambial strand have remained in intimate contact. (c) Vertical longitudinal section of a similar leaf margin. Growth of the leaf has elongated the original substomatal cavity giving rise to an elongate protonodule (pn) approximately 150 µm in length (delimited by small arrows—the large arrows indicate either end of the protonodule).

of infection was a roughly spherical 20 μm drop (Figs 8d, 9b and 10b) being extended and elongated into a roughly cylindrical "protonodule" some 150 μm in length and running parallel to and juxtaposed to the procambial strand (Figs 9c and 10b). Development of the leaf nodule from this point onward is intimately linked with the development both of the marginal vascular bundle and of the leaf. At this point, leaf development is rapid; each young leaf grows vigorously, expands and opens out, attaining in a few short weeks almost their mature size. Growth of the procambial strand/nodule parallels this vigorous leaf growth. During this growth period schizogeny of the procambial strand cells occurs; the schizogenous cavities become filled with material of fibrous and granular appearance which is secreted by the surrounding host plant cells (Fig. 10a,b). The procambial cells, small, compressed and cuboidal in earlier stages (Fig. 10) become enlarged and tubular in form with circular profiles in transverse section, and the material-filled intercellular spaces become more pronounced (Fig. 11a–c). Although the bacteria are active and the population increasing, they are unable to keep pace with this rapid nodule development, and therefore such bacterial cells as there are, become disseminated sparsely throughout the intercellular spaces of the young nodule (Fig. 11a). At this developmental stage, differentiation of a ring of vascular tissue around the young nodule also becomes apparent (Fig. 11c).

During the aforementioned rapid growth phase of the leaf, the nodule also reaches approximately its mature size of between 200 and 500 μm in diameter. The schizogenous development which characterized the expansion phase of the development of the nodule is now superseded by a period of lysigenous development (Fig. 11a–c). Lysis of the tubular cells in the inner region of the developing nodule occurs; the developing vascular tissue around the nodule periphery remains unaffected. The lysis of the nodule cells is characterized by the breakdown of the cellulose walls followed by a general degeneration of the cell contents; the eventual digestion of the cellular debris occurs in the intercellular spaces following the total collapse of the degenerating cell. While some of the cells in the nodule interior are being lysed, other tubular cells are unaffected and begin to secrete a dense granular material into the newly enlarged intercellular spaces (Figs 11b and 12a,c).

Further nodule development is characterized by an increasing definition of the various tissues which make up the nodule structure. The internal bacterial region with its remaining tubular cells becomes clearly distinct from the surrounding ring of developing vascular tissue (Fig. 12b). The tubular cells secrete large quantites of dense granular material into the ever increasing intercellular spaces (Fig. 12a,c). The bacteria, very sparse in earlier developmental stages begin to increase in number (Fig. 12c). Zones of clarity around the bacteria suggest that the secreted material is being utilized by the microsymbiont as a nutritional source.

As the developing nodule approaches maturity, differentiation between the external vascular sheath and the internal bacterial region becomes increas-

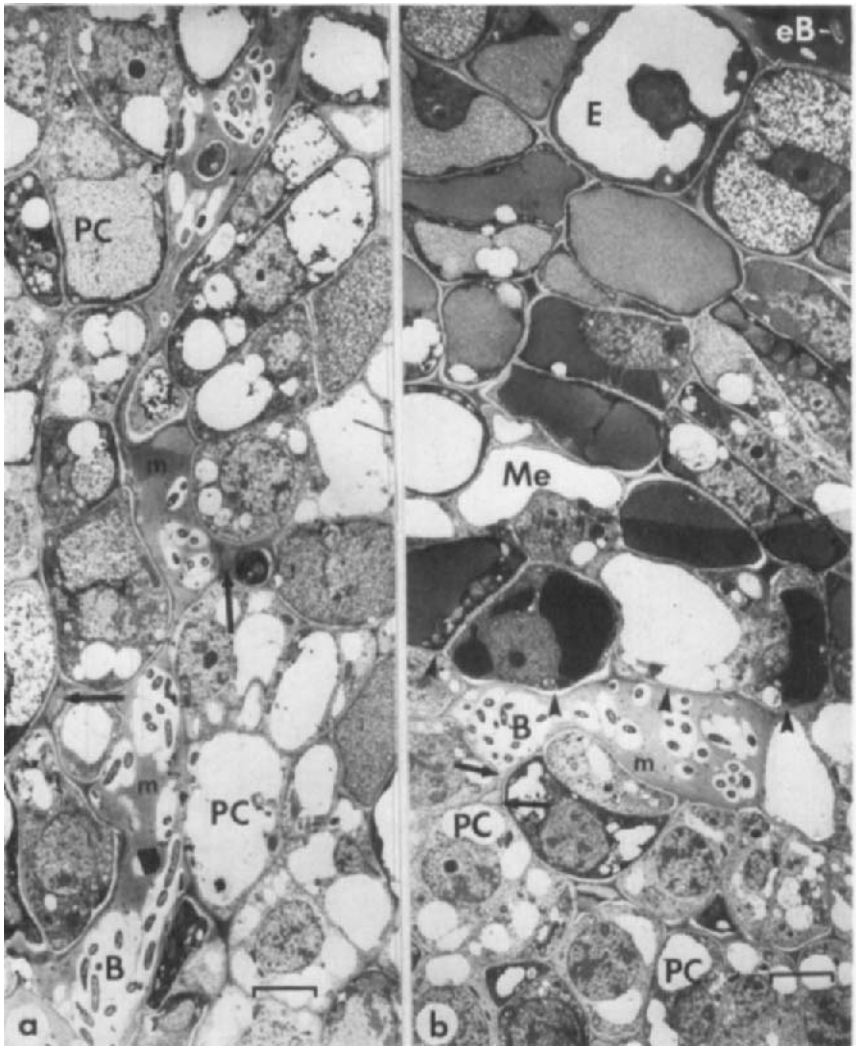

Fig. 10. (a) TEM photomontage of a longitudinal section through part of a young protonodule, corresponding to about the central 70–80 μm portion of the protonodule seen in Fig. 9c. The protonodule is filled with mucilage (m) in which the symbiotic bacteria (B) are liberally dispersed. The cells of the procambial strand (PC) some of which are beginning to show signs of elongation, lie on either side of the protonodule. Initial signs of schizogeny of the procambial cells is evident (arrows). Bar = 5 μm. (b) TEM photomontage of a transverse section through a young protonodule similar to that in (a) showing the bacteria (B) embedded in the mucilage. Above the protonodule is a region of young mesophyll cells (Me) topped by the epidermis of the leaf margin (E). Some bacteria (eB) can be seen in the external mucilage of the shoot tip chamber just above the leaf epidermis. The cells of the procambial strand (PC) are delimited from the mesophyll (Me) by the row of darts. Schizogeny of the cells of the procambial strand is beginning to be evident (arrows). Bar = 5 μm.

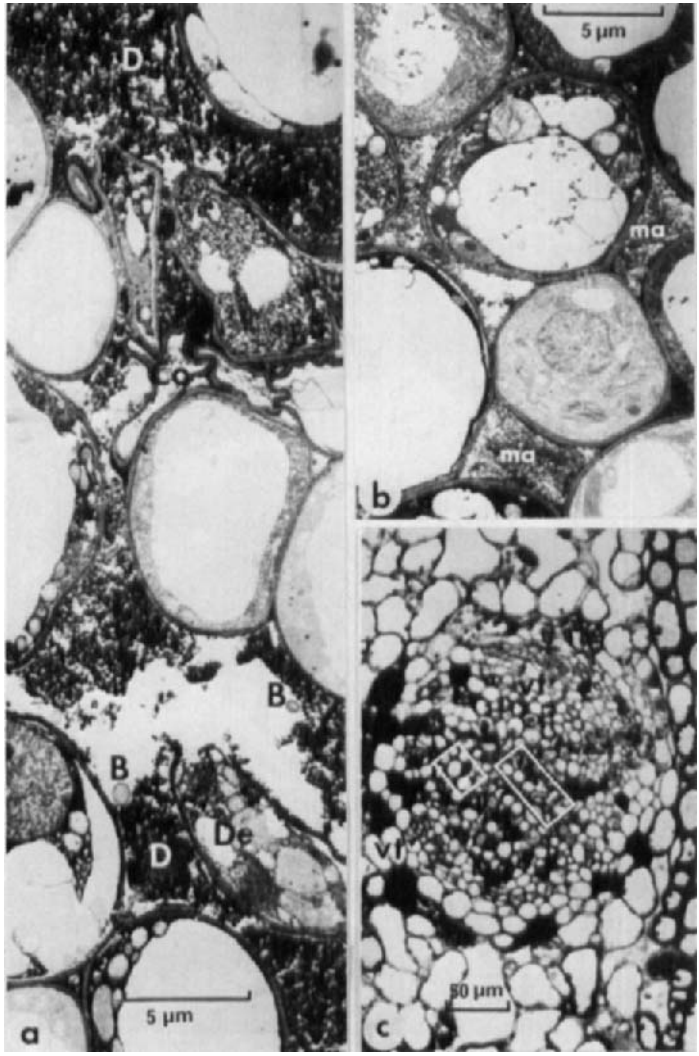

Fig. 11. (a) TEM of that centrally located part of a developing nodule similar to that enclosed by the large rectangle in Fig. 11c. Degenerating cells (De) and collapsed cells (Co) are commonplace indicating a shift to lysigenous development and cellular debris (D) is abundant in the intercellular spaces. The symbiotic bacteria (B) have become sparsely disseminated throughout the developing nodule. (b) TEM of a more peripherally located region of the developing nodule corresponding to the region delimited by the small rectangle in Fig. 11c. The cells here are not degenerating but appear healthy and apparently are secreting a granular or fibrous material (ma) into the intercellular spaces. (c) Light micrograph of a very young nodule developing within the procambial strand. The large and small boxed areas correspond to fig. 11a and b, respectively. Vascular tissue (vt) is beginning to differentiate in places around the periphery of the developing nodule.

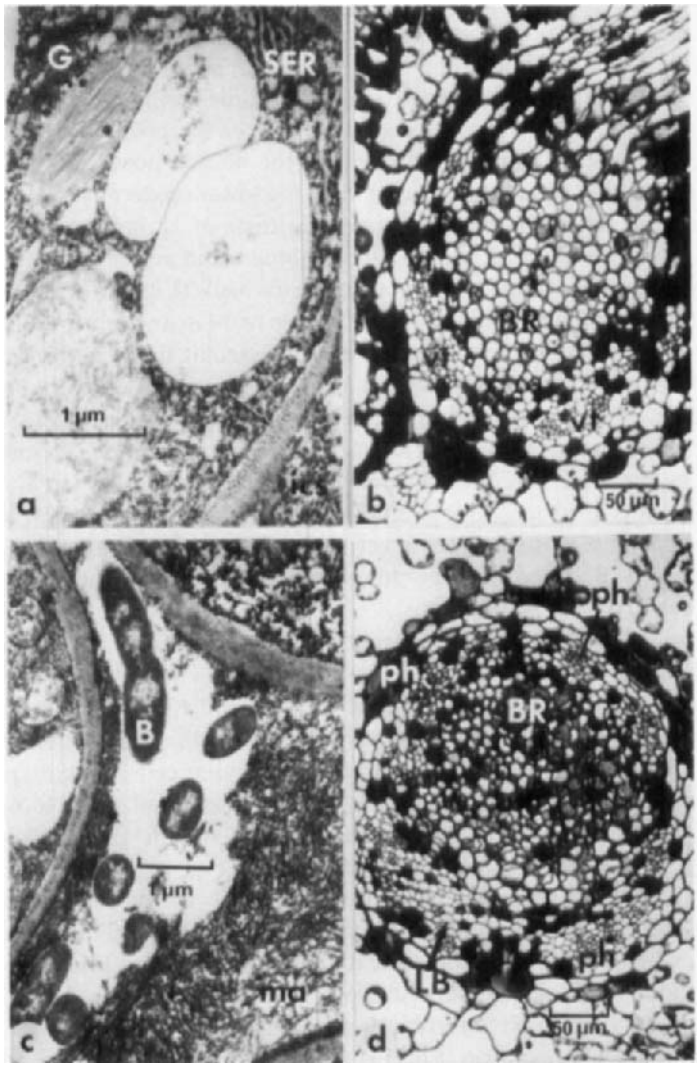

Fig. 12. (a) TEM detail of one of the peripherally placed healthy secretory cells shown in Fig. 11b. The granular/fibrous material found in the intercellular spaces (ics) can also be seen in the cell wall (arrow). These nodule cells contain well-developed smooth endoplasmic reticulum (SER) and numbers of Golgi bodies (G). (b) Transverse section through a young nodule at a mid development stage. The intercellular spaces in the bacterial region (BR) are becoming more pronounced. Discrete bundles of vascular tissue (vt) in the nodule sheath are becoming more clearly defined. Because of the slight tangential cut of this particular section it can be seen, in the upper right, that the nodule cells, circular in profile, are tubular in shape. (c) Detail of the intercellular spaces in a mid development nodule. The bacterial population (B) is increasing. The tubular nodule cells have secreted large quantites of the granular/fibrous material (ma). The zones of clarity around the bacteria are suggestive of enzymatic digestion of the material. (d) Transverse section through a young nodule at a late development stage. The intercellular spaces in the bacterial region (BR) have markedly increased and many of the tubular secretory nodule cells are losing their shape and integrity and apparently are collapsing. The vascular tissue in the nodule sheath is almost fully developed. One large main bundle (LB) forms which consists of xylem, phloem, schlerenchyma and other supportive cells while the other smaller bundles around the nodule consist mainly of phloem (ph).

ingly clear (Fig. 12d). The vascular tissue associated with the nodule consists of a large primary bundle and several smaller secondary bundles situated more or less at regular intervals around the nodule periphery. The prominent primary bundle is always located on that side of the bacterial region that is proximal to the leaf lamina and distal to the nodule pore and leaf margin; there, it is located basally, separated from the lower epidermis by only two or three cortical cells. The primary bundle consists of an innermost section of mainly xylem vessels, a central section of phloem and an outermost section of schlerenchymatous fibres and other supportive cells (Fig. 2c). The small peripheral bundles of which we have observed up to 14 in any one nodule, consist almost entirely of phloem tissue. All of this vascular tissue is embedded in a layer of flattened cortical cells which ensheath the central bacterial region of the nodule. It is interesting to note at this point that Miehe (1911) actually did see these vascular bundles which surround the nodule. As mentioned earlier, he omitted these "cambiform bundles" from his diagram and discussion. This was a rather unfortunate omission. Modern theory considers leaf nodules produce and translocate certain plant growth regulator substances (see Section V.B.2) and, as such, this peripheral vascular tissue may well have a role central to nodule function.

Mature *Ardisia* nodules are elongate structures, circular in transverse section. The boundary between the vascularized sheath and the central bacterial region is extremely sharp and distinct (Figs 2d and 13). Within the bacterial mass, few circular profiles of the tubular cells remain although there are many collapsed-looking cells. The remaining tubular cells, the collapsed cells and the cells of the nodule sheath are all connected in a loose three-dimensional network which is probably involved in the transport and exchange of metabolites to and from the nodule (Fig. 13).

During nodule development a steady increase in the amount of intercellular space within the nodule occurs. This space becomes invaded by the bacteria which are typical Gram-negative, gently curved rods some 2–5 μm in length and 0.4–0.75 μm in diameter. When the nodule is mature the bacteria assume many bizarre pleomorphic forms, a state in which they remain until their death at leaf abscission (Fig. 13). The initiation and development of leaf nodules in *Ardisia* is summarized diagrammatically in Fig. 14.

B. THE SYMBIOTIC CYCLE IN ARDISIA

In leaf nodule symbiotic systems, the endophyte is contained within, and maintained by, the host plant throughout all the stages of the host plant life cycle. Unfortunately, this rather unique feature of the symbiosis has received scant attention in the past from most workers who have concerned themselves largely with nodule anatomy and development or with the bacterial symbiont. A feature of the reproductive cycle component of this symbiosis

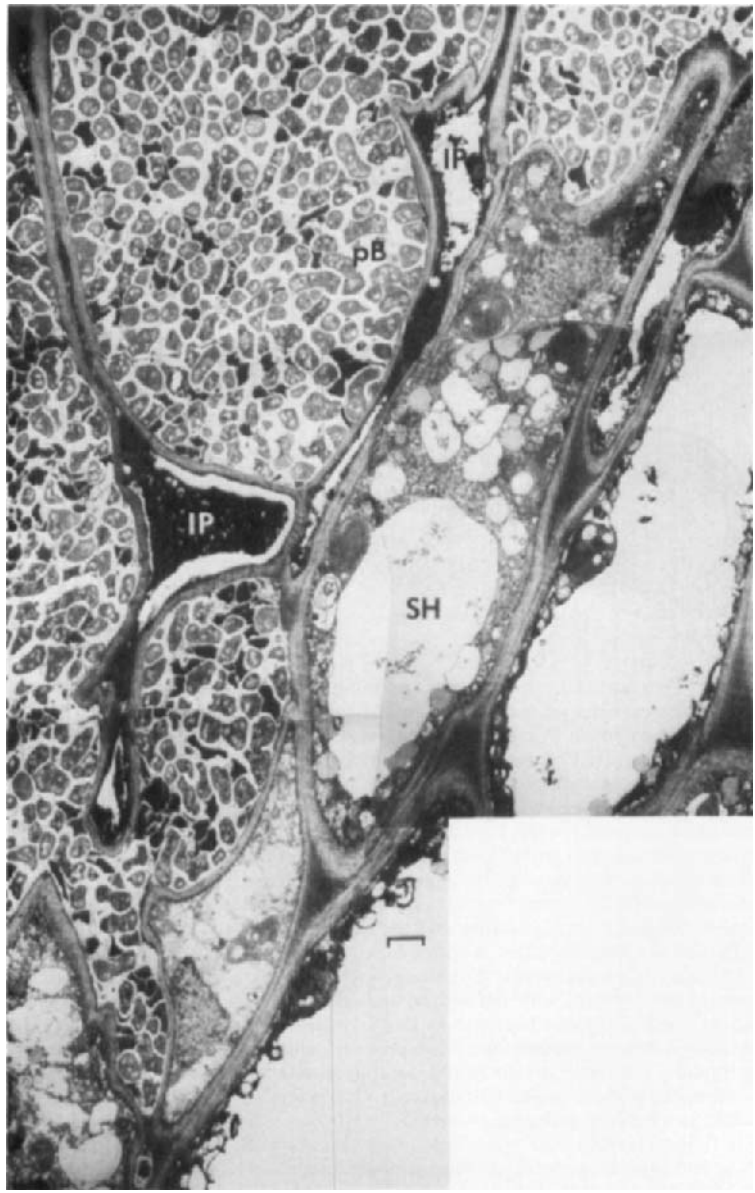

Fig. 13. Low magnification TEM photomontage of the peripheral region of a mature *Ardisia* nodule. The bacterial region, heavily populated with now pleomorphic bacterial cells (pB) is bounded by a sheath of flattened modified mesophyll cells (SH). Attached to the sheath are collapsed cells which penetrate into the nodule as invasive processes (IP). Although dead in appearance they presumably still function in the exchange of metabolites between host and bacteria (assuming that exchange is still occurring at this developmental stage). The very electron dense shapes interspersed among the bacteria, are dead and dying bacterial cells. Bar = 2 μm.

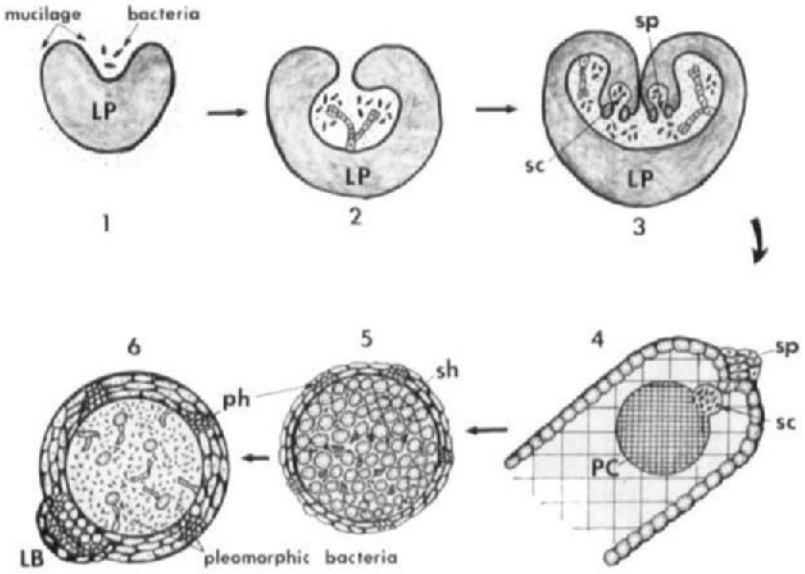

Fig. 14. Diagrammatic summary of the initiation and development of leaf nodules in *Ardisia*. 1. A crescent-shaped leaf primordium (LP) is initiated from the apical meristem. The primordium is surrounded by mucilage containing bacteria. 2. The distal lamina-forming arms of the leaf primordium (LP) grow towards each other enclosing some mucilage and bacteria. The role of maintenance of the bacteria is taken over by the primordium evidenced by the development of secretory trichomes from the adaxial protoderm of the primordium. 3. The lamina-forming arms now grow inward towards the adaxial protoderm of the primordial leaf. At the tips of these arms stomatal pores (sp) and underlying substomatal chambers (sc) develop. Some bacteria in mucilage flow through the pores into the chambers thus initiating leaf nodules. 4. Proliferation of the cells around the pore (sp) pushes the substomatal chamber (sc) deeper into the tissue of the leaf margin ensuring that the bacterial mass and the developing procambial strand (PC) are in intimate contact. A period of schizogeny of the procambial cells occurs, allowing the bacteria to disseminate sparsely throughout the procambial strand. 5. After the period of schizogenous development, a period of lysis and cell death occurs, allowing more intercellular space to be occupied by the bacteria. At this stage in development a distinct sheath (sh) of flattened cells is forming around the nodule periphery embedded in which are small developing vascular bundles composed mainly of phloem (ph). 6. At maturity the interior of the nodule is mainly composed of pleomorphic bacterial cells. Only a few live and collapsed host cells remain, presumably involved in exchange of metabolites between host and symbiont. The vascular sheath is fully developed and comprises a large bundle (LB) which is composed of xylem, phloem, sclerenchymatous fibres and other support cells, and a number of small, mainly phloem, bundles arranged around the nodule periphery embedded in the sheath.

which dogged earlier workers is the strict economy exercised by the host plant on the numbers of bacteria maintained in extranodular locations; this has severely limited the observations that could be made using the light microscope. Thus the concept of a cyclic symbiosis put forward by earlier investigators was arrived at more by phenomenological reasoning and speculation than by direct observation. The electron microscope has made direct observation more attainable; however, the complexities and intricacies of the microsymbionts' pathways through the reproductive cycles of the host plants are such that information gaps still remain.

The first suggestion that the symbiosis might be cyclic was made by Miehe (1911b) who found bacteria in the seed of *Ardisia* between the "embryo and endosperm at the radicle pole". He was unable to account for the presence of these bacteria on any ontogenetic basis and therefore speculated that this was a new and peculiar type of relationship in which the bacteria somehow pass through the reproductive organs of the host plant into the seed and from there to the next generation. Until recently the only study in which an attempt was made to test this hypothesis was that of De Jongh (1938) who studied the life cycle in *A. crispa*. He found that in lateral shoot tips, inflorescence primordia develop within a bract lined with secretory trichomes and containing bacterial mucilage. As each flower in the inflorescence developed, the carpels approached one another trapping some bacterial mucilage within the developing flower and depositing the bacterial cells in a cap-shaped mass on top of the placenta. He proposed that as the integuments came together during ovule development, some bacteria became trapped in a similar fashion to the entrapment during closure of the carpel. Thus the bacteria were inside the ovule where they could be positioned between the embryo and the endosperm near the radicle. He speculated that when the seed germinated the bacteria remained inside when the radicle and hypocotyl pushed through the micropyle; the young shoot tip then followed and, as it exited the micropyle, the "bacterial film" covered the shoot tip and axial buds of the cotyledons thus closing the cycle. The unfortunate aspect of such a phenomenological description of the transfer of the bacteria through the host plant's reproductive structures is that it is of necessity simplistic and rather inaccurate; at the ultrastructural level it can be shown that a more complex and active sequence of plant–microbe interactions occur to ensure the safe passage of the microsymbiont through the host reproductive cycle (Miller *et al.*, 1984a; Miller and Donnelly, 1987).

1. Transfer of the Symbiont to the Floral Shoot

Flowering occurs in nodulated *Ardisia* species in the second and subsequent years after the emergence of the seedling. As an integral part of each year's growth, several lateral shoots arise from the axils of the main stem. Symbiotic bacteria are maintained in all axillary meristems and, when the new lateral shoots develop from these meristems, bacteria become incorporated

into each new shoot tip by the same mechanism described for the main shoot tip (see Section II.A.1). These new lateral shoots develop three to five leaves and then become dormant; the bacteria continue to be maintained in the dormant shoot tip by secretions from the stellate trichomes. During the next growing season, the shoot tips re-activate and flower development is initiated.

The first visible event in shoot reactivation is the appearance of a new young leaf. Instead of normal development and expansion however this young leaf remains small, functioning as a protective bract for the inflorescence primordium. Like a normal leaf the adaxial surface of the bract is populated with secretory stellate trichomes; these trichomes secrete mucilage into the chamber which the rolled-up morphology of the bract creates about the shoot tip meristem. As in a normal shoot tip, this mucilage provides nutrients for the maintenance of the bacterial population. Each florescence primordium, which contains initials for up to about 30 individual flowers, develops within this chamber filled with bacteria-laden mucilage. Flowers are initiated from the primordium sequentially over a discrete time period and therefore flowers at many developmental stages may be found in any single inflorescence. Each individual primordial flower develops within a small bract. After the bract has been initiated, each individual flower begins to develop from the inflorescence promordium; the calyx develops more rapidly than the rest of the primordial flower. The calyx then completely surrounds and encloses the rest of the flower primordium, trapping some of the bacteria-containing mucilage inside as it does so. Transfer of the bacteria from the dormant shoot tip to the primordial flower is summarized diagrammatically in Fig. 15.

2. Incorporation of the Bacteria into the Reproductive Organs

Once every primordial flower has developed its enclosing calyx, the young inflorescence expands out of the bract and each individual flower expands out of its individual bract. The calyx of each individual flower has taken over the role of enclosing and supplementing the inoculum which will eventually be used to infect the ovary (Fig. 16a). The developing corolla also functions in the production of mucilage to keep the bacterial population thriving (Fig. 16a,b). Some of this bacteria-laden mucilage now becomes located adjacent to the primordial placenta, being positioned there by the movement of the developing carpel/stamen initials. As development of the flower proceeds, the outermost portion of the carpel/stamen initial cleaves off to produce the stamens while the innermost portion fuses to form the carpel (Fig. 16c). The carpel almost entirely encloses the kidney-shaped placenta except at the apex of the placenta, immediately below the base of the style, where a small conical chamber has been formed (Fig. 16d). This chamber contains the bacterial inoculum with which the ovules will become infected (Fig. 17). The most significant aspect of this stage of the journey of the bacteria through the host

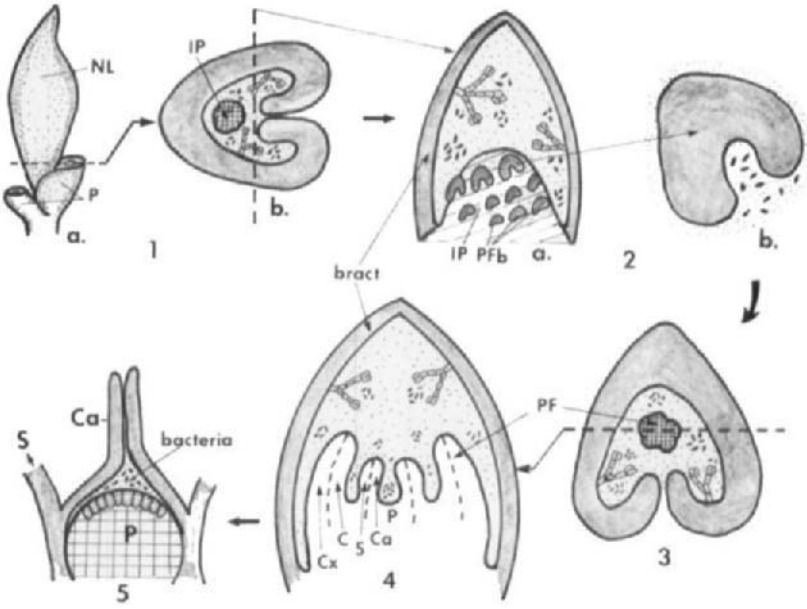

Fig. 15. Diagrammatic representation of the mechanism by which the symbiotic bacteria are transferred from the dormant vegetative shoot tip into the flowers. 1a. The dormant shoot tip is similar in structure and functional organization to any other shoot tip on the host plant (cf. Fig. 3). During the previous season, lateral shoots arose which yielded between three and five leaves. The petioles of the last two leaves are shown (P). On reactivation in the flowering season, a new leaf (NL) develops. 1b. Transverse section through the re-activated shoot tip. The new leaf (NL) does not grow into a normal leaf but remains as a small protective bract into which the inflorescence primordium (IP) grows. Trichomes on the adaxial surface secrete mucilage which maintains the bacterial colony. This bacterial mucilage bathes the inflorescence primordium. 2a. Longitudinal section through the same shoot tip. The inflorescence primordium (IP) gives rise to numerous individual flower bract primordia (PFb). 2b. Detail of the flower bract primordium. Like a true leaf primordium it entraps some bacteria and mucilage between its primordial lamina-forming arms. 3. The bract's lamina-forming arms grow together forming a chamber filled with bacteria and mucilage secreted from the trichomes arising from its adaxial surface. The true flower primordium (PF) now grows into this protected chamber. 4. Longitudinal section through the developing flower chamber represented by diagram 3. As the primordial flower develops, joint calyx/corolla (Cx, C) initials and stamen/carpel initials (S, Ca) are formed. Bacteria-filled mucilage is everywhere in the bract chamber, including between the stamen/carpel initials above the as yet undifferentiated placenta (P). 5. As the flower develops the stamen/carpel initials split apart, the outer portions becoming the stamens (S—not illustrated) and the inner portions fuse to form the complete carpel (Ca). The bacteria in mucilage which were between the stamen/carpel initials are thus trapped and deposited as a conical-shaped mass on top of the developing placenta (P).

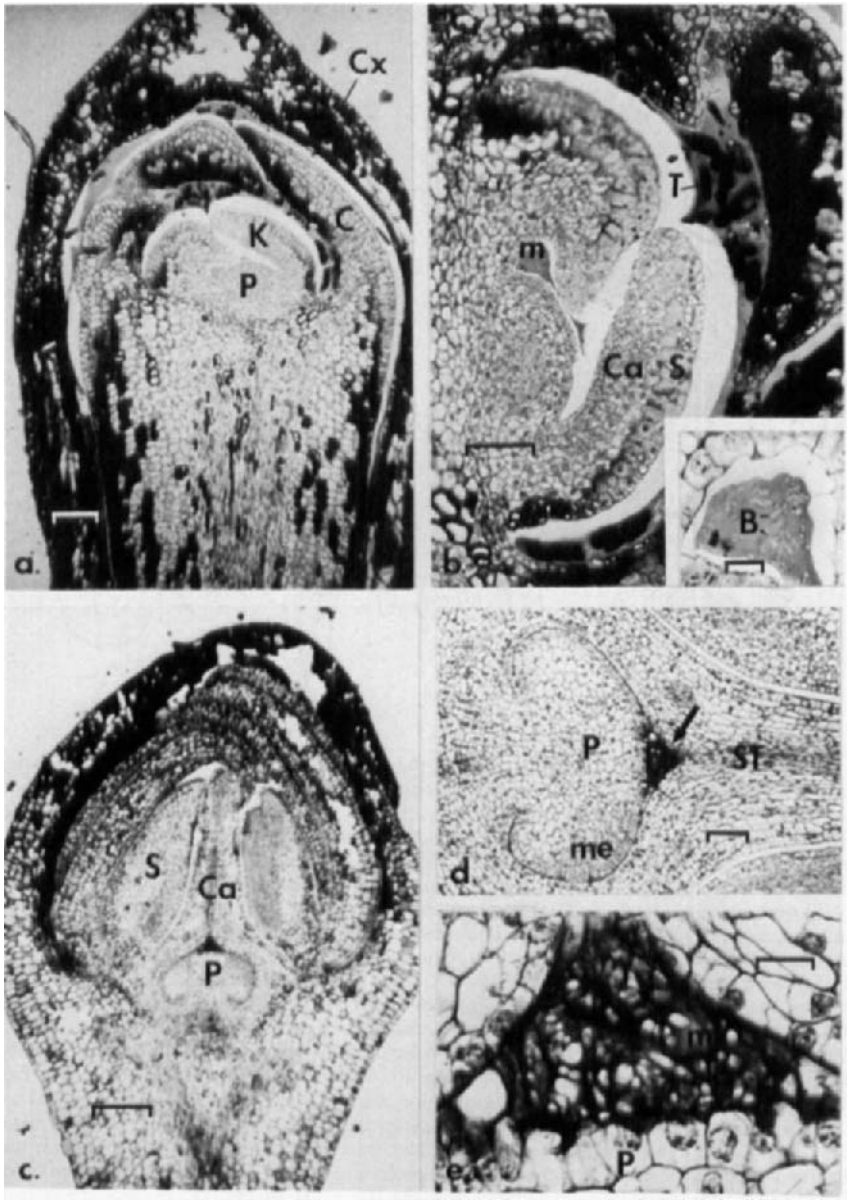

Fig. 16. (a) LM of a longitudinal section through a flower of *Ardisia crispa* at an early developmental stage. The calyx (Cx) is well developed at this early stage and encloses the young developing flower parts. Trichomes on the inner surface of the calyx and on parts of the corolla (C) supplement the supply of mucilage keeping the bacterial partner well nourished. Beneath the developing corolla lies the joint carpel/stamen primordium (K) and beneath it, the primordial placenta (P). Bar = 100 μm. (b) Detail of the central portion of the young flower shown in (a). Mucilage (M) has been enclosed on top of the primordial placenta by the development of the carpel/stamen primordium (Ca/S). Mucilage secreting trichomes (T) populate the inner surface

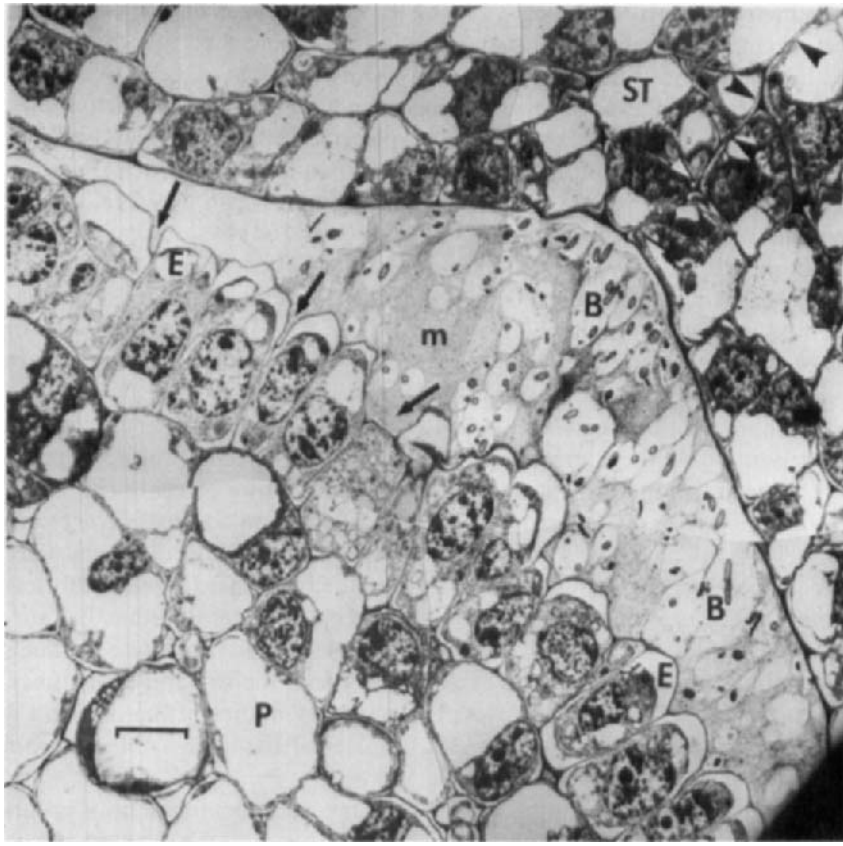

Fig. 17. Low power TEM photomontage of the conical chamber which arises by the closure and fusion of the carpel arms to form the style (ST) above the placenta (P). Where the carpel arms have fused to form the style is indicated by darts. During the enclosure some of the symbiotic bacteria (B) in mucilage (m) have become trapped in the small conical chamber. The cells of the placental epidermis (EP) have become columnar in shape and possess pronounced periplasmic spaces, a feature indicative of secretory activity. At certain points, the vertical adjoining walls between the columnar epidermal cells are beginning to detach from one another and thus forming grooves on the placental surface (arrows). Bar = 5 μm.

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of the corolla. Inset: oil immersion detail of the trapped mucilage showing the presence of bacteria (B). Bar = 50 μm; inset bar = 10 μm. (c) LM of a transverse section through an *Ardisia* flower at a more advanced development stage. The joint carpel/stamen primordium has split, the outer portion developing into stamens (S) while the inner portions fuse to form the style of the carpel (Ca). Bar = 200 μm. (d) Detail of the central region of the flower shown in (c). The carpel and placenta (P) are closely appressed except at the base of the style (ST) and apex of the placenta where a small densely staining conical chamber has been formed (arrow). The noticeably meristematic region (me) on the placenta will develop into one of the ovules. Bar = 50 μm. (e) Oil immersion detail of the small conical chamber atop the placenta (P). The unstained patches in the densely staining mucilage (m) correspond to abnormally large capsules surrounding the bacteria. Bar = 10 μm.

reproductive organs is the behaviour of the cells of the placental epidermis. They become columnar in form and develop pronounced periplasmic spaces and large electron-translucent vacuoles which can often be seen to be contiguous with the plasmalemma, features suggestive of secretory activity. In various places the vertical walls of the placental epidermal cells begin to detach from each other forming grooves on the placental surface (Fig. 17). As the flower develops and opens out, the placenta, which was totally meristematic when the bacterial cap was present (Fig. 16d) has developed up to four ovules (Fig. 18a). The placental apex is now flattened and large indentations are found on its surface (Fig. 18b). These indentations are deep mucilage-filled channels, the mucilage being derived from the epidermal cells of the placenta which are now highly secretory (Fig. 18c). The bacteria are found in association with the mouths of these channels.

The changes in the surface morphology of the placenta are very evident in Figs 19a and b. In the young placenta (Fig. 19a) the placental surface is relatively smooth, being only faintly grooved. In the mature placenta (Fig. 19b) the grooves have widened and deepened and now form a reticulate network of mucilage-filled channels which traverse the placental surface. Our studies have shown that the bacteria are always preferentially associated with these channels. The channels terminate at the margins of the placenta that surround the ovules and it is here that the mucilage produced by these channels is vented onto the surface of the ovule. From there, the bacteria-laden mucilage can easily enter the micropyle. The secretory nature of the placenta is thus a crucial element in the successful transfer of the microsymbiont from the floral axis to the ovule.

In summary, the following sequence of plant–microbe interactions ensuring infection of the ovules is suggested. After closure and fusion of the carpel, the bacteria in mucilage are placed in a small conical chamber on top of the placenta. As the flower matures, secretory grooves begin to develop on the surface of the placenta which then deepen to form a network of channels over the entire placental surface. The bacteria in the conical chamber become deposited in these channels. The mucilage secreted from the cells lining the channels flows along the channels, carrying the bacteria with it, to the placental margins surrounding the ovules. The mucilage then flows out over the surface of the ovules, physically directed toward the micropyle by the radiative pattern of the cells of the ovule surface. This infected mucilage then flows through the micropyle and into the embryo sac, thus positioning the microsymbiont in a situation where it can be incorporated into the seed.

It is at this point in the life cycle, after the bacteria have entered the micropyle, that a significant gap in our knowledge of the symbiosis arises. That is, by what mechanism is the bacterial partner placed near the epicotyl of the embryo whence it will infect the next generation of host plants? De Jongh (1938), following an earlier description of embryogeny in *Ardisia*, claims that the embryo develops from the wedge-shaped mass of cells flanked by the integuments

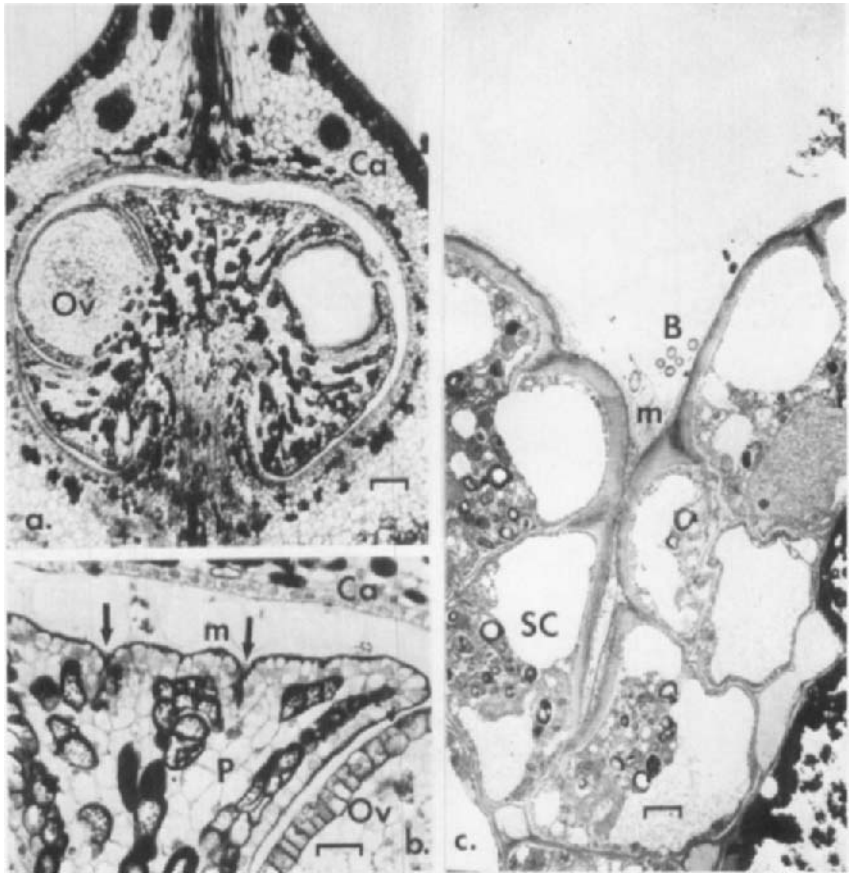

Fig. 18. Transverse section through the basal portion of the carpel (Ca) of a mature *Ardisia* flower showing the placenta (P) and ovules (Ov) within the ovary. The small conical chamber containing the bacteria is no longer evident, the base of the style and apex of the placenta being now flattened and appressed. Bar = 100 μm. (b) High magnification LM detail of the upper surface of such a mature placenta (P). The grooves which began to develop at earlier stages have now deepened and widened to become distinct channels on the apical surface of the placenta (arrows). The placental surface is separated from the carpel (Ca) by a thin layer of mucilage (m). Ovule, Ov. Bar = 20 μm. (c) TEM photomontage of one of the placental channels. They can be up to about 30 μm. deep and are filled with mucilage (m) which is produced by the surrounding secretory cells (SC). The symbiotic bacteria (B) are located in the upper portion of the channel. Bar = 2 μm.

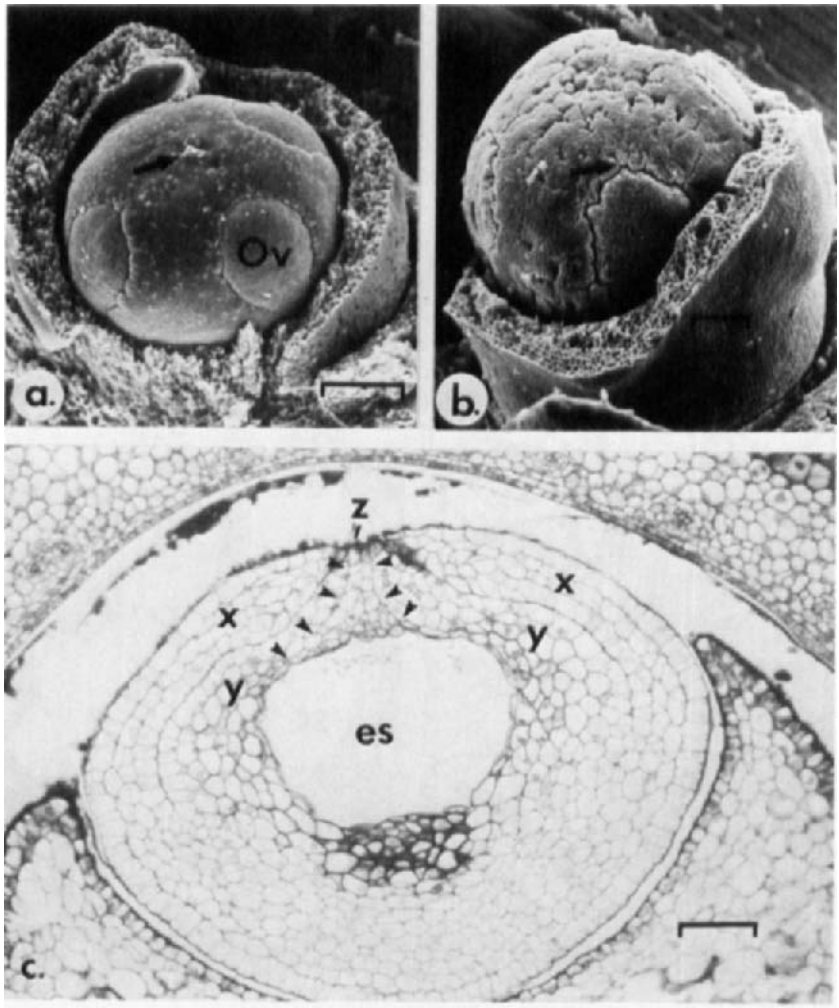

Fig. 19. SEM of the surface of the placenta from a young *Ardisia* flower. The surface is still relatively smooth and remnants of mucilage from the conical chamber are evident (arrow). Ovules (Ov) have developed within the placenta. Bar = 100 μm. (b) SEM of the surface of the placenta from a mature *Ardisia* flower. The surface is traversed by a network of deep channels which terminate at the placental margin around the ovules (arrows). The mucilage which is produced by the cells lining the channels, carries the symbiotic bacteria out from the channels, over the ovule surface and into the embryo sac through the micropyle. Bar = 100 μm. (c) LM of a transverse section through an ovule. The inner integument (y) and outer integument (x) meet at the micropyle (z) and enclose the embryo sac (es). The exact mechanism of embryo formation has not been determined; one theory suggests that the embryo develops from the wedge-shaped mass of cells (delimited by darts) which forms one flank of the inner integument, and as it does so, incorporates the symbiotic bacteria into the developing epicotyl. Bar = 200 μm.

of the ovule (see Fig. 19). However, the problem with De Jongh's account is that it places the bacteria in the wrong location for successful inoculation of the next generation as will be seen in the following section.

3. Transfer of the Symbiont to the Next Generation

If the transmission of the bacteria from one host plant generation to the next is to be fully appreciated, an understanding of the location and movement of the micro-organisms within the germinating seeds is essential. The accepted transmission mechanism, based on only one observation made by Miehe (1911) of a bacterial mass located near the radicle, was summed up by De Jongh (1938) thus:

> The bacterial film at the radicle pole seems to remain at their original location when the root pushes through. This root reaches an appreciable length before it is followed by the hypocotyledon. Later the vegetative point follows . . . the bacterial film now covers the vegetative point and the axial buds of the cotyledon. The bacterial cycle of *Ardisia* is closed.

This explanation seemed rather curious to us. It did not seem reasonable, even from a phenomenological point of view, that bacteria which are always associated with shoot tips during the life of the host plant should, in the seed, be associated with the embryonic root. The concept of the bacterial inoculum "waiting" for the vegetative shoot tip, rather than being expelled when the radicle germinated also seemed to defy physical logic. A more searching examination of the conditions in the embryo sac was therefore necessary.

The fruit of *A. crispa* is a drupe some 7–9 mm in diameter which contains a single seed 4–5 mm in diameter (Fig. 20). The dormant embryo is a small cigar-shaped structure 2–2.5 mm in length, radially placed within the seed and lying transversely to the longitudinal axis of the drupe (Fig. 20). The sac in which the embryo is located is filled with mucilage which separates the embryo from the surrounding tough, horny endosperm. The radicle of the embryo lies adjacent to the micropyle. Bacteria are found in this mucilage, not just near the radicle, but distributed throughout the entire embryo sac.

The cotyledons are small juxtaposed crescent-shaped structures. In transverse section, they enclose a small S-shaped cavity which, in common with the rest of the embryo sac, is filled with mucilage (Figs 20 and 21). Small stellate trichomes arise from the adaxial surface of the cotyledons and project into this cavity. Located in the mucilage within this inter-cotyledonary cavity is the colony of symbiotic bacteria which will infect the next generation of host plants (Figs 20 and 21).

At the beginning of germination, the radicle expands and pushes its way through the micropyle to form the primary root. During this time the cotyledons also expand and the S-shaped chamber increases in volume (Fig. 21). There now develops from the embryonic meristem at the base of, and within the S-shaped chamber between the cotyledons, the primordium of the first

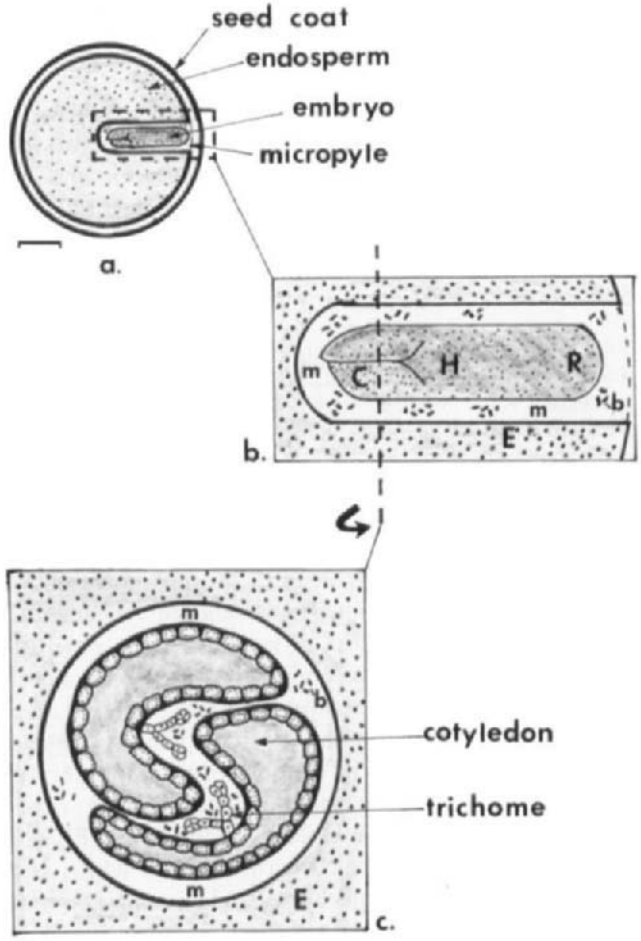

Fig. 20. Diagrammatic representation of the gross morphology and internal organization in a seed of *Ardisia*. (a) Longitudinal section through an *Ardisia* seed. Bar = 1 mm. (b) Detail of that part of (a) enclosed by the dashed line (the seed coat has been omitted). The embryo lies in an embryo sac which is completely filled with mucilage (m) containing the symbiotic bacteria (b). The embryo sac is surrounded by a tough horny endosperm (E) except at the micropyle where only a loose membrane separates the embryo sac from the seed coat. Cotyledons, C; hypocotyl, H; radicle, R. (c) Transverse section through the cotyledons of the embryo shown in (b) and rotated through 90 . The cotyledons are two small crescent-shaped primordial leaves which enclose between them an S-shaped chamber. This chamber is filled with mucilage derived from trichomes on the adaxial protoderm of the cotyledons. A thriving colony of symbiotic bacteria reside in the mucilage in the S-shaped chamber. It is these bacteria which will become the "starter culture" for the next host plant generation. The other bacteria distributed throughout the embryo sac are superfluous and will die when the seed germinates. Endosperm, E; bacteria, b; mucilage, m.

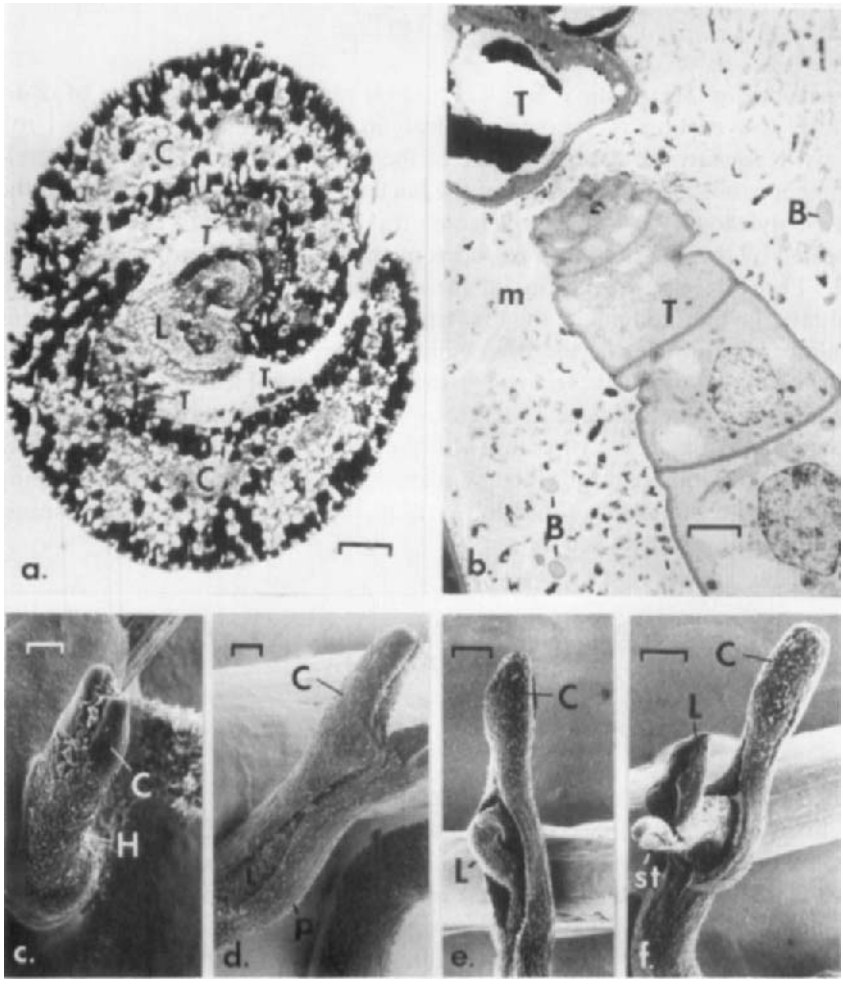

Fig. 21. (a) LM of a transverse section through an embryo of *Ardisia* at a very early stage in germination of the seed. The first true leaf (L) develops in a mucilage filled S-shaped cavity between the two cotyledons (C). This cavity is filled with bacteria-containing mucilage secreted from trichomes (T) on the adaxial surface of the cotyledons. This first true leaf of the new generation becomes infected with bacteria in the same way as all leaves in the shoot tips of mature *Ardisia* plants do (cf. Fig. 8), i.e. infected mucilage invades pores which open at the tips of the new developing leaf. Bar = 200 μm. (b) TEM of part of the S-shaped cavity showing a trichome (T), the mucilage (m) and some of the symbiotic bacteria (B). (The "scratched" appearance of this micrograph results from hardened electron dense, tannin deposits abundant in the cotyledons falling out during sectioning and damaging the cutting knife edge). Bar = 2.0 μm. (c)–(f) SEM series of germination of an *Ardisia* embryo. (c) Dormant embryo removed from the seed case. Cotyledons, C; hypocotyl, H. This embryo is at the same developmental stage as that shown in Fig. 20. Bar = 200 μm. (d) early in germination the cotyledons (C) enlarge and the cotyledonary petioles (p) elongate. Between the cotyledons the epicotyl is beginning to produce the first true leaves (L) of the new host plant. This growth causes the cotyledon petioles to part. The embryo shown in (a) is at the stage in germination shown here. Bar = 200 μm. (e) With further development, the rapidly growing epicotyl and young first leaf (L) bends and backs out of the intercotyledonary chamber through the split between the petioles of the cotyledons (C). Bar = 500 μm. (f) The entire epicotyl has now expanded out of the space between the cotyledons and their petioles. The first true leaf (L) has opened out revealing the first young shoot tip (st) of the new host plant. Bar = 500 μm.

true leaf (Fig. 21). As the young leaf primordium develops, the arms of tissue which will eventually become the leaf lamina bend backwards and roll inwards toward the adaxial surface of the growing primordium. This development causes a small amount of the bacteria-laden mucilage present in the intercotyledonary chamber to become trapped in the small chamber being created by these lamina-forming arms of the new first leaf primordium (Fig. 21). Pores develop at the tips of the arms and bacteria enter the pores to initiate the first nodules of the first true leaf of the young seedling. Into this chamber the next leaf will grow, which in turn develops a closed chamber into which the successive leaf will grow. The process of leaf initiation, growth and development and bacterial enclosure follows this pattern throughout the entire life of the new host plant (Miller *et al.*, 1984a). Thus, at a very early stage in the ontogeny of the new plant when the shoot tip is developing between the embryonic cotyledons within the seed, the symbiont is transferred to the next host generation.

III. LEAF NODULE SYMBIOSIS IN PSYCHOTRIA (RUBIACEAE)

A. INITIATION, DEVELOPMENT AND STRUCTURE OF LEAF NODULES

The presence of bacterial leaf nodules in rubiaceous plant species came to light in a manner similar to the discovery of nodules in *Ardisia*. Trimen (1894) first described the occurrence of "small knob-like excrescences" on the leaf surface of certain rubiaceous species in Ceylon. He considered this to be a valuable taxonomic feature but was quite unaware that he had discovered bacterial nodules. It was Zimmermann (1902) who first described these foliar structures in *Pavetta* species as "bacterial nodules" and who was first to study them in detail. As well as the structure of the mature nodule, Zimmermann described how bacteria entered the leaf through early-forming stomata while the leaf was still inside the bud; he did not consider the possibility that the bacteria might be maintained in the shoot tip on a permanent basis.

Von Faber (1912) studied leaf nodule development and structure in both *Pavetta* and *Psychotria* species. Although his proposed model of nodule development differed from that of Zimmermann, he agreed that nodules were initiated by bacteria entering the leaf through precocious stomata. Von Faber was the first to make the interesting observation that the nodule pores in *Psychotria* open on the abaxial leaf surface, while in *Pavetta* the pores open on the adaxial surface. The significance of this difference is not yet known, but will undoubtedly be related to variations in the structural and functional characteristics of the vegetative shoot tips between the two genera.

Like studies on *Ardisia*, study of the symbiosis in nodulated rubiaceous

hosts was limited by the power of the light microscope and therefore significant advances in our understanding of these relationships awaited the introduction of the electron microscope. Recently therefore, much has been learned concerning the initiation, development and structure of leaf nodules in *Psychotria* spp. (Lersten and Horner, 1967; Whitmoyer and Horner, 1970; Miller *et al.*, 1983b,c).

1. Morphology and Role of the Shoot Tip

As was the case in *Ardisia*, the organizational morphology and functional role of the rubiaceous shoot tip is paramount to the maintenance of the symbiotic relationship. In the shoot tip of *Psychotria*, leaf development is decussate. Each pair of new leaves is preceded in development by two pairs of fused stipules (Fig. 22). The fused stipules arch over the developing leaf primordia, thus protecting them in an enclosed chamber. Arising from the adaxial surface of these stipules are some large simple trichomes and numbers of brush-like dendroid colleters (Fig. 22b,d). The arrangement of these colleters can be seen in the scanning electron micrograph of the adaxial surface of a fused stipule pair shown in Fig. 22c. In the shoot tip of *Psychotria*, the adaxial surfaces of the stipules lie in close contact with the abaxial surface of the developing leaf; a thin expanse of curved connecting tissue provides a hollow between the stipules into which the main vein of the young leaf fits.

The dendroid colleters are multiseriate and multicellular and secrete mucilage which bathes the developing leaves and fills the entire shoot tip. The long branch cells of the colleters contain masses of Golgi bodies and extensive sheets of rough endoplasmic reticulum, suggesting that the mucilage is of a protein/carbohydrate type (Fig. 23). That the mucilage is used as a source of nourishment for the bacteria and as a vehicle for their successful infection of the leaf was first suggested by Horner and Lersten (1968). Evidence for this suggestion has been gathered by Lersten (1974a,b, 1975) who has shown that most nodulated rubiaceous species possess secretory dendroid colleters whereas virtually all non-nodulated species possess only standard trichomes.

2. Initiation, Development and Structure of Leaf Nodules

In each shoot tip, the abaxial surfaces of the laminae of each leaf are bathed in bacteria-containing mucilage. At a very early stage in leaf development, a number of stomatal-like pores are initiated, apparently at random, in the epidermis of the leaf lamina (Fig. 24). Some of the mucilage, containing a few cells of the symbiotic bacteria, flows through each pore into each substomatal chamber. Rubiaceous leaf nodules have thus been initiated. Some of the cells which surround the pore are now stimulated to divide periclinally (Fig. 24). With continued leaf growth, anticlinal divisions now occur which push the guard cells and the bacteria-filled sub-stomatal chamber deeper into the developing leaf lamina (Fig. 24). This first small nodule is some 45 μm in diameter and is already crammed full of bacteria. The early developmental

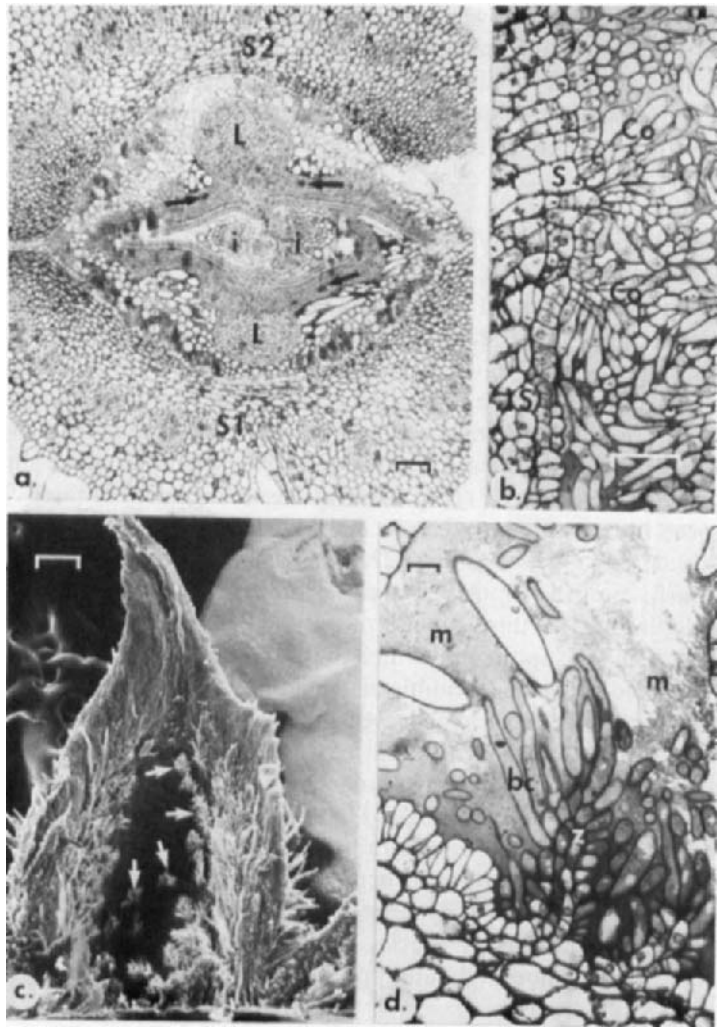

Fig. 22. (a) Transverse section through a vegetative shoot tip of *Psychotria kirkii*. Two pairs of fused stipules (S1,S2) enclose a young pair of leaves (L). The inner surfaces of the stipules are heavily populated by large simple trichomes and numerous dendroid colleters. Circular and elongate profiles of the simple trichomes are noticeable but the much smaller profiles of the colleter cells are not discernible at this low magnification. The densely stained material interspersed among the trichome profiles is the mucilage secreted from the colleters. Located between the two young leaves are the initials (i) for the next pairs of leaves and stipules. Several young nodules are visible in the developing laminae of the young leaves (arrows). Bar = 200 μm. (b) Detail of some young dendroid colleters (Co) arising from the adaxial epidermis of a stipule (S). Bar = 50 μm. (c) SEM of the adaxial surface of one fused pair of stipules where numerous brush-like colleters (arrows) can be seen. Bar = 500 μm. (d) LM of a longitudinal section through a mature dendroid colleter. Colleters are multicellular and multiseriate. They consist of a two to four cell thick stalk (Z) from which radiate numerous elongate branch cells (bc). These colleters secrete the surrounding mucilage (m) in which the shoot tip's bacterial colony is maintained. Bar = 20 μm.

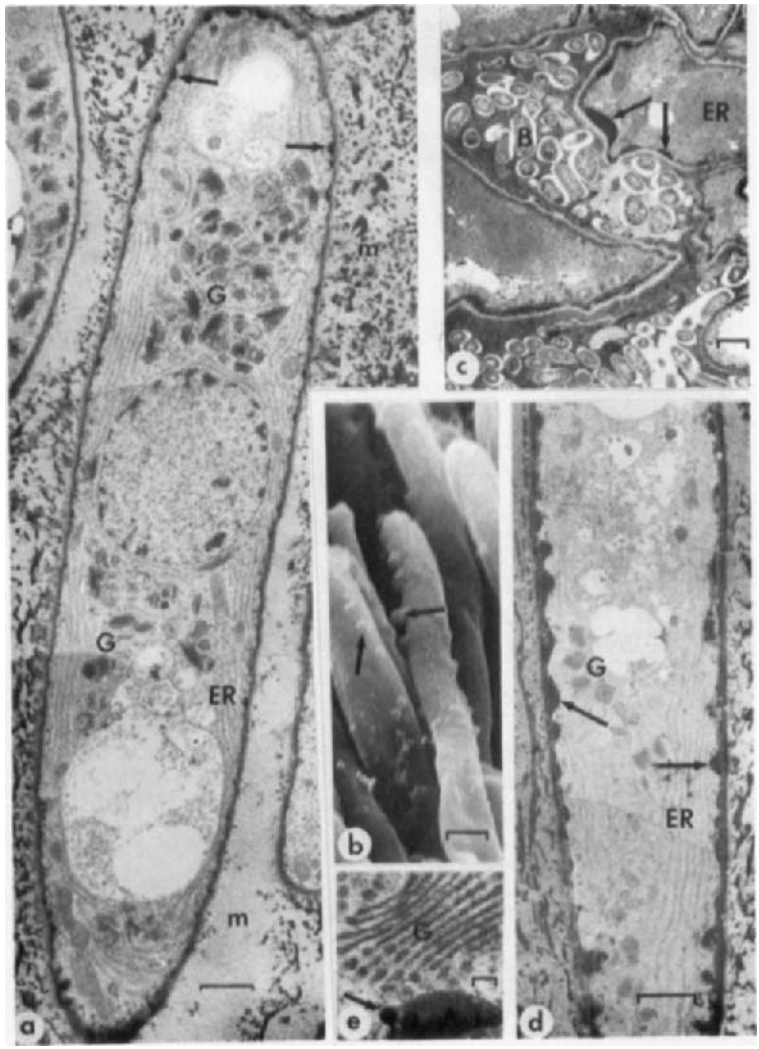

Fig. 23. (a) TEM photomontage of a branch cell from a dendroid colleter of *Psychotria kirkii*. These cells contain large numbers of Golgi bodies (G) and sheets of rough endoplasmic reticulum (ER) perinuclearly and in the peripheral cytoplasm. Electron dense blebs of material build up between the cell wall and the plasmalemma for eventual export from the cell (arrows). The mucilage (m) in the shoot tip chamber has a very heterogeneous appearance (electron density) ranging from black to light grey. Bar = 2 μm. (b) SEM of some colleter branch cells. The blebs of accumulated material are seen here in the process of being exported from the cell (arrows). Bar = 5 μm. (c) Colleter cells from *Psychotria punctata*. The colleter cells from this species are characterized by large ovoid stacks of rough endoplasmic reticulum (ER). Again large accumulations of electron dense material build up behind the cell wall for export from the cell (arrows). Numerous bacteria (B) can be seen in the extracellular mucilage. Bar = 1 μm. (d) TEM photomontage of a similar colleter branch cell to that shown in (a) but this time stained by a carbohydrate specific PA-TSC-SP procedure. The ground cytoplasm, endoplasmic reticulum (ER) and other cellular organelles have not stained appreciably. However the Golgi apparatus (G) and the periplasmic accumulations of material (arrows) have stained well as has some of the extracellular mucilage. It would seem that the mucilage is a carbohydrate/protein type, the components of each perhaps being secreted separately, hence the heterogeneous appearance in electron density found both in routinely stained and specifically stained sections. Bar = 2 μm. (e) Detail of a Golgi body (G) close to the plasmalemma of a colleter branch cell. Golgi derived vesicles (arrow) can be seen fusing directly with the accumulations of material (A) that build up in the periplasmic space beneath the cell wall. Bar = 0.1 μm.

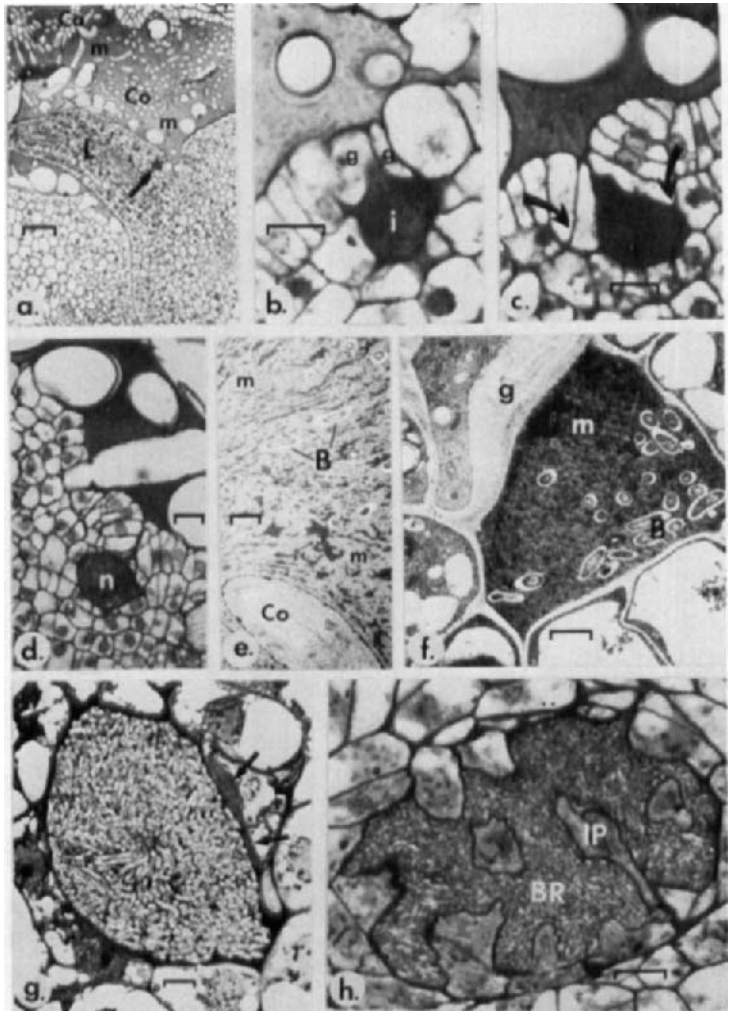

Fig. 24. Early stages in the initiation and development of leaf nodules in *Psychotria*. (a). In the shoot tip the lamina (L) of each young leaf is bathed in mucilage (m) secreted from the colleters (Co) which populate the adaxial surface of the surrounding protective stipules. In the protoderm of the young leaf lamina early forming stomata occur and some of the mucilage containing bacteria flows into the sub-stomatal chamber thus initiating a leaf nodule (arrow). Bar = 50 μm. (b) High magnification LM of the newly initiated nodule arrowed in (a) showing the stomatal guard cells (g) and sub-stomatal chamber containing the bacterial inoculum (i). Bar = 10 μm. (c) After entry of the infected mucilage the epidermis surrounding the stomatal pore undergoes periclinal division, pushing the sub-stomatal chamber deeper into the leaf tissue (arrows). Bar = 10 μm. (d) Anticlinal divisions of these cells then occurs which further pushes the young nodule (n) even deeper into the leaf tissue and effectively cutting it off from the external environment. Bar = 10 μm. (e) TEM of the mucilage (m) in the shoot tip showing the symbiotic bacteria (B). The concentration of bacterial cells in the shoot tip mucilage is generally rather low. However, at those points where early forming stomata occur in the young leaf epidermis, the local concentration of bacteria is relatively high, suggesting that the signal which induces the formation of the stoma originates from the bacteria when a "threshold" colony size is reached. Colleter cell, Co. Bar = 2.0 μm. (f) TEM of the serial section of the newly initiated nodule shown in (b). After entry into the substomatal chamber the mucilage (m) always appears more dense, both with LM and TEM stains. This is probably due to water being absorbed from the mucilage by the surrounding plant cells, thus effectively concentrating the mucilage. Note that one of the cells lining the young nodule is a guard cell (g) evidenced by its thickened cell wall. Bacteria, B. Bar = 2.0 μm. (g) TEM of the serial section of the young nodule shown in (d). The symbiotic bacteria have increased markedly in number, and now the enlarged sub-stomatal chamber is almost completely full of the microorganisms. Even although the young nodule has been pushed deep into the leaf tissue, the guard cells are still associated with the nodule, again evidenced by their thick cell walls (arrow). Bar = 2.0 μm. (h) High magnification LM of a young nodule which has commenced schizogenous development. Schizogenous development is described in the legend to Fig. 25. Bacterial region, BR; invasive process, IP. Bar = 10 μm.

events which have occurred up till now have taken place within the enclosed chamber-like shoot tip formed by the protective fused pair of stipules. Further growth makes the young leaves expand out of the shoot tip, pushing the stipules apart.

Growth and development of the nodule is now closely linked with growth and development of the leaf. A procambial strand develops near each young nodule, and as nodule development begins a sheath of somewhat flattened mesophyll cells forms around the periphery. The bacterial population continues to expand and this expansion pressure causes some of the cells which line the young nodule (which in the very beginning are cells lining the substomatal chambers) to be split apart from each other along their middle lamellae (Fig. 25). Whether this splitting is caused purely by physical expansion of the bacterial population or whether some enzymatic digestion is involved as well is not yet known.

Some of the innermost cells of the sheath appear to project into the young bacterial mass. This is due to the splitting or schizogeny, not only of the adjoining cells of the innermost layer, but of the cells of the innermost layer and the layer behind it. As the bacterial population expands into the intercellular spaces thus created, strings of cells of the innermost layer swing out into the bacterial mass to become an invasive process. Once the innermost layer has been "shaved off" the second layer is now open to attack and so some of the adjoining cells in that layer then become split apart. This process of nodule growth and expansion occurs in close concert with growth and expansion of the leaf; indeed, leaf development does not take place without the bacterial partner. It is at this stage in development that the crucial input from the bacteria to the host seems to occur. On its own the host plant is unable to proceed with normal cell division and differentiation; the bacteria somehow harness the host plant's gene expression and regulation, allowing the leaf to develop as the nodule develops. It has been shown that during this very early stage in nodule development, intense levels of RNA build up in the bacterial mass (Whitmoyer and Horner, 1970) indicating a high level of bacterial activity. While a proportion of this RNA will be mRNAs associated with bacterial growth and division, the mRNAs which are involved in the production of the growth regulation substances will also be present. This should provide a convenient point to begin the study of bacterial function with respect to the symbiosis using modern molecular techniques.

As both leaf and nodule continue to grow, this schizogenous cleavage process of the innermost cell layer continues, the sheath layers being continually renewed by divisions within the outer layers of the sheath itself. Although leaf growth and development is being dictated by the bacteria, final cell size and number is presumably a set parameter of the host plant genome; therefore when host plant cell division stops, so too does nodule growth.

When mature, rubiaceous leaf nodules are flattened spherical or cylindrical structures ranging from 500 to 2000 μm in diameter and, depending on genus

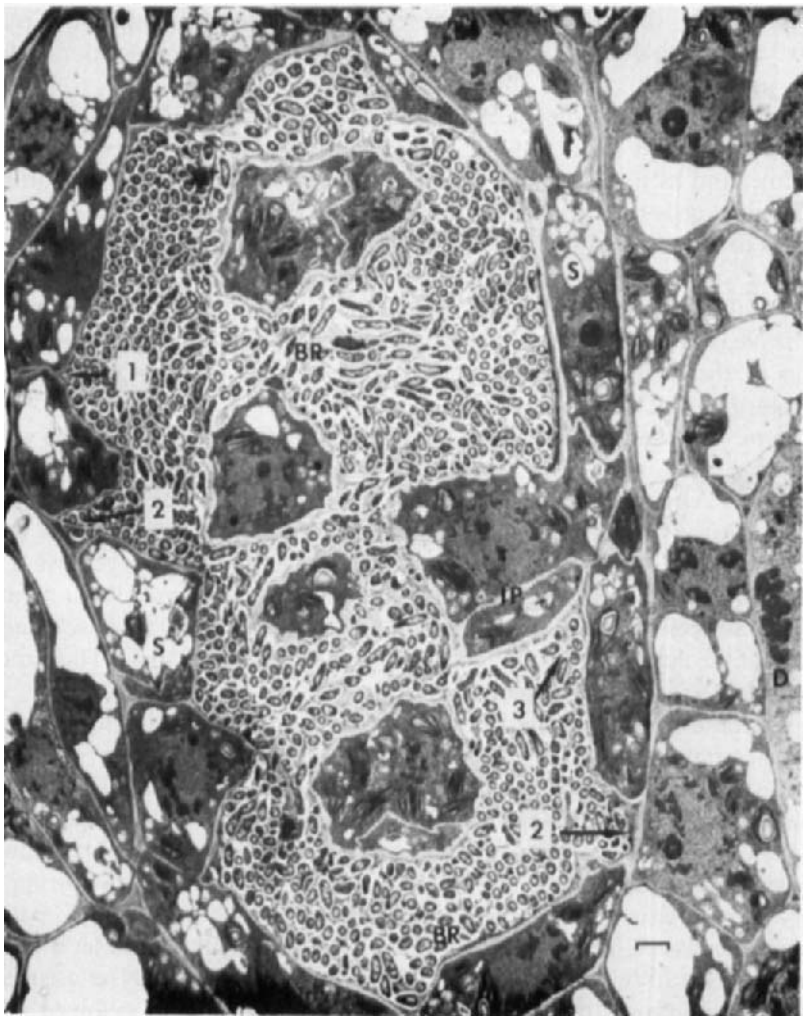

Fig. 25. TEM photomontage of a young nodule of *Psychotria kirkii* at a very early developmental stage. The bacterial region (BR) is surrounded by a sheath of flattened mesophyll cells (S). The invasive processes (IP) which project into the bacterial mass come about by the schizogeny of the cells of the innermost layer of the sheath. The schizogeny occurs in three steps. Step 1: Adjoining cells of the innermost layer of the sheath become loosened and split apart along their joint middle lamella. Step 2: Bacterial cells enter the split between the cells, perhaps by the pressure of the rapidly expanding bacterial population, and push the adjoining cells apart. Step 3: Loosening of the walls between the innermost and second layers of sheath cells now occurs. Again bacterial cells expand into the split between the two layers of cells causing a string of innermost cells to "swing out" into the bacterial mass thus creating an invasive process (IP). Cell divisions (D) in the outermost layers of the sheath ensure that the developing nodule has a continuous supply of sheath cells for use in the schizogenous process. Bar = 2 μm.

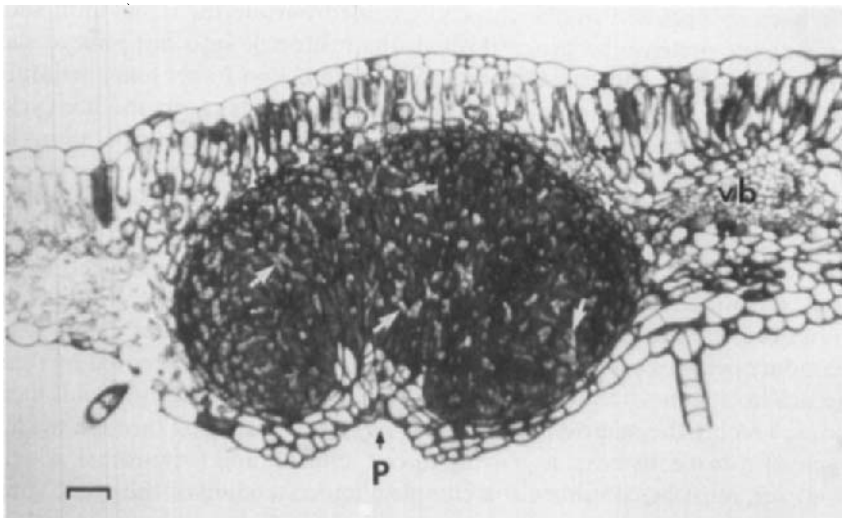

Fig. 26. Fully grown and mature *Psychotria* nodules are kidney-shaped in median transverse section through the pore region (P) which is the site of the original stoma where the infection event which led to this nodule occurred. Each mature nodule is supplied by a vascular bundle (vb). The schizogenous developmental process has given rise to a three dimensional, interconnected and anastomosing reticulum of cells (arrows) which act as the surface over which exchange of metabolites between the microsymbiont and the host plant takes place. Bar = 100 μm.

and species, located apparently at random on the lamina, midrib or petiole (von Faber, 1912, 1914; Van Hove, 1972; Van Hove and Kagoyre, 1974; Lersten and Horner, 1976). A three-dimensional network of invasive mesophyll pervades the bacterial region and this cellular network acts as an interface over which metabolites are exchanged between the symbiotic partners (Fig. 26).

B. THE SYMBIOTIC CYCLE

The first study in which an attempt was made to follow the microsymbiont through the reproductive cycle of a nodulated rubiaceous host species was that of von Faber (1912). He examined the flowers of the host plant *Pavetta zimmermanniana* at various developmental stages. He found that the primordial inflorescence develops in a mucilage-filled chamber delimited by the stipules. As successive floral organs are initiated, the developing flower becomes dish-shaped and some bacteria in mucilage become passively trapped in each flower as the carpels close. Bacterial cells trapped within the flower in this way are very few in number. Von Faber also found bacteria in the seed between embryo and endosperm, and he reasoned that somehow the micro-organisms must enter the ovule during flowering. He speculated that

the bacteria enclosed by the carpels remained outside the ovule until such time as the pollen tube broke through the micropyle into the embryo sac, thus allowing the bacteria access to this location. Von Faber had great difficulty in detecting bacteria at this most crucial of stages in the life cycle. Apparently, after much searching through a large number of preparations he found some bacteria on the micropyle of only two ovules. His observations were enough to convince von Faber that the symbiosis in *Pavetta* could be described as "hereditary".

Even with the enhanced power of the electron microscope, detecting the bacteria in the reproductive stages of rubiaceous hosts still remains difficult. It has become clear to the present author, as it did to von Faber, that a strict economy perhaps even greater to that found in *Ardisia*, is exercised by rubiaceous host plants on the size of bacterial populations in extra-nodular locations. Tracing the microsymbiont at the ultrastructural level through the life cycle of rubiaceous hosts is proving to be a difficult and tedious task which, however, must be continued if a complete understanding of the cyclic component of this relationship is to be understood.

1. The Distribution of Bacteria During Flowering

During the first year of growth, young *Psychotria* host plants develop zero to several lateral shoots, and these, as well as the main shoot, produce pairs of leaves in a decussate manner on a continuous basis (Fig. 22a). Bacteria are maintained in all of these shoot tips in mucilage secreted from dendroid colleters. During the second year of growth, flower development is initiated. Each inflorescence arises by the differentiation of an axillary bud (Fig. 27). Initiation of the inflorescence primordium from the axillary bud occurs at a very early stage in the development of the leaf whose axillary bud is to become that inflorescence; at the point when the inflorescence is initiated that leaf is still one of a pair enclosed by the shoot tip chamber formed by two pairs of fused stipules (Fig. 27). Prior to the initiation of the inflorescence, two pairs of fused stipules, or, more correctly now fused bracts, develop which form a small chamber inside which the primordial inflorescence will grow. As the bracts enlarge and arch over the axillary bud, they enclose some of the mucilage containing a few bacterial cells; dendroid colleters arise from the adaxial surface of the bracts, taking over the role of bacterial maintenance from the colleters of the vegetative shoot tip.

The inflorescence initiates as an elongate stalk of cells which arises from the meristem and grows into the chamber formed by the bracts (Fig. 28a). Each flower initiates from this meristematic stalk as a raised, almost spherical bleb of cells. This bleb begins to flatten as the calyx begins to develop; at this early stage the calyx develops secretory dendroid colleters at its apex (Fig. 28b). Secretions from these colleters undoubtedly assist in the transfer of the bacteria into the developing flower. Further development of the flower sees initiation and growth of the stamens and the carpel arms (Fig. 28c). The

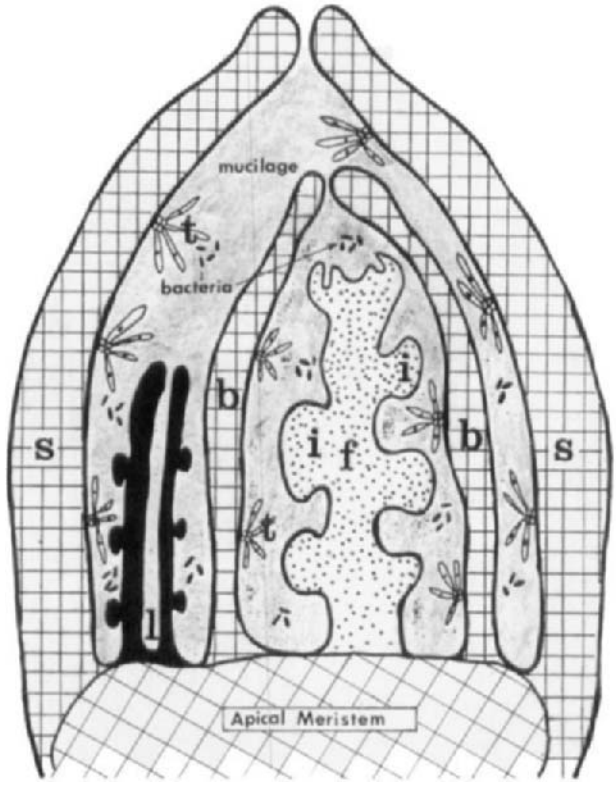

Fig. 27. Diagrammatic representation of floral initiation in a vegetative shoot tip of *Psychotria*. In the shoot tip, a pair of leaves (l) has developed normally from the apical meristem and are growing in the mucilage-filled apical chamber formed by the protective pairs of fused stipules (S). When the signal for flowering is received by such a shoot tip, that part of the apical meristem which was about to be set aside as the axillary bud of one of the developing leaves, begins to initiate an inflorescence primordium (f). As at the beginning of regular leaf development, the first organs to grow during development of the inflorescence are the protective stipules, or more properly now, protective bracts. Like stipules, these protective bracts are lined with secretory dendroid colleters (t). As the bracts develop from the axillary bud initial, they enclose some bacteria-filled mucilage from the vegetative shoot tip chamber. By the time the bracts have developed and arched over to form a protective chamber above the inflorescence primordium, the bract colleters have taken over the role of maintenance of the colony of symbiotic bacteria. The primordial inflorescence grows as an elongate stalk of cells into this mucilage-filled chamber. Each flower initiates from this primordial inflorescence as a roughly spherical bleb of cells (i).

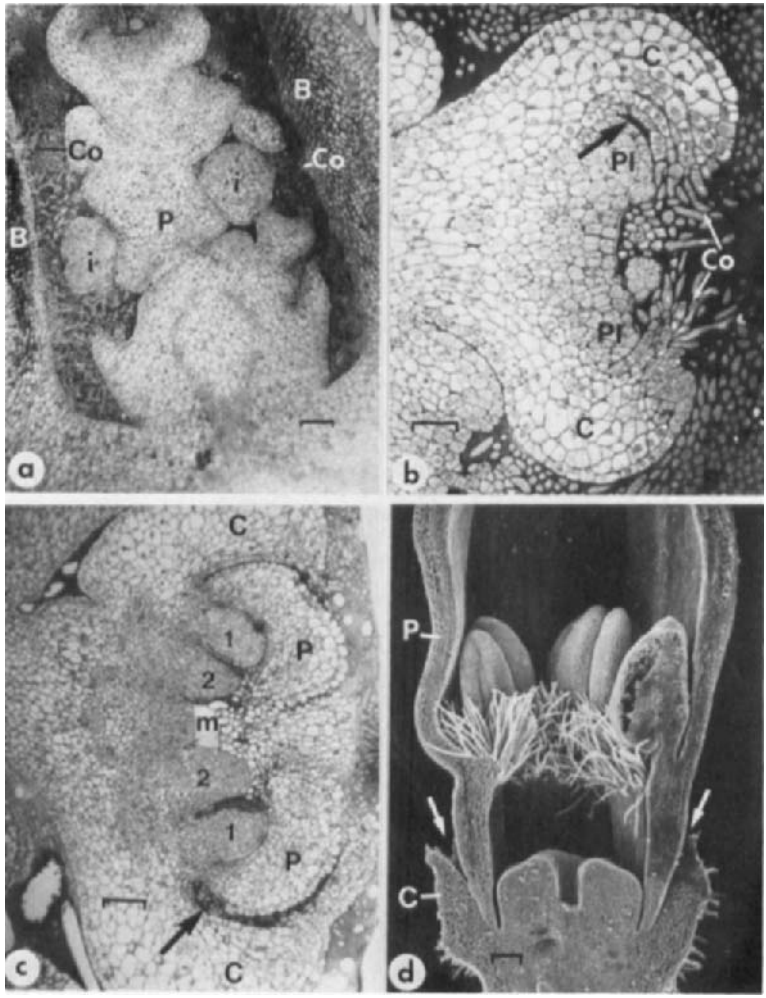

Fig. 28. (a) Low power longitudinal section through a shoot tip at the commencement of flowering showing the inflorescence primordium (P) in the mucilage filled chamber formed by the surrounding bracts (B) (cf. Fig. 27). The densely staining mucilage in the chamber is derived from the many dendroid colleters (Co) lining the bracts. The flower initials (i) are more or less spherical blebs of cells arising from the stalk of the inflorescence primordium. The most highly developed young flower is at the top of the stalk. Bar = 200 μm. (b) Detail of the primordial flower at the top of the inflorescence stalk in (a). As each initial beb starts to develop into a flower it flattens and the calyx (C) begins to develop rapidly. Even at this early stage dendroid colleters have arisen from the protoderm at the tips of the developing calyx. The corolla initials are just evident at this stage (PI). Some bacterial mucilage becomes trapped between the initials of the calyx and corolla (arrow). Bar = 100 μm. (c) With further development, the calyx (C) and corolla (P) are now well advanced. The stamens have been initiated (1) as has the carpel (2). A small amount of mucilage (m) has been deposited in the space above the as yet undifferentiated placenta. A considerable amount of bacterial-laden mucilage is found between the corolla and calyx (arrow). This infected mucilage leads to the formation of calyx nodules. Bar = 100 μm. (d) Scanning micrograph of a well developed but as yet unopened *Psychotria* flower. The calyx (C) remains relatively small in comparison to the corolla (P). Calyx nodules occur on the upper, inner side of the calyx at the base of the corolla (arrows). Bar = 200 μm.

calyx and corrolla grow considerably at first and arch over the developing stamens and carpel (Fig. 28c). A small amount of mucilage becomes trapped between the carpel arms immediately above the developing placenta. Some of the mucilage is trapped between the calyx and base of the corolla (Fig. 28d). Since the calyx is a modified leaf it can give rise to stomatal pores. Some bacteria-laden mucilage enters through these pores early in calyx development and calyx nodules are initiated. Nodules of the calyx have been reported for *Pavetta* species (Zimmermann, 1902) and for *Psychotria* species (Miller *et al.*, 1984b). The function or significance of calyx nodulation is not understood at present.

This is as far as this author has been able to trace the microsymbiont through the life cycle of *Psychotria*. Much work is still required to clarify the mechanism whereby the symbiont is transferred into the embryo sac. Von Faber (1912) speculated that when the mature flower was pollinated, the bacteria which were sitting on the micropyle (and the difficulty von Faber had in finding these must be remembered), were pushed into the embryo sac by the advancing pollen tube. Miehe (1911) had proposed a similar mechanism for the entry of the bacteria into the ovules in *Ardisia*. Unfortunately Miehe was unaware of the work of Jaensch (1905) who had shown that embryo development in *Ardisia* was apogamous; pollen germination is absent and apomictic embryos develop from the wedge-shaped mass of cells on the inner integuement of the ovule (see Fig. 19). As such, transfer of the bacteria into the ovule by a pollen tube would therefore be impossible in *Ardisia*. It was shown in Section II.B.2 that transfer of the microsymbiont into the *Ardisia* ovule is effected by a flow of infected mucilage from the secretory placenta. It is not yet known whether pollen germination occurs in nodulated rubiaceous hosts, whether they are capable of apomictic embryo formation or whether some other mechanism, such as placental secretion, is involved in the transfer of the symbiont into the embryo sac.

2. *The Seed*

The fruit of *Psychotria* is a subglobose drupe up to 9 or 10 mm in diameter; each drupe contains two semiglobose seeds 4–5 mm in diameter and 3–4 mm thick. The embryo is a small elongate structure 2–2.5 mm in length radially placed, more or less in the dorsal periphery at the commissural face of the seed (Fig. 29a,b). It lies within a small embryo sac separated from the horny endosperm by a layer of mucilage. At the base of the adaxial surfaces of the cotyledons, which comprise about half the length of the embryo, there is a small spherical to ovoid chamber (Fig. 29c). In *Ardisia*, a similar intercotyledonary chamber contained secretory trichomes, mucilage and the symbiotic bacteria. In the *Psychotria* embryo, the chamber contains mucilage but, so far, and after serially sectioning six embryo sacs, we have never been able to detect any bacteria in this location. This could be due to an unlucky selection from the host plant of six naturally occurring bacteria-free seeds or, and

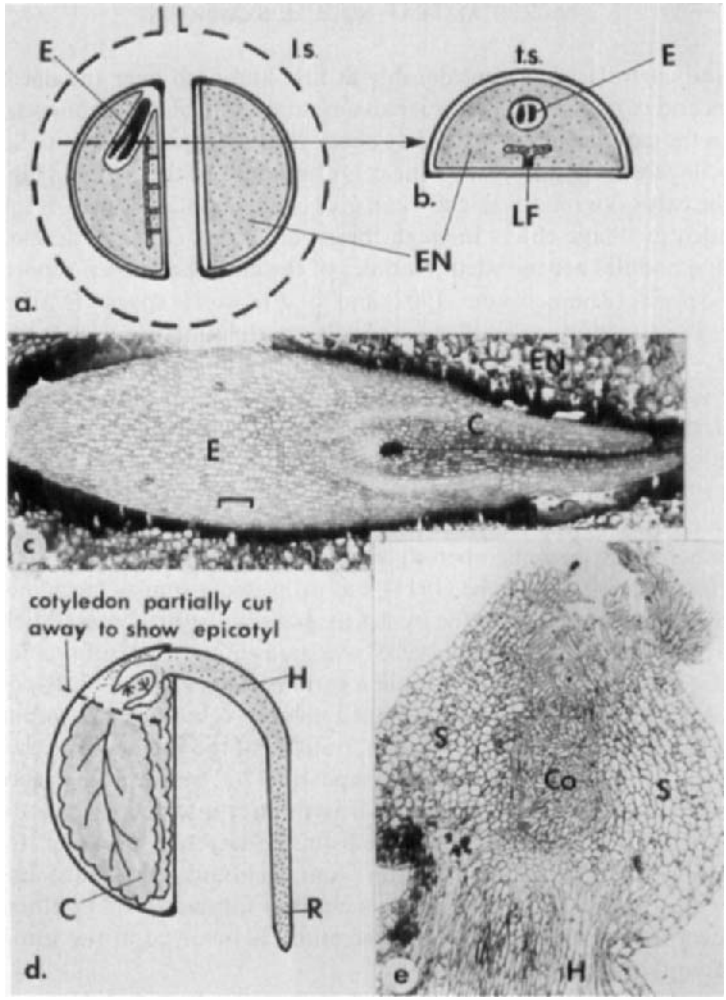

Fig. 29. (a) Diagrammatic representation of a longitudinal section through a sub-globose drupe of *Psychotria* showing the two semi-globose seeds which it contains. The embryo (E) is radially placed more or less in the dorsal periphery of the commissural face of the seed. It lies in a small mucilage filled embryo sac, surrounded by a tough horny endosperm (EN). (b) Transverse section through one seed at the point indicated by the dashed line in (a), showing the embryo (E) surrounded by endosperm (EN). A feature of the *Psychotria* seed which is of unknown significance with respect to the symbiosis is the presence of a longitudinal fissure (LF) in the endosperm arising from the internal commissural face of the seed. (c) Light micrograph of a *Psychotria* embryo (E). It is separated from the endosperm by a densely staining layer of mucilage. At the base of the cotyledons (C) there is a small ovoid mucilage filled cavity. Searches to date however have failed to reveal bacteria in this location. Bar = 100 μm. (d) Diagrammatic representation of a germinating *Psychotria* seed showing the root-forming radicle (R), the expanded hypocotyl (H) and the well developed cotyledons (C). In between the cotyledons the epicotyl has developed. The epicotyl consists of the first two pairs of fused stipules, the adaxial surface of which is populated with secretory dendroid colleters. (e) Light micrograph of such an epicotyl as shown in (d). The hypocotyl (H) is surmounted by two pairs of fused stipules (S). The shoot tip chamber, in which the first true leaves of this next generation of the host plant will grow, is filled with mucilage and secretory dendroid colleters (Co). This is the first stage in seed germination that this author has been able to detect the presence of the symbiotic bacteria.

more likely, the bacterial inoculum for infection of the next generation of *Psychotria* plants is located elsewhere in the seed. There is a longitudinal fissure arising from the internal commissural face of the seed (Fig. 29a,b). Perhaps the microsymbiont is to be found in this location and therefore this structure should be examined for the presence of bacteria.

Germination in *Psychotria* is epigeal. At the beginning of germination the radicle grows out to form the primary root. The hypocotyl increases in diameter and the cotyledons begin to develop and expand as the endosperm is utilized. Growth of the hypocotyl continues, elongating greatly to form the stem of the young seedling and at the same time bringing the seed case with the cotyledons still enclosed above ground. While the cotyledons develop inside the seed case the epicotyl develops between them (Fig. 29d). The first two pairs of fused stipules arise from the meristem, forming the first shoot tip chamber of the new host plant generation (Fig. 29d,e). As in mature shoot tip chambers described earlier, the adaxial surfaces of these first stipules are similarly populated with secretory dendroid colleters (Fig. 29e). With further development, the first shoot tip enlarges and the first two primordial true leaves begin to grow. It is at this stage in development that the author has first been able to locate the symbiotic bacteria during seed germination. It must be stressed, however, that all this development has taken place within the seed indicating that the bacteria are enclosed somewhere in the seed and do not originate from the external environment. Where the bacteria reside in the dormant seed prior to infecting the epicotyl remains a question only further ultrastructural research will answer. With further growth, the hypocotyl straightens and the cotyledons expand and open out, breaking open and discarding the seed case and revealing the new young shoot. The bacterial population in this young shoot tip will be succesfully transferred into all other shoot tips and axillary buds in the host plant and, on flowering, will once again be safely transmitted to the next host plant generation.

IV. LEAF GLAND SYMBIOSIS IN DIOSCOREA (DIOSCOREACEAE)

The symbiotic relationship between bacteria and members of the monocotyledonous genus *Dioscorea*, the true yams, differs in many respects from the true leaf nodule symbiosis found in the two previously described dicotyledonous plant families. Symbiotic bacteria enter into and multiply within, a large pre-existing gland or nectary which constitutes the apex or acumen of the host plant leaf; distict leaf nodules within the leaf lamina or on the leaf margin formed in response to bacterial invasion do not occur. A photograph of such a glandular acumen is shown in Fig. 1 and a transverse section through the gland in Fig. 30. These large glandular leaf apices were first reported in the West African rainforest yam *Dioscorea macroura* Harms. by

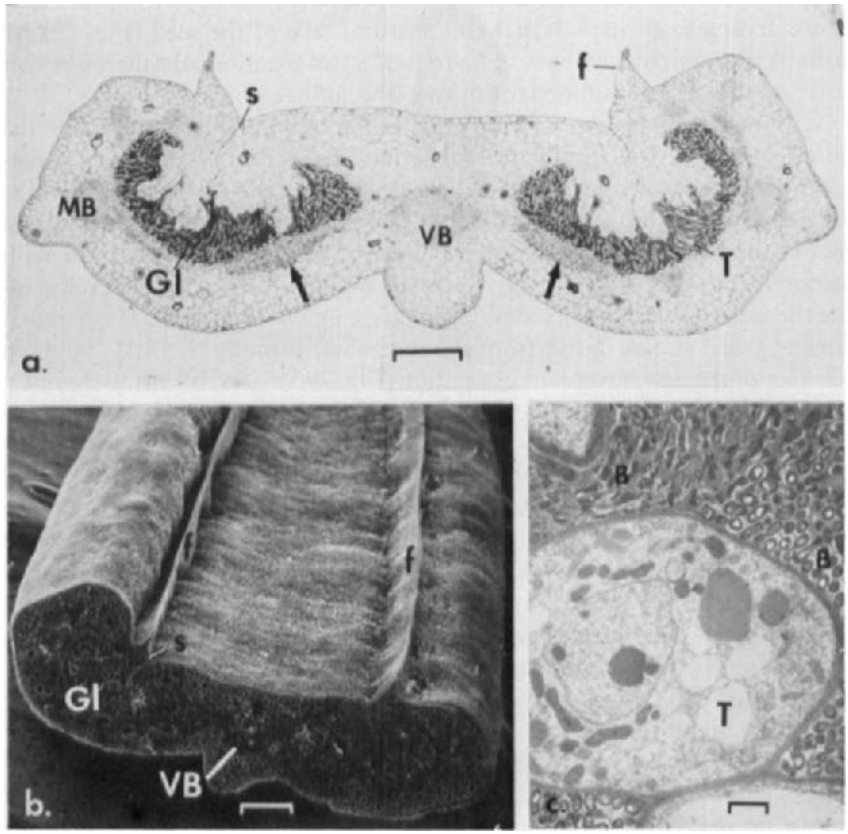

Fig. 30. (a) LM montage of a transverse section through the glandular acumen or "drip tip" of *Dioscorea sansibarensis*. The bacterial glands are the two densely staining, roughly kidney-shaped structures (Gl) situated on either side of the main vascular bundle (VB). The glands are well supplied with vascular tissue; at regular intervals along the length of the acumen, transverse veins linking the main bundle (VB) to the marginal bundles (MB) form the floor of the bacterial glands (arrows). Arising from the floor of the glands are numerous multicellular vermiform trichomes (T); these trichomes project into the gland lumen and probably represent the interface over which nutrients and other substances are exchanged between host and microsymbiont. An apparently occluded slit or duct (s) runs from the roof of the gland to the upper external surface of the acumen. The slit is formed during development of the gland (see Fig. 31); its significance, if any, is not yet understood. On either side of the acumen flanges of tissue (f) are present which presumably function in the water disposal activity of the tip. Bar = 250 μm. (b) SEM of part of a leaf acumen. The location of the section and the direction of view are shown by a dotted line and arrow respectively in Fig. 1d. Bacterial glands, Gl; slits, s; flanges, f; main vein, VB. Bar = 250 μm. (c) TEM of part of a bacterial gland showing the symbiotic bacteria (B) and part of a vermiform trichome cell (T). The trichome cells contain many plastids, mitochondria, dictyosomes and endoplasmic reticulum and are involved in the secretion of a mucilage-like substance which nourishes and maintains the bacterial colony. Bar = 2.0 μm.

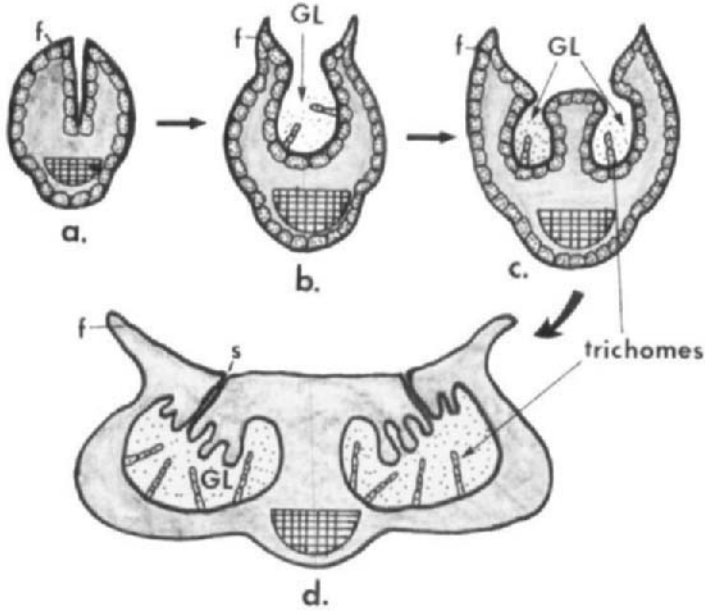

Fig. 31. Diagrammatic representation of stages in the development of an acuminate leaf gland of *Dioscorea sansibarensis*. The cross-hatched region in each diagram represents the main vascular bundle. (a) Transverse section through a very young developing leaf acumen. An invagination or split occurs from the upper epidermis leaving two flanges (f) of tissue on either side. (b) With further development the invagination deepens and widens to become a flask-shaped cavity (GL). Secretory trichomes begin to develop from the inner surface of the gland while the flanges (f) of tissue begin to elongate. (c) The central portion of the floor of the flask-shaped cavity elevates to the level of the upper epidermis forming a ridge tissue which bisects the original cavity and as such gives rise to two young glands (GL). The inner epidermes of the tissue flanges (f) begin to bulge and grow inward towards the raised ridge of tissue. The trichomes continue to develop and secrete mucilage. (d) At maturity the epidermis of the raised ridge of tissue and the epidermis of the inwardly growing flange (f) become closely appressed as an occluded slit (s) sealing the lumen of the gland (GL) off from the external environment. The epidermis of the flange and raised ridge become highly convolute giving the roof of the gland a very irregular appearance. The trichomes, which are secretory and vermiform in nature, fill the gland full of mucilage in which the symbiotic bacteria multiply. Infection of the gland with bacteria presumably takes place when the gland is open to the external environment but yet when there are trichome secretions to attract and maintain them; as such infection must occur at the stages in development represented in (b) and (c).

Uline (1897) who speculated that they functioned as hydathodes. Gentner (1904) considered that since these acuminate glands constituted an efficient "drip-tip" at the leaf apex, the glands themselves must act as reservoirs for the collection and storage of water. It was Orr (1923) who first showed that the glands were filled with "nitrogen-fixing bacteria" and that much of the development of the gland occurs very early before the young leaf unfolds. The first indication of the glands' appearance is in the upper epidermis of the developing leaf acumen where a simple invagination occurs between two flanges of tissue (Fig. 31a). The invagination deepens to become a flask-

shaped cavity (Fig. 31b). As development proceeds the central portion of the floor of the flask-shaped cavity elevates to the level of the upper epidermis, forming a ridge of tissue which bisects the original cavity (Fig. 31c). Secretory vermiform trichomes also develop from the floor of the cavity and grow into the cavity lumen. During these developmental stages the lumen of the cavity has been open to the external environment. As the acumen develops to maturity the epidermis forming the roof of the gland becomes highly convolute, and becomes closely appressed to the epidermis beneath the tissue flanges. This effectively seals off the gland from the external environment, there only being a very narrow fissure or slit between the closely appressed epidermal tissue; the seal is made even tighter by the deposition of cutin in the fissure. The mature glands are kidney-shaped in cross-section and are filled with trichomes, their secretions and the symbiotic bacteria.

Schaede (1929) who next investigated the *Dioscorea* acumen was of the opinion that the bacteria were not symbiotic, but rather parasitic on the yam. This view was held by Behnke (1984) who, in a review on plant trichomes, described certain aspects of the bacterial leaf glands of *D. macroura* at the ultrastructural level. However we have recently shown (Miller and Reporter, 1987) that uninfected bacteria-free plants grow more slowly and produce usually only one yellow-green leaf per node in contrast to the vigorous growth habit of infected plants which produce two deep green leaves per node. However it may be that the parasitic views of Schaede (1939) and Behnke (1984) should not be discounted entirely; in subsequent work on *D. sansibarensis* the author and colleagues (unpublished) found a specimen which showed poor growth typical of our uninfected plants but which was surprisingly infected. On top of that we have isolated two different organisms from the glands of different *D. sansibarensis* specimens (see Section V.A.3). It may be that these glands are somewhat "promiscuous" and can be infected by a range of different phyllosphere inhabitants. As such, the possibility that a range of relationships ranging from mild parasitism through commensalism to beneficial symbiosis may occur within these *Dioscorea* hosts. Unfortunately, this line of research was halted by the closure of the Charles F. Kettering Laboratory, Yellow Springs, Ohio, where it was being conducted. This aspect of the yam symbiosis should be vigorously pursued; although nitrogen fixation has not been detected in the symbiosis, the introduction of a bacterium which will reside in the gland and which has been engineered to fix nitrogen or produce pesticides or extra powerful growth promoters, etc., could have considerable agronomic implications for *Dioscorea* which is a relatively undeveloped, but yet valuable tropical food crop.

In mature leaves of *D. sansibarensis*, the long acuminate tips can, in well established plants, reach a length of up to 120 mm. In cross section the bacterial glands are kidney-shaped structures located within the tissues of the acumen (Fig. 30). Towards the apex of the acumen, two glands are found which run the entire length of the acumen from the tip to the edge of the leaf

lamina. Transverse sections of the acumen taken serially away from the tip towards the leaf lamina show that the glands bifurcate once or twice giving rise to four or even six glands running parallel to each other along the upper portion of the acumen. The glands are completely lined with cuticle; numerous simple multicellular trichomes, which are not cutinized, arise from the lower epidermis and project into the lumen of the gland. Presumably exchange of metabolites between the yam and the bacteria occur over the walls of these trichomes (Fig. 30).

Another major difference between this association and the two dicotyledonous leaf nodule associations lies in the question of the cyclic relationship. The life cycle of these yams does not follow the predictable monoecious pattern of vegetative growth, flowering, seed set, seed germination and subsequent vegetative growth of the next generation such as is found in the two leaf-nodulated dicotyledonous families. In fact, sexuality in the yam is dioecious and rather degenerate. Flowering is uncertain as is the ratio of males and females in any given seed sown population; none of our *D. sansibarensis* specimens have ever flowered. Perennation is much more frequently achieved both by subterranean tubers, and in some species such as *D. sansibarensis*, also by aerial axillary tubers or bulbils. No information concerning the presence or distribution of the bacteria at many of the stages in the yam life cycle is yet available. The author has sectioned primordial buds from dormant aerial bulbils and has failed to find any bacteria. In addition surface sterilized tubers and bulbils give rise to uninfected plants which subsequently become infected on exposure to non-sterile conditions, e.g. the glasshouse. In the light of this, and until further evidence points to the contrary, it may be assumed that there is no cyclic component to this symbiosis, each host plant in any particular generation being infected from the environment as it grows.

V. THE MICROSYMBIONT AND SYMBIOTIC FUNCTIONS

A. IDENTITY OF THE MICROSYMBIONT

Ever since Zimmermann's discovery in 1902 that the leaf nodules of *Pavetta* species contained bacteria there have been numerous attempts to isolate, culture and identify the endophyte from many of the nodulated species from all three plant families involved in leaf symbiosis. Suggestions as to the real identity of the symbiont have been as numerous and diverse as the isolation attempts themselves. Because of the difficulty in obtaining reliably bacteria-free plants no one has been able to satisfy Koch's postulates and thus show unequivocally that isolate and endophyte are one and the same. Although many investigators have attempted to circumvent Koch's postulates by using

TABLE II
Cumulative list of claimed endophytes isolated from leaf-nodulated plants

Proposed identity of endophyte	Host plant (Family initial)	Investigators
Bacillus foliicola	*Ardisia* (M)	Miehe, 1913b; De Jongh, 1938; Hanada, 1954; Rodriguez-Periera et al., 1972
Bacterium foliicola	*Ardisia* (M)	De Jongh, 1938; Zeigler, 1958; Yamada, 1960
Xanthomonas hortoricola	*Ardisia* (M)	Hanada, 1954
Phyllobacterium myrsinacearum	*Ardisia* (M)	Knösel, 1968
Rhizobium sp.	*Ardisia* (M) *Psychotria* (R)	Gordon, 1963
Chromobacterium lividum	*Ardisia* (M) *Psychotria* (R) *Pavetta* (R)	Bettelheim *et al.*, 1968
Mycobacterium rubiacearum	*Psychotria* (R) *Pavetta* (R)	von Faber, 1914; Rao, 1923
Bacterium rubiacearum	*Pavetta* (R)	Zeigler, 1958
Flavobacterium sp.	*Psychotria* (R)	Adjanohoun, 1957
Phyllobacterium rubiacearum	*Pavetta* (R)	Knösel, 1968
Klebsiella rubiacearum (*pneumoniae*)	*Psychotria* (R)	Silver *et al.*, 1963; Centifanto and Silver, 1964
Bacillus sp.	*Psychotria* (R) *Pavetta* (R)	Yamada, 1960
Agrobacterium sp.	*Psychotria* (R) *Dioscorea* (D)	Fletcher and Rhodes-Roberts, 1979 Miller (unpublished)
Pseudomonas sp.	*Dioscorea* (D)	Miller (unpublished)

sophisticated biochemical, bacteriological and serological techniques, these techniques have tended simply to confirm the identity of the symbiont that each individual investigator has proposed. Thus there is still considerable disagreement over the identity of the endophyte or endophytes involved in these symbioses. A cumulative list of the genera proposed as the leaf nodule endophyte is given in Table II.

1. Isolations from Myrsinaceous Hosts

Miehe (1911b, 1913b) attempted to isolate the endophyte from a number of locations from the host plant *Ardisia crispa*. He reported success only by culturing excised embryonic plantules on neutral pea agar. He named his isolate *Bacillus foliicola*. De Jongh (1938), using similar isolation protocols as Miehe, isolated a micro-organism from a suspension of crushed plantules which he also identified as *Bacillus foliicola* Miehe. However, he considered this to be in breach of accepted international nomenclature rules at that time

and renamed the isolate *Bacterium foliicola* Miehe. The investigations of Zeigler (1958) and Yamada (1958, 1960) have subsequently claimed success in isolating the symbiotic bacteria from *A. crispa*; both are in agreement with De Jongh that the *Ardisia* endophyte is *Bacterium foliicola* Miehe. In addition Yamada (1960) reported the isolation of *Bacterium foliicola* from *A. punctata* nodules while Rodriguez-Pereira *et al.* (1972) isolated an organism from the embryo of *Ardisia crenata* which they claim is identical in all respects to the description offered by Miehe of *Bacillus foliicola*. Hanada (1954) also obtained *Bacillus foliicola* from *A. crispa*; most of Hanada's work however concerned the isolation of the bacteria from the nodules of *A. hortorum* which he identified as *Xanthomonas hortoricola*. Knösel (1962) claimed the successful isolation of the microsymbiont from both *A. crispa* and *A. crenata*. Knösel considered that all nodulated plants of the family Myrsinaceae were infected with the same bacterium and thus put forward the new generic name and specific epithet, *Phyllobacterium myrsinacearum* to describe the symbiont in this family. Gordon (1963) claimed successful isolation of the endophyte from *A. crispa* and, on the basis of its cultural characteristics, tentatively classed it as a species of *Rhizobium*. Bettelheim *et al.* (1968) using a variety of cultural techniques and fluorescent labelled antibodies claimed that their isolate from *A. crispa* is *Chromobacterium lividum* (which under modern nomenclature is presumably *Janthinobacterium lividum*).

2. Isolations from Rubiaceous Hosts

Von Faber (1912, 1914) was the first to report success in isolating the microsymbiont from rubiaceous hosts. He isolated micro-organisms from *Pavetta zimmermanniana*, *Pavetta indica* and *Psychotria bacteriophila* all of which he considered to be the same and which he identified as *Mycobacterium rubiacearum*. Rao (1923) also claimed success in the isolation of a bacterium from *Pavetta indica* and he agreed with von Faber's suggestion of *Mycobacterium rubiacearum*. Zeigler (1958) isolated what he called *Bacterium rubiacearum* from the nodules of *Pavetta zimmermanniana*, the same genus to which he assigned the microsymbiont of *Ardisia*. Adjanohoun (1957) claims to have isolated the endophyte from the nodules of *Psychotria calva* and identified it as a *Flavobacterium* species.

In keeping with his studies on the endophyte from *Ardisia*, Knösel (1962) assigned the microsymbiont of rubiaceous host plants to his genus *Phyllobacterium*, isolating *Phyllobacterium rubiacearum* from the nodules of *Pavetta zimmermanniana*. Gordon (1963) isolated a number of bacterial strains from *Psychotria*, all but one of which he considered to be contaminants. However, one type of micro-organism was isolated consistently and Gordon took this to be the endophyte. On the basis of cultural characteristics he could not place it in any known taxon with any degree of certainty and thus provisionally placed his presumed endophyte in *Rhizobium* even although it fixed atmospheric dinitrogen in pure culture. Silver *et al.* (1963) and Centifanto

and Silver (1964) isolated a micro-organism from *Psychotria bacteriophila* which they claim to be *Klebsiella rubiacearum*. Using serological techniques, DNA base ratios and DNA hybridization techniques they found that their isolated endophyte was identical to *Klebsiella pneumoniae* (Schroeter) Trevison.

Bettelheim *et al.* (1968) extending the work of Gordon (1963) isolated micro-organisms from ten different *Psychotria* and *Pavetta* host plant species. By using a variety of cultural and serological techniques they claimed that the endophyte in all of these host plants is *Chromobacterium lividum*. Since they also identified the *Ardisia* symbiont as *Chromobacterium lividum* they imply that their isolated organism is universal to all nodulated species of families Myrsinaceae and Rubiaceae. Yamada (1970, 1972) isolated bacteria from the nodules of *Pavetta lanceolata* and *Psychotria capensis*, characterizing them both as *Bacillus* species. Since the latter host plant named is a non-nodulated species some of Yamada's claims must remain questionable. Fletcher and Rhodes-Roberts (1979) have made many attempts to isolate the endophyte from leaf nodules of *Psychotria* using an array of techniques. These investigators isolated 12 *Agrobacterium*-like organisms, all of which have unfortunately subsequently died. Since then over 100 attempts have been made to try to re-isolate these bacteria but without success (M. E. Rhodes-Roberts, personal communication).

3. Isolation Attempts from Dioscorea

Little attention has focused on this symbiosis. The only known attempt to isolate the symbiont from *Dioscorea* before this author's attempt has been that of Orr (1923). Orr isolated a Gram-positive, rod-shaped organism from the leaf glands of *D. macroura*. He did not attempt to assign his isolate to any bacterial taxon. We have made several attempts to isolate the bacteria from the acuminate leaf glands of *D. sansibarensis*. Although all of these attempts have seemed successful, they have consistently given rise to the isolation of two different Gram-negative micro-organisms. One of these has been provisionally identified as an *Agrobacterium*, possibly a biotype of *Agrobacterium radiobacter* (M. E. Rhodes-Roberts, personal communication). The other isolate has not as yet been fully characterized but is more than certainly a non-fluorescent *Pseudomonas* species.

4. Remarks on Isolation Attempts and Identifications

Clearly, the identity of the leaf nodule symbionts is one of the most confused issues surrounding these symbiotic relationships. Every new isolation attempt adds more to the confusion, this author being no exception by adding *Pseudomonas* as a possible contender to the growing list of possible endophytes. However, this list can be whittled down somewhat by considering the circumstances of each identification. In their review concerning the nomenclature of bacteria from nodulated rubiaceous and myrsinaceaous

hosts, Horner and Lersten (1972) discount many of the proposed microsymbiont genera for a number of valid reasons. *Bacterium* was discounted by rules of nomenclature; *Rhizobium*, the legume root nodule symbiont, by generic definition; *Bacillus* is discounted as it excludes non-endospore forming bacteria and *Mycobacterium* because it excludes Gram-negative bacteria. This left as the possible endophyte, *Chromobacterium*, *Phyllobacterium*, *Xanthomonas* or *Klebsiella*. This author finds difficulty in accepting any of these genera as the taxa to which leaf nodule bacteria, at least in rubiaceous and myrsinaceous hosts, should be assigned. Firstly, Bettelheim *et al.* (1968) claimed on the basis of immunological labelling, that their isolate, *Chromobacterium lividum*, was the universal endophyte of all leaf nodulated plants. On the basis of fine structure alone, the concept of one organism being the endophyte in all species is improbable; comparisons of the bacterial structure for some leaf nodule hosts is shown in Fig. 32. Also, since *Chromobacterium lividum* produces pigments, most usually purple, one would expect to be able to detect it in nodules or at least at some point in the life cycle of the host. No pigments have ever been detected by this author at any life cycle stage in any host plant he has ever studied. Further, De Ley *et al.* (1978) showed that the isolate of Bettelheim *et al.* (1968) was not *Janthinobacterium* (= *Chromobacterium*) on the basis of comparisons between their rRNA cistrons.

Knösel (1962) erected the genus *Phyllobacterium* to include two species, *P. myrsinacearum* and *P. rubiacearum*, which he claims are the symbionts from myrsinaceaous and rubiaceous hosts, respectively. The main objection to Knösel's isolates being the endophyte is that they were "isolated" from mature leaves. Relatively early in leaf development, the endophyte becomes pleomorphic; a condition notorious for non-viability. As such, leaf nodule bacteria cannot be isolated from mature leaves. Many investigators, including this author, have had singular lack of success in isolations using mature leaves, the only bacteria usually appearing on isolation plates being common phyllosphere contaminants. Interestingly, Gillis and De Ley (1980) showed, on the basis of DNA/rRNA hybridization studies, that *Phyllobacterium* belongs in the RNA group of taxa associated with the phyllosphere in which many of the contaminants commonly associated with leaf nodule isolations belong. A similar argument to the above also applies to *Xanthomonas* and *Klebsiella*, both of which were claimed to have been isolated from mature leaves and both of which are commonly found on the phyllosphere.

In the author's opinion it seems that looking for a simple answer to the problem of the identity of the endophyte in rubiaceous and myrsinaceous hosts is perhaps misguided. We tend to look at these cyclic leaf symbiotic relationships in the same light as the more common plant pathogenic or root symbiotic associations. However there is one very large underlying difference between the micro-organisms involved in the latter and those involved in leaf nodulation. Plant pathogens and the root symbionts *Rhizobium* and *Frankia* exist freely in the environment and invade host plants for better or worse, as

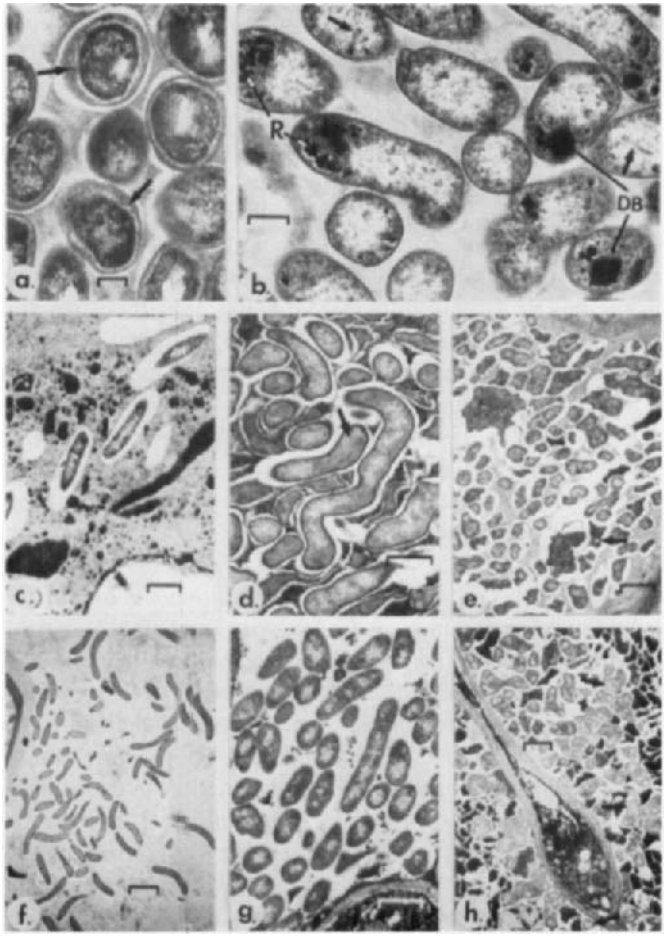

Fig. 32. (a) Bacteria from an acuminate leaf gland of *Dioscorea sansibarensis*. These bacteria are Gram-negative and are coccoid to ovoid in shape, rarely rod-like and range in size from about 0.3 to 0.9 μm in diameter. The most distinct feature of these bacteria lies in their wall ultrastructure; although displaying typical Gram-negative wall structure of the outer layer, intermediate layer and inner cell membrane, these bacteria possess a very thick, grossly enlarged intermediate layer (arrows). Bar 0.1 μm. (b) The symbiotic bacteria from the leaf nodules of *Psychotria punctata* are small fat Gram-negative rods some 0.7–1.0 μm in diameter by 1.5–2.0 μm in length. Cells typically contain several α-glycogen rosettes (R) and at least one large electron dense crystalline body (DB). They display pronounced nucleoid regions which often contain small tubular inclusion bodies (arrows). Bar = 0.5 μm. (c) Bacteria from the shoot tip of *Psychotria kirkii* var. *kirkii*. The cells are surrounded by the heterogeneous protein/carbohydrate mucilage secreted from dendroid colleters (see Fig. 23) and are typically straight or gently curved rods up to 0.5 μm in diameter and 2.5 μm in length. Each bacterium is Gram-negative (based on wall structure) and possesses a distinct electron-translucent capsule. The bacterial cytoplasm contains no discernible structures but does exhibit distinct nucleoid regions. Bar = 1.0 μm. (d) The bacteria from a developing leaf nodule of *Psychotria kirkii* almost become filamentous, having lengths sometimes up to and even exceeding 10 μm. In common with bacteria from the shoot tip, these young nodule bacteria possess a distinct capsule. Large multilamellate stacks of membranes are commonly found in the bacterial cytoplasm (arrow). Bar = 1.0 μm. (e) The

they become available. Leaf nodule bacteria are confined to the host plant; host plants do not become "infected" from the external environment. In fact, it is probable that the external infection event which led to the establishment of these relationships occurred several millions of years ago. Therefore it is not unreasonable to expect that leaf nodule bacteria may bear little resemblance to modern free-living plant associated bacteria although they are probably ancestrally and evolutionarily related. Given the length of time in which leaf nodule bacteria have potentially been involved in the close cyclic relationship with their hosts, it would again be not unreasonable to expect that the endophyte has evolved to be very specialized and highly adapted to fit such a restrictive and controlled ecosystem as a higher plant life cycle. Thus we should perhaps begin to consider leaf nodule bacteria more in terms of being *Rickettsia*-like or *Mycoplasma*-like organisms, or perhaps even in the same class of highly adapted, highly specialized bacteria found as intracellular endosymbionts in protozoa, insects and invertebrates.

The exception which proves the rule can be found in the genus *Dioscorea*. The leaf gland symbiosis, as was shown in Section IV, is considerably different from the relationships that exist in myrsinaceous and rubiaceous hosts. First, the relationship does not appear to be cyclic and secondly, sterile grown plants are bacteria-free and become easily infected when exposed to the external environment. That is, in this case, infection of each generation occurs from the external environment. In keeping with this, isolation of the proposed endophytic *Agrobacterium* or *Pseudomonas* bacteria is relatively simple and straightforward.

Because of the crucial central role these bacteria play in the development of their hosts, their isolation, culture and identification is mandatory for future research activity. The fundamental difficulty in showing that the isolate is the endophyte is that reliably bacteria-free plants are not readily available. A certain number of dwarfed plants, known as cripples, occur naturally in very small numbers in every batch of seeds. These plants do not develop normally; the first two or three leaves are produced normally but then growth and differentiation ceases and the shoot tips degenerate to callus. The seedling can stay in this static bacteria-free condition for up to 3 years before it

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bacteria in a mature *Psychotria* nodule are distinctly pleomorphic with diameters of up to 4.0 μm. Although still largely featureless, some cells can be found which accumulate one or two lipid droplets (arrow). Bar = 2.0 μm. (f) Bacteria from between embryo and endosperm in the seed of *Ardisia crispa*. They are Gram-negative, long curved rods 0.5 μm in diameter and up to 5.0 μm in length. Although not visible at this magnification, most of the bacterial cells contain mesosomes when in this seed-borne location. Bar = 2.0 μm. (g) During early nodule development the bacteria are straighter rods and now exhibit fairly pronounced nucleoid regions. Mesosomes are now absent. Bar = 1.0 μm. (h) Bacteria in mature *Ardisia* nodules are noticeably pleomorphic. Several distinct nucleoid regions exist in every pleomorphic cell, each of which has at its centre a small electron dense nucleoid inclusion body (arrows). The black shapes interspersed among the bacteria are dead pleomorphs. Bar = 2.0 μm.

turns yellow and dies. However a variable proportion revert after 6 months to 1 year, sending out a lateral shoot which becomes the main shoot and which contains bacteria. It is assumed that if the microsymbionts fail to enter the ovule during flower development, then a bacteria-free cripple occurs. If not enough bacteria enter the ovule to constitute a "threshold" colony, if the colony gets wrongly placed within the embryo sac, or if many of the bacteria in the colony die in the seed for some varying reasons, a cripple which eventually reverts occurs. It is presumed that it takes several months for such a damaged colony to grow to functional colony size at the location where the growth stimulating effect is exerted; when the threshold level is reached, a lateral shoot is induced and the cripple becomes a revertant. Many methods of producing bacteria-free plants in quantity using heat, cold, irradiation antibiotics and tissue culture have been tried with varied and sometimes limited success (Miehe, 1919; von Faber, 1912, 1914; De Jongh, 1938; Yamada, 1955a–d, 1956, 1964; LaMotte and Lersten, 1972). The rapidly expanding sophistication of modern molecular biology techniques, however, open up the possibility that endophyte-specific DNA probes can be constructed from isolates which may allow re-infection experiments with unreliable bacteria-free plants to be bypassed.

B. FUNCTION OF THE RELATIONSHIP

1. Nitrogen Fixation

Symbiotic relationships between higher plants and bacteria are known best from the root nodule associations between *Rhizobium* and members of family Leguminosae and actinorhizal associations between the actinomycete *Frankia* and a diverse group of woody plants from several plant families (Stewart, 1966). In all of these associations the contribution of the endophytic bacteria is the supply to the host plant of biologically fixed nitrogen. In his 1966 review Stewart tells us:

> Hellriegel & Willfarth ... in 1886–8 ... reported their findings as showing that legumes which bore on their roots nodules inhabited by a bacterium assimilated elemental nitrogen whereas those without nodules did not. This was followed by the discovery in 1892 by Noble, Schmidt, Hiltner and Hotter that nodulated plants of the non-leguminous angiosperm *Elaeagnus* showed much better growth in nitrogen deficient medium than did corresponding non-nodulated plants.

In 1902, Zimmermann discovered bacterial leaf nodules. It is therefore not surprising, in light of what was known about plant–microbe interactions at the time, that both von Faber and Miehe made the unfortunate, yet understandable and still widely-held assumption that these leaf nodule associations simply constituted another, albeit peculiar, class of nitrogen fixing symbiosis; indeed it was the same basic assumption which resulted in this author, as a graduate student in a laboratory concerned with symbiotic nitrogen fixation research, being directed into the study of leaf nodules.

Working on the nitrogen fixation assumption, von Faber (1912, 1914) isolated the endophyte from nodulated rubiaceous plants and, using the Kjeldahl method of nitrogen estimation, showed that his isolate was capable of fixing nitrogen and so convincing von Faber that the symbiosis, at least as far as rubiaceous hosts were concerned, was a nitrogen-fixing one. This was to be the first of many attempts throughout the century to prove that a nitrogen fixing capability exists in leaf nodule symbiosis. Miehe (1914, 1919) however produced a different set of findings for myrsinaceous hosts, claiming that his isolate from *Ardisia* did not fix nitrogen because his Kjeldahl analysis was negative and the endophyte would not grow on nitrogen deficient media. Miehe thus disagreed with von Faber's earlier conclusions, himself concluding that his own earlier hypothesis was wrong and that nitrogen fixation was not involved in the symbiosis.

Němec (1932) agreed with Miehe's conclusion that nitrogen fixation was not a factor in the *Ardisia* symbiosis since root formation, which requires large amounts of nitrogen, takes place normally in bacteria-free seedlings. De Jongh (1938) was unable to culture his isolate from *Ardisia* on nitrogen-free medium and speculated that nitrogen fixation may not be involved in the symbiosis. However De Jongh was aware of the dangers of drawing conclusions from observations on the physiological activities expressed by bacteria in artificial culture and then applying these conclusions to the situation *in vivo*. Claims of nitrogen fixation by isolates of the endophyte from rubiaceous nodules by von Faber (1912, 1914) and Rao (1923) and from the bacterial leaf tip glands of certain members of the monocotyledonous genus *Dioscorea* prompted De Jongh to point out that "Löhnis succeeded in showing that the root symbiont of the Leguminosae is incapable of fixing nitrogen in pure culture". He suggests that this should be borne in mind when considering the claims of von Faber, Rao and Orr.

Hanada (1954), using a low nitrogen medium and Kjeldahl analysis, claimed his isolate from *Ardisia* did fix nitrogen, while Yamada (1953, 1954) in preliminary studies indicated that the endophyte of *A. punctata* might fix nitrogen but not that of *A. crispa*. In a later more extensive work, Yamada (1960) failed to detect nitrogen fixation in his isolates using nitrogen-free media and Kjeldahl analysis. The use of the $^{15}N_2$ method yielded negative results from both isolates and living host plants. Gordon (1963) and Bettelheim *et al.* (1968) claim positive results for isolates from *Ardisia* using the $^{15}N_2$ method. Hofstra and Koch-Bosma (1970) using Kjeldahl analysis, the $^{15}N_2$ method and acetylene reduction found no fixation took place in *Ardisia* plants regardless of whether the plants were kept in the light or in the dark.

After von Faber the next worker to examine the nitrogen fixing capacity of leaf nodulated rubiaceous species was Rao (1923). He isolated bacteria from the nodules of *Pavetta* and "*Chomelia*" (Bremekamp, 1960, later properly identified Rao's second plant as another *Pavetta* species) and claimed to successfully culture them on nitrogen-free mannitol medium. Bond (1959) failed

to detect nitrogen fixation in detached leaves of *Psychotria bacteriophila* using the $^{15}N_2$ method. Gordon (1963) and Bettelheim *et al.* (1968) used the $^{15}N_2$ method on bacterial isolates from *Psychotria* and *Pavetta* and, as in *Ardisia*, they concluded that nitrogen fixation was occurring. Silver *et al.* (1963), Centifanto (1964) and Centifanto and Silver (1964) used both Kjeldahl analysis and the $^{15}N_2$ method to show that their isolates fixed nitrogen in pure culture. After further study, Silver subsequently withdrew his claim for the occurrence of nitrogen fixation in *Psychotria* nodules (Becking, 1976). A number of recent studies using the entire range of nitrogen fixation detection techniques have shown there to be no positive evidence of nitrogen fixation taking place in leaf nodulated plants (Löhr, 1968; Becking, 1971; Silvester and Astridge, 1971; Van Hove, 1976).

In a preliminary study using *Pavetta* species, Grobbelar *et al.* (1971) reported that nitrogen fixation was detected by the $^{15}N_2$ method, acetylene reduction and by growth experiments in nitrogen deficient soil. However these results were withdrawn in a later paper (Grobbelar and Groenewald, 1974) when on re-investigating of a total of seven *Pavetta* species and two *Psychotria* species using the $^{15}N_2$ method and acetylene reduction, their findings were such as to encourage them to claim that nitrogen fixation was not in fact occurring. About the same time Yamada (1972) employing Kjeldahl analysis and nitrogen-free culture media could not demonstrate nitrogen fixation from isolates of the endophyte of *Pavetta lanceolata*.

In summary then, studies carried out to detect nitrogen fixation in myrsinaceous plants have all proved negative as have the majority of investigations into the nitrogen-fixing capacity of the isolated and cultured presumed endophyte. All the studies, with two exceptions, carried out on rubiaceous hosts *in vivo* to demonstrate nitrogen fixation have been negative; the two exceptions which gave positive results were later recanted. The majority of investigations into the nitrogen-fixing capacity of isolated and cultured presumed endophytes from rubiaceous plants have all yielded positive results.

In a recent review, Silver (1977) indicates that nitrogen fixation is probably not a feature of these leaf nodule associations and Postgate (1980) actively excludes leaf nodule symbiosis from the "accepted" list of nitrogen-fixing systems.

Over the last 8 years this author has used the acetylene reduction technique to monitor the leaf nodulated plants *A. crispa*, *Psychotria kirkii* var. *kirkii*, *Psychotria punctata* and *Dioscorea sansibarensis* for nitrogen fixation. All stages in the host plant life cycle including nodules, shoot tips, seeds, seed germination, flowering and fruit set have been tested. Ethylene production has never been detected in any of these host plants at any stage of their life cycles.

2. *Plant Growth Regulators*

A fascinating aspect of leaf nodulated plants is that all evidence to date

shows that if the bacterial partner is lost, differentiation, development and growth of the host plant ceases and subsequently its death ensues (Miehe, 1919; De Jongh, 1938; Gordon, 1963; Miller and Reporter, 1987). In the case of *Ardisia* both Miehe and De Jongh have shown that bacteria-free seedlings develop normally up to the second or third leaf stage; after that the shoot tip degenerates to callus and growth and differentiation cease. The seedling, some 3–6 cm in height, stays in this state for up to 3 years before finally becoming chlorotic and dying. Von Faber (1914) and Gordon (1963) have shown, in *Pavetta* and *Psychotria*, respectively, that bacteria-free seedlings develop normally until the fourth pair of leaves whence all development and growth ceases. These cripples also eventually die. Exposure of mature *Psychotria* plants to air temperatures of 45–50°C for 4–6 h (conditions which can be found in any graduate student's glasshouse on a warm summer day when he forgets to open the window and turn on the fan!) can cause death of the symbiotic bacteria while sparing the host plant. In this case growth and development does not entirely cease immediately; each shoot tip gives rise to several pairs of bizarre, distorted leaves and curious mixtures of mixed leaf and flower structures. After a while these disfigured leaves die, the shoot tips become necrotic and fall off, and the host plant dies.

In his final paper on the bacterial symbiosis in *A. crispa*, Miehe (1919) reasoned that since *Ardisia* plants could not grow without the bacteria and the bacteria were not fixing nitrogen, then there must be a stimulative effect, a "Reizwirkung", emanating from the bacteria. He unfortunately did not pursue this line of enquiry. Based on his experiments with buds from crippled plants, Němec (1932) concluded that in *A. crispa* a hormonal substance excreted by the bacteria must be capable of inducing normal growth and development in the host. De Jongh (1938) was the first person specifically to treat induced and natural cripple plants with a known growth regulator substance to ascertain whether the crippled symptoms could be reversed. It was known to De Jongh that dwarf hybrids of *Epilobium hirsutum* L. could be stimulated in growth by the application of "heteroauxin" (β-indolylacetic acid). In view of this he applied to the buds of *Ardisia* cripples a number of different concentrations of heteroauxin, two to four times a day for 20–60 days. The results were all negative, the only change being an increase in root growth.

Yamada (1960) grew rape plants, *Brassica napus* L., in bacterial suspensions of *Bacterium foliicola* and noted enhanced growth over his controls. He compared this growth to that of a series of plants grown in a number of different concentrations of IAA and gibberellin. Yamada concluded that the effect of the bacterial suspensions upon the rape seedlings was closer to that of IAA than that of gibberellin. Using a number of analytical techniques, including several different bioassays in addition to paper chromatography and spectrophotometric analysis, Yamada confirmed that the growth-promoting substance present in the bacterial suspensions of *Bacterium folii-*

cola was IAA. In a later study, Yamada (1972) failed to detect IAA from the cultured *Bacillus* species isolated from both *Pavetta lanceolata* and *Psychotria capensis*.

On the application of gibberellic acid to crippled *Psychotria punctata* plants, Silver *et al.* (1963) found that the cripple symptoms were modified but not reversed. Their conclusion was that another as yet unknown growth-promoting substance was involved in the symbiosis. Becking (1971) applied a number of concentrations of a range of plant growth regulators including IAA, gibberellin, kinetin and benzyladenine to the shoot tips of crippled *Psychotria mucronata* plants. All yielded negative results except that the gibberellic acid treated plants showed a little enhanced growth over the controls. However, Becking (1971) noticed that in senescent leaves the area around the nodules remained green. He carried out a chlorophyll retention test by placing slices of nodules from these senescing leaves on top of young oat leaves. The oat leaves remained green in the areas under the nodule slices, indicating the activity of a cytokinin-like compound. Further to this Becking showed by autoradiography that directional transport of ^{14}C-labelled α-amino-isobutyric acid from shoot tip to leaf nodules occurred adding further support to the view that cytokinins were involved.

Rodrigues-Pereira *et al.* (1972) isolated and cultured a micro-organism from *Ardisia crenata* which they claimed had identical characteristics to Miehe's (1914) *Bacillus foliicola*. Using extracts from this and from the strain of *Chromobacterium lividum* isolated by Bettelheim *et al.* (1968), they were able to show enhanced growth in both radish cotyledon and soybean callus bioassays. These extracts were also able to produce great malformations in pea seedlings identical to the malformations induced in such seedlings when infected with the known cytokinin-producing organism *Corynebacterium fascians*. The conclusions of Rodrigues-Pereira and his colleagues were that both *Bacillus foliicola* and *Chromobacterium lividum* produced cytokinin.

The first investigators to analyse nodules *per se* for the presence of cytokinins were Edwards and LaMotte (1975). Small discs of both nodulated and non-nodulated areas of young leaves of *Psychotria punctata* were punched out and subjected to a cytokinin extraction and purification technique. These purified extracts were assayed using the radish cotyledon and soybean callus bioassays. Edwards and La Motte (1975) showed that the extracts from nodules exhibited at least 100-fold higher cytokinin activity than extracts from regular leaf tissue and so they concluded that the function of the nodules was in the production of cytokinins. This author applied the technique of Edward and La Motte to *Ardisia* nodules (Miller, 1982) and was only able to detect slightly higher cytokinin in leaf nodule extracts compared to extracts from regular leaf tissue.

IV. CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Leaf nodule symbioses unfortunately are perhaps the least researched and the most overlooked of all the higher plant–microbe interactive systems, both historically and to the present day. The reasons for this are several. First, the species involved, albeit several hundred, are indigens of the hot, humid tropics and are of no apparent commercial or agricultural importance. The exception to this is the yam, *Dioscorea*. Why this bacterial symbiosis (wrongly presumed throughout this century to be nitrogen fixing) in what is a relatively important tropical crop genus has not been investigated for its potential ability to improve the crop, remains to the author somewhat of a mystery. Secondly, apart from *Dioscorea*, the species involved in the symbioses are evergreen woody dicots, a group of plants notoriously difficult to work with in comparison to annual herbs such as legumes and other crop species which are involved in symbiotic or pathogenic relationships with micro-organisms. To compound this, an investigator needs to grow his own supply of host plants; this requires time, patience and considerable hot, humid greenhouse facilities or having an interested and sympathetic large Botanic Garden nearby. *Psychotria* seeds cannot be ordered from a seed merchant with the same degree of ease as soybeans or corn! Thirdly, so much basic information is missing in our understanding of these relationships that potential investigators are almost certainly bound to be put off from venturing into what appears at first sight to be very murky water. Classic examples of such information gaps are: (a) some of the mechanisms involved in the transfer of the microsymbiont through the life cycle of the host are unknown; (b) the real identities of the symbionts; and (c) the possible benefits the microsymbiont is providing the host are also unknown.

I firmly believe, however, that the time is ripe for the scientific community to re-evaluate these complex relationships in terms of what we actually *do know* about them. We know that these associations are cyclic; the bacteria travel as constant companions of the host plant throughout not only its entire life cycle, but also through the life cycle of every one of its descendants. It causes concern to many people that pesticides, nutrients, growth regulators and bacterial "super-strains" or even genetically altered microbes are released into the environment with abandon. Yet, distributed between the bacteria and host plants in these leaf nodule relationships is the genetic material which allows bacteria and plants to intimately co-exist on a permanent basis. This has the potential to be an ideal packaging system for the delivery to plants of systemic pesticides, fixed nitrogen and growth promoters. Given the rapidly expanding sophistication and power of molecular genetic manipulation and engineering it is not out of line to envisage a genetically altered crop plant, engineered to accept a cyclic symbiont which would deliver to the plant any or all of the substances mentioned above. This would

mean that growth promoters, nutrients and pesticides could be directed at specific host plants without the substances or the bacteria being released into the environment at large. This may seem at present far fetched, fictional and fraught with insurmountable scientific and technical problems. However, reports of functional transgenic creatures are increasing in number and complexity daily; take for example the recent successful introduction of the luciferin/luciferase genes into *Nicotania*; 20 years ago to many the concept of tobacco plants glowing in the dark courtesy of fire-fly DNA would have smacked of nothing short of sorcery!

What advances in our ability to genetically alter higher plants will occur over the next 20 years? When one looks at the rapidly increasing ability and power of gene identification, splicing and transfer techniques the answer to that question is that genetic engineering of higher plants will probably accelerate by leaps and bounds over this period of time. Time, however, is something that leaf nodulated species may not have: a frightening number of species are being wiped out or brought to the verge of extinction by the wholesale destruction of what until recently have been relatively safe and secure tropical habitats. It is of concern to me that the possibility exists that many leaf nodulated species will be lost, as many of them are members of the fastest disappearing ecosystem in the world—the tropical rain forests.

We also know that, for the dicot host plants, the relationship with the microsymbiont is *totally obligate*; if the host loses its bacterial partner, almost all visible gene expression in the plant ceases. Growth and differentiation come to a halt, the shoot tips degenerate to callus and the plant dies; it is unable to grow on its own. Thus these host plants are essentially naturally occurring minus "something" mutants, and this "something" is likely to be a plant growth regulator (PGR). In fact, all the evidence we have to date points to cytokinin as being the PGR involved. Higher plant mutants deficient or reduced in other PGRs are currently available, but a cytokinin minus mutant is not available. Indeed, such a mutant would probably never be noticed, since without the ability of cell division it would never grow. Leaf nodulated plants will not grow either in the absence of their microsymbiont; however provide them with the microbe and normal growth and development resume. Essentially they could be regarded as both "cytokinin plus" and "cytokinin minus" as far as gene expression is concerned; killing the bacteria by simple heat treatment of the host plant turns what was a normal plant with normal levels of PGRs into a helpless specimen unable to continue normal cell division and differentiation. Therefore these leaf nodulated plants offer a potentially productive and elegant system for the study of the role of cytokinins in plant growth and development.

Another interesting oberservation, discussed in Secton III.A.2, is the dramatic rise in bacterial mRNA within the young nodule when the most rapid phase of growth and development of the leaf and nodules occurs. Since some of this mRNA will code for the proteins directly involved in the production

of the PGR being synthesized by the bacteria, and since it is prokaryotic mRNA and thus easily separable for host plant mRNA, a very convenient point for molecular studies of these bacteria with respect to their function in the symbiosis is identified.

Thus, there are several areas of potentially exciting and rewarding research waiting to be conducted on leaf nodule systems. On a more immediate level, however, some of the more basic questions which still remain, must first be answered. Many of the mechanisms involved in transferring the symbiont through the various stages of the host life cycle have been identified. However, serious information gaps exist, particularly in the seed-borne stage of the cycle. We must know exactly how the bacteria are maintained at all stages in the host life cycle and we must know precisely all the mechanisms involved in the transfer of the microsymbiont through all of the stages of the cycle. These are questions which only a good deal of exacting microscopy will answer. Another basic question is what are the microsymbionts and what are their relationships to other bacteria? This problem needs to be addressed, and if Koch's postulates cannot be satisfied the traditional way, then new molecular techniques will have to be employed to verify whether an isolate is truly the endophyte.

Perhaps the most urgent question begging an answer is what exactly is being supplied to the host plant by the bacteria? Is it raw cytokinin? If so, which one; is it one already identified or will it be a novel one? Is the plant's cytokinin synthetic pathway completely missing or is the bacteria supplying not cytokinin, but an intermediary metabolite? Or is the plant producing a cytokinin which is ineffective and which the bacteria metabolize into a functional form? These are some of the questions which further research on leaf nodule systems must answer first.

The purpose of this chapter was to document our present level of understanding of these bizarre symbiotic relationships. If it is considered to have achieved that aim then the author would be satisfied. However it would be more rewarding, if, after reading this chapter and discovering some of the intriguing questions these relationships pose, other scientists were stimulated to bring to bear their own particular talents and expertise on these facinating symbiotic systems. The rewards for all could be many.

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Fracture Properties of Plants

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I. INTRODUCTION

There is a number of reasons for studying the mechanisms controlling the structural integrity (largely expressed as fracture properties) of plants. The

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first is in terms of the plant itself—the plant has to be able to withstand the mechanical effects of wind, water and gravity and grow in such a way that its integral parts remain intact and do not split open unannounced. This may sound a trivial statement, but it is the experience of engineers that it is much easier to design something which breaks than something which doesn't (Gordon, 1976) especially when, as with plants, there is an upper limit (if only an implied one) to the amount of material available for construction. To survive, the plant must therefore have mechanisms for resisting fracture (the initiation and propagation of cracks). By contrast, the plant can encourage and direct fracture by laying down abcission and dehiscence layers which are very brittle. The fracture properties of plants are also important for the animals which feed upon them. Mechanical properties constitute a significant factor in palatability which, for man, extends to mechanical properties during and after various processes in preparing plants as food.

A plant may also cease to be able to carry the loads required for survival, due to structural failure. This may involve fracture but can also involve other forms of deformation, both elastic (i.e. recoverable) and plastic (i.e. permanent). It seems very probable that a large part of the mechanical design of plants is concerned with structural stability and the plant's ability to suffer local damage without incurring structural failure. In a plant, with its hierarchical design (cellulose—cell-wall—cell—tissue—organ—plant), it is not always easy to know whether one should consider a particular stability/strength problem as one in materials or structures. In general, behaviour under a tensile load will depend only on material properties whereas a compressive or shear load will depend on structural properties as well. More subtle differentiation will become apparent later in this review.

For both the materials scientist and the botanist it is of interest to draw some general principles from the data. It is already possible to calculate breaking strengths and stiffnesses of a limited number of plant organs simply from their morphology (e.g. the longitudinal stiffness of a grass leaf is directly and linearly proportional to the total cross-sectional area of sclerenchyma in the leaf). With more data it will be possible to draw some general conclusions regarding the durability of plants which will have significance both in the practical areas of agriculture and horticulture and will also throw light on more of the evolutionary pressures to which plants are exposed.

It is important at an early stage to differentiate stress, strain, stiffness, strength and toughness. Stress is expressed as force per unit area ($N\,m^{-2}$ = Pa = 10 dynes cm^{-2}). Strength is the stress at which the sample breaks and so has the same units as stress. Since strain is a pure number (relative change in shape, e.g. increase in length per unit length), stiffness (resistance to deformation) has the same units as stress and, in tension, is numerically equal to the stress required to double the length of the test piece. Strength and stiffness are commonly confused, leading to talk of "elastic strength", which is

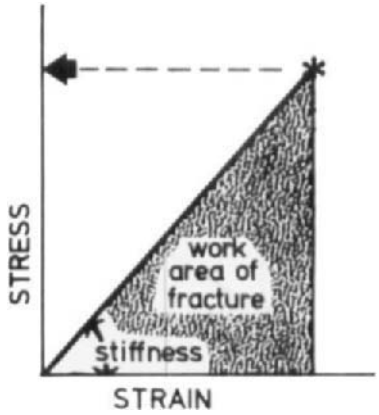

Fig. 1. Simple interrelationships of stress (force/area), strain (change in length/original length), strength (stress at failure, large arrow), stiffness (stress/strain) and toughness (area beneath the curve, shaded).

more or less nonsense. Figure 1 shows all these parameters. A more complete account is found in Vincent (1982b). Since toughness, which is closely related to resistance to fracture, is measured as work, a strong material is not necessarily a tough one. Fracture involves the failure or cracking (not necessarily the breaking into pieces) of a material. A crack must first be initiated. Griffith (1921) showed that small imperfections can act as initiators, but that for a given stress level, these imperfections must be above a certain, or critical, size. Such cracks are commonly called "Griffith cracks". The initiation of a crack therefore depends to some extent on strength. Fracture of the molecular bonds at the tip of the crack also depends on the strength of these bonds, but the shape of the crack tip, especially if it is sharp, has the effect of concentrating stress in that region, thus mediating the propagation of the crack. Once initiated, the crack can be propagated in one of three ways: by tension (giving crack-opening), by shear (giving edge-sliding or in-plane shearing) or by transverse shearing (giving out-of-plane shearing or tearing) (Fig. 2). In order that a crack can be propagated through the material, energy must be provided in the form of strain (stretching or shearing). In a brittle material, which requires relatively little energy for fracture, a large part of the strain energy finds its way to the crack tip and the fracture surface is relatively smooth with little apparent texture. An example is glass, which has a high shear stiffness and produces fractures with a shiny, and therefore smooth, surface. Toughness can be increased by a number of mechanisms, all of which increase the amount of energy required for fracture and all of which can be present in a tough material. Mechanisms include the following:

1. The strain energy is unable to reach the crack tip, either (a) being dissipated by, for instance, plastic yield and failure of the material remote from

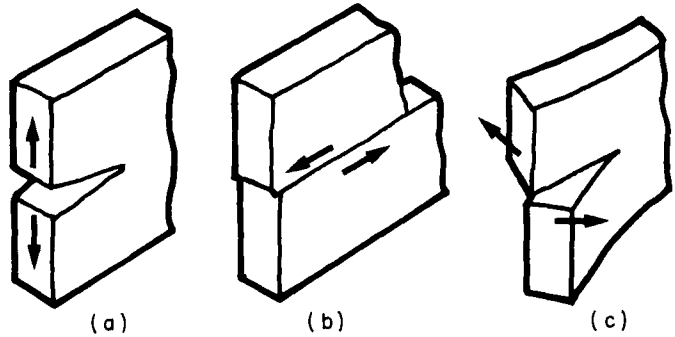

Fig. 2. The three modes of fracture: (a) tension; (b) edge-sliding or in-plane shear; (c) tearing or out-of-plane shear.

the crack or, (b) not being transmitted at all since the shear stiffness of the matrix material is too low (evidenced by a J-shaped stress–strain curve, common in soft tissues; Mai and Atkins, 1989).

2. The total energy required for cracking is raised, e.g. the fracture surface is very convoluted and therefore of large area or the material at the crack tip deforms plastically.

3. The stress at the crack tip is de-focused by, for example, increasing its radius of curvature (Fig. 3) or by the Cook–Gordon effect (Fig. 4).

4. As the crack opens, fibres or filaments extend across it dissipating energy by their own deformation or by friction as they pull out from the bulk of the material.

The fractured surface of cells usually appears quite clean at high magnifi-

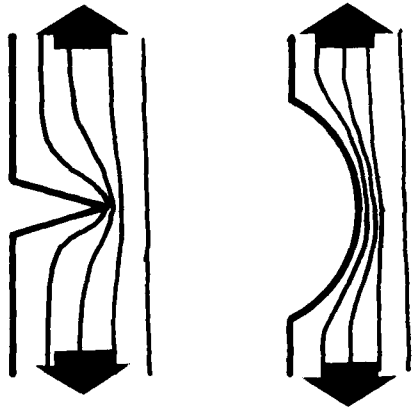

Fig. 3. The effect of a sharp notch in concentrating stress at its tip. The narrow lines represent transfer of stress through the material. The concentrating effect can be avoided by allowing the tip of the notch to become rounded, as happens in very stretchy materials. See Gordon (1976) for a fuller account.

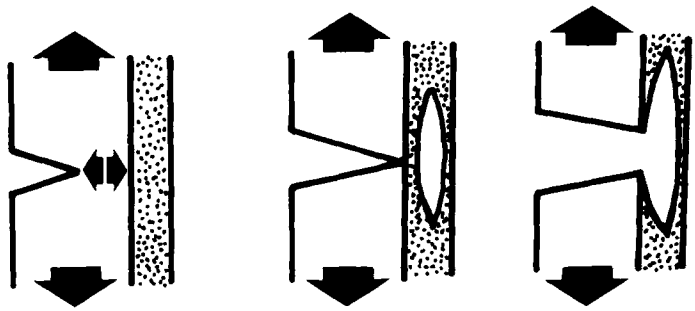

Fig. 4. The Cook–Gordon crack-stopping mechanism in a material under tension (large arrows). Ahead of the crack there is always a small stress orthogonal to the main one (small arrows). Given a weak interface ahead of the crack (the shaded area), this small stress can open up a crack-blunting hole. See Gordon (1976) for a fuller account.

cation which suggests brittleness, but Considine (1982) observes that the cell walls of grape skin fail by a viscous flow mechanism.

II. TECHNIQUES FOR MEASURING FRACTURE PROPERTIES

In order to be able to measure the fracture properties of plant tissues it is necessary to use "proper" engineering and materials science methods. Botanists have frequently found this difficult, using inappropriate or even nonsensical units of measurement such as grams force per gram dry weight, though there are instances where such an approach can point to generalizations (Blahovec, 1988). Even so, Blahovec found it impossible to show a simple relationship without the use of the double-log plot, a transformation which is notoriously reliable in producing straight lines from otherwise intransigent data. It would make better sense to investigate individual plants and mechanisms. There are also numerous papers where the sums are obviously wrong with numerical values, for example, stiffness, up to a thousand times too low (e.g. Batal *et al.*, 1970; Hankinson and Rao, 1979). This general difficulty with comprehension may not always be the fault of the botanist since it can be difficult to enlist the services of an engineer! There are problems both of the complexity and credibility of the subject matter. It can also be difficult for a botanist to communicate with an engineer since the assumptions, vocabularies and jargon of the two disciplines can be so different. In addition, fracture mechanics is not always well understood even by engineers, let alone the added complications with viscoelasticity and fibrous materials which occur in nearly all biological materials.

One can use a number of simple fracture tests. These give repeatable and very similar results in terms both of units and of the magnitude of the values. The uniformity and cross-compatibility of these results is important for two

reasons. If two different types of test give the same result, then the property being investigated is very probably a real one, is a function of material rather than structure and is not just a result of the type of experiment, the method of measurement or the expression of the results. By contrast, if two different tests give different results, this may be due either to inadequate understanding of the nature of the experimental technique or it may be that the test piece is failing in two different ways in the two tests indicating some influence from the structures within the test piece. For instance, a test involving cutting could give the same figure for fracture toughness in two plant materials, but a test involving a free-running crack might give a higher figure for one of the materials. Under such circumstances one would suspect a structural difference between the two materials, for instance that the tougher material had fibres which were cut through in the first test but were pulled out in the second test. Such differences can be confirmed by examining the fracture surface.

Fracture toughness can be measured and calculated in various ways. For each of the test geometries described below there is a specific mathematical solution which makes assumptions about the material and the test and allows the calculation of toughness from a number of more or less simple measurements. General information is available from Atkins and Mai (1985). However, biological materials frequently transgress these assumptions being anisotropic, very stretchy, inhomogeneous or of an odd shape. There is a pragmatic way of coping with these problems which may well be easier than the more "respectable" mathematical approach. The area enclosed by the force–deformation curve, which a mechanical test generates, represents work (force × distance). So with a little care it is possible to measure that area, hence the work, and express it in terms of the area of material cleaved. It is very tempting, but very foolhardy, to deduce that in a tensile test of a linearly elastic material, the energy to break is the area of a triangle (Fig. 5), i.e. 0.5 × force × distance.'However, this type of test cannot account properly for the energy already stored in the specimen during the test. This is best illustrated by the behaviour of a specimen in such a test: frequently there is a loud noise at failure and bits of the test piece may be violently ejected. These events imply that the test piece contained stored elastic strain energy which was not dissipated by the measured fracture. Therefore the simple graphical estimate will frequently give an estimate of toughness which can be orders of magnitude too high. To choose an extreme example, the work of fracture of glass is of the order of $1\ J\ m^{-2}$, but the amount of energy stored in a typical specimen just before fracture is so great that the (force × distance) approach would give an answer 1000 times higher than this.

Great care is necessary to ensure that the curve encloses *only* the fracture energy and does not include this stored elastic strain energy. The easiest way to ensure this, but one which is not always practicable, is to use a test in which the crack grows in a stable fashion (such as the wedge test, the

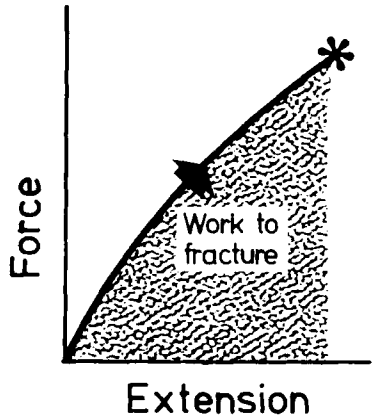

Fig. 5. Simple graphical determination of the work to fracture. If the test piece breaks suddenly this method (given by the shaded area) will give an overestimate, perhaps up to 1000 times the real work of fracture.

"trouser-tear" test, the double cantilever beam test or the notched beam test—see below) and the test piece is unloaded (i.e. returned to its original length or shape, or until the recording device shows that no load is still being applied) before the test piece has broken into two pieces (Fig. 6). In a few instances, where the material is relatively tough, it is possible to use this technique even where the propagation of the crack is normally unstable. An example is a tensile test of a notched strip of apple tissue. If this is tested at a sufficiently slow rate of extension such that the force has fallen to zero just as the crack has travelled across the specimen and it has completely broken, then there can be no strain energy left and the area under the curve represents only the energy used for fracture. In this manner any elastic strain energy is

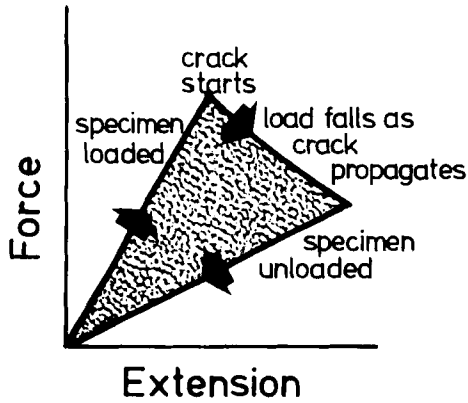

Fig. 6. A better way of determining work of fracture graphically. The specimen is unloaded slowly, so all the work enclosed by the curve (shaded area) is used by propagating the crack and there is no elastic energy left in the specimen at the end of the test.

discounted from the final reckoning, and the energy which the force–deformation curve encloses is that required to propagate the crack. The great advantage of this graphical technique is that it is entirely independent of any mathematical model and the assumptions involved in generating such a model. It is therefore one of the few ways in which measurements can reliably be made on complex plant structures and materials. All the tests described below can be dealt with using this graphical approach (commonly called the 'work-area' method), so there is a remarkable dearth of mathematical formulae in this review.

There is another problem. Any piece of plant tissue will have a number of imperfections (scratches, nicks, notches, cuts, and "natural" openings such as stomata), whose size, nature and distribution are difficult to control or predict. Depending on the nature of the material, these imperfections can affect, or even direct, the mechanisms of failure. For instance, they can initiate a crack. This is because any imperfection will have the effect of concentrating stress around it, more especially at sharp corners (Gordon, 1976). Smooth corners and edges are important in controlling fracture. One strategy is to confine the deformation to a very small area, effectively limiting failure to a small zone. This is achieved in the "trouser-tear" test and by techniques involving cutting or wedging. A second strategy is to introduce an imperfection larger than any of those already in the test piece. This is commonly done by notching or cutting the specimen.

A. MACHINERY

A great variety of machines for testing materials is reported in the literature. The "universal" test machines made by Instron are so universal that many workers talk of an "Instron" test. There are, of course, many other makers of test machines. With any such machine it is important that it is considerably stiffer than the test piece, and it is the subtleties of design in this area which make it most advisable to use a proprietary machine rather than a home-built one. A "soft" machine will, in the limit, be uncontrollable without special precautions, giving rise to an unpredictable and unmeasurable exchange of strain energy between the machine and the test sample. This is especially important with fracture tests, where strain energy and its partitioning within the test sample is so important (Atkins and Mai, 1985). The commonest mistake is to put a coil spring in a series with the test piece and estimate force from the displacement of the spring. It is highly likely that the spring will store more strain energy than the test piece, which is not important until fracture starts, but after that—catastrophe! In the absence of a "proper" test machine a dead-weight system, such as hanging a bucket from the test piece and gradually filling it with water or sand (e.g. Delf, 1932), can be used for measurements of strength, but this is rarely adequate for measurements of fracture

toughness, since as the specimen breaks it becomes more compliant and the constant force translates into an increasing stress as the cross-sectional area drops. Fracture measurements are best made with a constant rate of displacement (Atkins and Mai, 1985).

B. TEST MORPHOLOGY

1. Pulling

The simplest test morphology is the tensile test. However, there are some traps for the unwary. When a strip of material is extended it usually becomes narrower. At the ends where the specimen is clamped into the machine this narrowing is prevented and the material will appear stiffer as a consequence. In order to overcome this effect it is necessary to have the sample eight to ten times longer than wide. These "end effects" may then be small enough to be ignored, though it is often still necessary to measure changes in length from the central zone of the specimen rather than from the deflections recorded by the test machine. A counsel of perfection would be to have a waisted or dumb-bell-shaped specimen. The nature of clamping at the end can also be a problem due to the cellular nature of most plant material. The cells collapse when the clamp closes and the material is irreparably damaged and weakened at the ends, leading to spuriously low readings of force. There are various solutions to this problem, depending on the nature of the tissue. For instance with apple or potato parenchyma slices, sticking the specimen onto aluminium end tabs with "super glue" and holding the tabs in the clamps; with the lamina of *Laminaria*, wrapping the ends of the specimen in absorbent tissue before clamping. The tensile test is probably suitable only for flat specimens—excised strips, or leaves or laminae—although stems can be tested if suitably modified clamps are used.

The tensile test on a notched specimen can provide both the work to fracture (toughness) and information about the ease of propagating a crack through the material. The notch directs the fracture so that the test piece does not break at the clamps; depending on the material it may also cause a stress concentration. In a plot of strength versus the relative length of the notch (expressed as a fraction of the total width of the specimen) a straight line (Fig. 7a) indicates a "notch-insensitive" material which can sustain damage without being greatly weakened. Grass and *Laminaria* lamina fall into this category (Vincent, 1982a; Vincent and Gravell, 1986). If the line falls lower (Fig. 7b) it indicates that the material is "notch-sensitive" and can be weakened by the presence of small imperfections. This is potentially dangerous for the integrity of the material since a small crack may severely weaken the material. There is a problem when using this test in calculations of fracture toughness in that the formulae used are suitable only for isotropic homo-

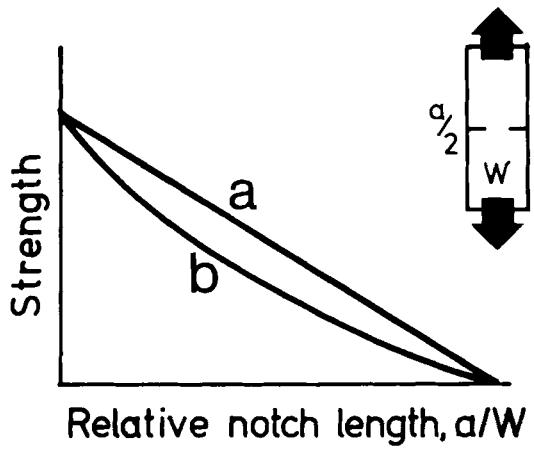

Fig. 7. A simple test of notch sensitivity. Strength (calculated on the nominal cross-section area) is reduced by cutting into the specimen thus reducing the load-bearing section area. If this is the only effect the cut has (curve a) then the material is "notch-insensitive". But if the strength is reduced in greater proportion than this reduction (curve b) then the cuts are causing stress concentrations and the material is "notch-sensitive".

geneous materials at low strain. Plant materials fulfill neither of these criteria, but the formulae still seem to work in that they give sensible answers. There are other parameters available from these and other tests, such as the stress–intensity factor, K. These can give useful information and their derivation and discussion about them is dealt with by Atkins and Mai (1985). Care should always be exercised when using engineering formulae, derived for specific sets of assumptions, with data from biological materials, which rarely conform to these assumptions.

1. Single-edge notch:

$$E\,R = \sigma^2\, W(7.59(c/W) - 32(c/W)^2 + 117(c/W)^3)$$

2. Double-edge notch:

$$E\,R = \sigma^2\, 2W(\tan((\pi c)/(2W)) + 0.1\sin((\pi c)/W))$$

3. Centre notch:

$$E\,R = \sigma^2\, 2W\tan((\pi c)/(2W))$$

where E is stiffness, R is fracture toughness, σ is stress at break, c is the length of the notch (for edge-notches) or half the length of the notch (for a centre notch) and W is the width of the specimen. The equation is best solved graphically by rearranging the expression such that R can be determined as the slope of a straight line.

There is a whole class of very useful tensile tests grouped as double cantilever beam. The specimen is prepared as Fig. 8a and loaded and unloaded as

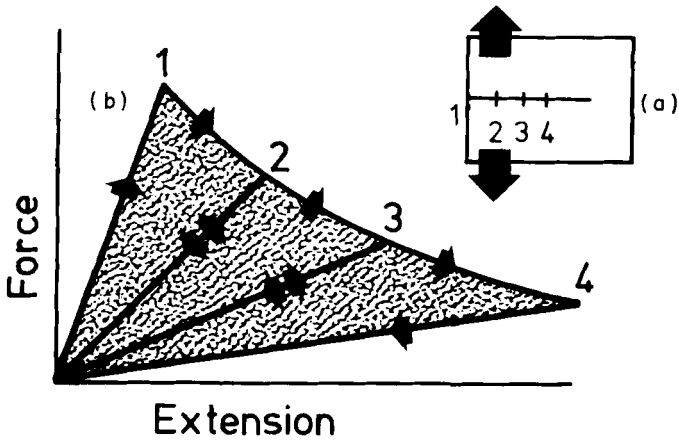

Fig. 8. A development of the work-area experiment. Loading and unloading the specimen (a) and allowing the crack to progress from 1 to 2 to 3 to 4 in three cycles generates the curve shown in (b), from which three separate estimates of work to fracture (curve area/area of fracture) can be made.

indicated, noting the distance through which the crack has been propagated. The resulting force–deflection curve (Fig. 8b) gives an enclosed area from which the toughness can be measured graphically. If the specimen is loaded and unloaded a number of times, each time the advance of the crack being noted, then effectively one can perform a number of tests on the same specimen. Each separate area on the force–deflection curve, being associated with a separate crack surface, can be treated in isolation. The crack can usually be controlled fairly easily, to the extent that very inhomogeneous materials such as orange skin and similarly layered materials can be tested. Various subtleties in the preparation of the specimen and the way of loading it can make the experiment and calculation easier (see Atkins and Mai, 1985).

Another test which can be performed with a simple tensile test machine is the "trouser-tear" test (Fig. 9). This gives a transverse shear fracture. The specimen is prepared as a strip and a starter crack cut longitudinally. The two ends of the "trouser legs" of the specimen are pulled apart to propagate the crack. The force–deflection trace gives a plateau representing the force required for propagation; the work is obtained from the area under the curve (Fig. 10). If the test piece is unloaded before it has fractured totally, the elas-

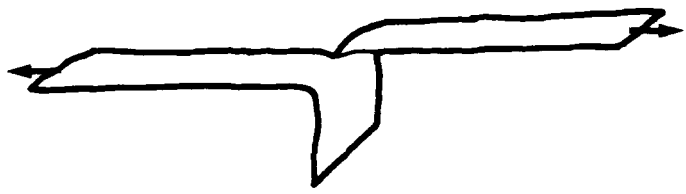

Fig. 9. Morphology of a "trouser-tear" test.

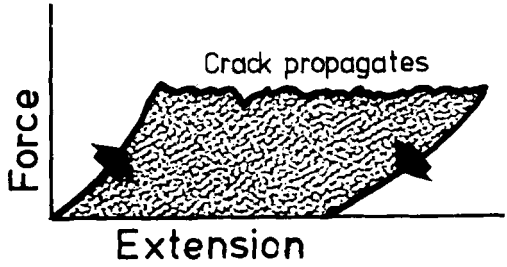

Fig. 10. Force–extension curve from a trouser-tear test. Once again, the area enclosed by the curve (shaded) represents the work to fracture.

tic strain energy stored in the two "legs" of the test piece can be removed from the calculation. However, this test is of use only where the fracture surface is relatively simple. It produces very poor results with grass leaf since the area fractured is variable due to the way the diverse cell architecture directs the crack out of the vertical. It works well with *Laminaria* lamina where the crack has less structure to deflect it.

2. Wedging and Cutting

There is another standard fracture test which involves crack-opening and has the same geometry obtained in a tensile fracture test. Strain energy is fed into the sample by a wedge forcing the two "ears' of the sample apart (Fig. 11a), which thus store the strain energy (Atkins and Mai, 1985; Khan, 1989). The advantage of this is that the test piece can be small (less than a few millimetres in any dimension) and does not have to be attached to a mounting. The specimen is cut only in the first part of the test when the wedge is entering the sample. In the later stages of the test there is sufficient strain energy to initiate and maintain a free-running crack within the material. The amount of strain depends on the included angle of the wedge—a larger angle forces the two ears of the specimen further apart thus imparting more strain energy. Thus a wider wedge has to penetrate less in order to build up sufficient strain energy for a crack to propagate. The amount of strain energy also depends on the stiffness of the material, stiffer material storing more energy for the same amount of strain. Once the crack has begun to propagate ahead of the tip of the wedge, the force required to continue pushing the wedge into the material (and hence the amount of energy being fed into the material) drops and the stored strain energy is used up by the advancing crack tip (Fig. 11b). It is possible to use this test in a purely comparative way, comparing forces, deflections and energies directly without the need for calculation. Since the test relies on strain energy storage within the sample, it is essential that all samples for such comparison be of the same size and geometry.

A test which is related to the wedge test uses an instrumented microtome (Atkins and Vincent, 1984; Willis, 1989). Instead of storing strain energy in

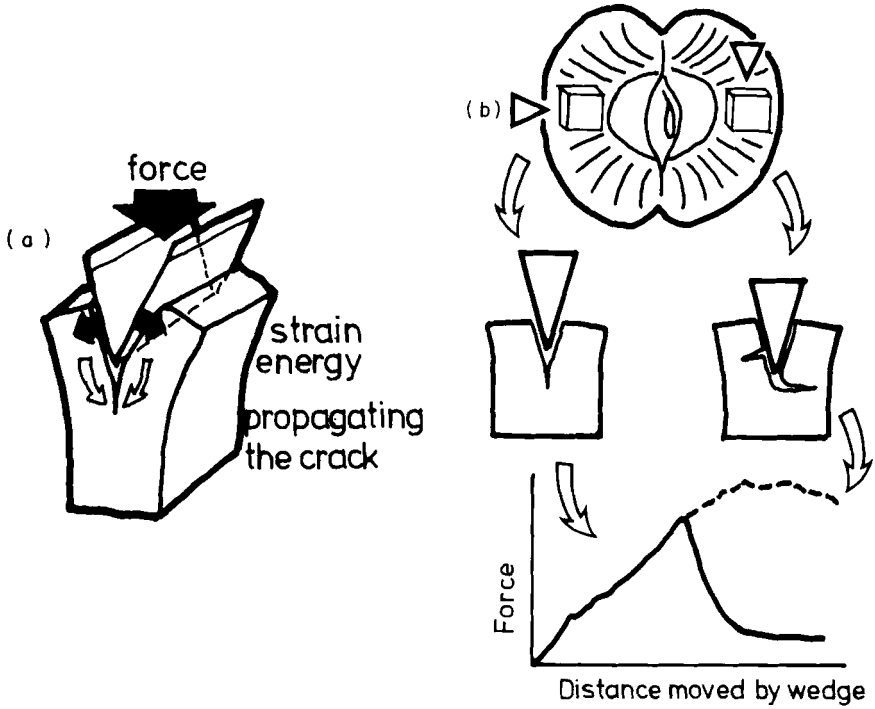

Fig. 11. The wedge test—a crack-opening test directly equivalent to a tensile test. (a) The force on the wedge is transmitted to the two "ears" of the specimen, forcing them out such that they store strain energy which can be fed to the advancing crack once the failure stress has been reached; (b) the wedge test applied to apple showing failure modes in "brittle" and "tough" directions with associated traces.

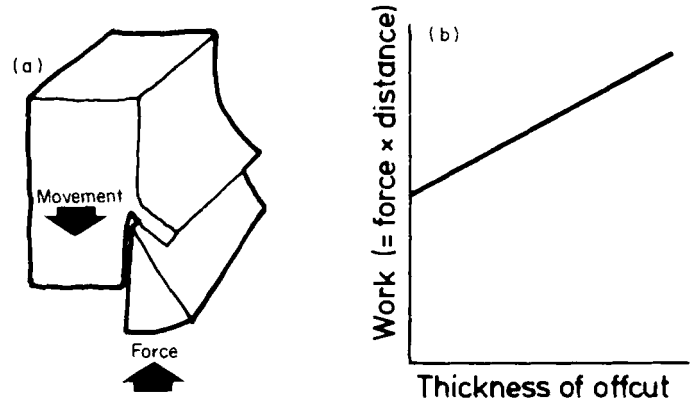

Fig. 12. Work of cutting. (a) The test piece moves past the knife blade generating a force as a slice is removed. The force is composed of work of cutting and work of curling; the latter varies with the thickness of the offcut. Thus the work of cutting at zero thickness of the offcut can be estimated graphically (b).

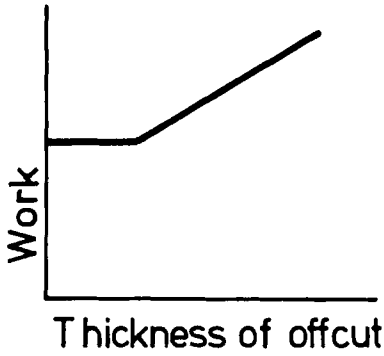

Fig. 13. Work of cutting a cellular material. At a finite section thickness the cell walls are so damaged that they present no resistance and require no work of curling, leaving only the work of cutting as a plateau. From Atkins and Vincent (1984).

the test piece and allowing a free-running crack to develop, the microtome blade cuts throughout the test. The work which the blade does in cutting is revealed as a downward force which can be detected by transducers in the knife mounting. The blade is performing two jobs: it is driving a crack through the material and it is also bending or curling the section away from the block (Fig. 12a). In order to partition the force into these two components (work of cutting and work of curling) it is necessary to plot total work against section thickness. This produces a variety of responses with different materials, the general response being linearly increased force with increasing thickness of the section (Fig. 12b). The work of cutting (which is now a component of the work of fracture) is found by extrapolating this line to zero thickness, where there can be no work of curling. The force so determined is divided by the width of the specimen to give a value for the work of cutting. With a cellular structure such as is found in plants, the section ceases to curl off the blade at a thickness equivalent to about three cells and the force–thickness curve comes to a plateau at the same section thickness (Fig. 13): at this dimension the structure of the cells ceases to have any significant shear stiffness. The fracture work measured by this method represents a minimum: since the crack has its course through the material defined almost completely by the knife (assuming optimal cutting conditions) it is not possible for toughening mechanisms to be expressed which work by deflecting the crack. Thus wood has the same work of fracture in all directions when measured with the microtome, but a free-running crack across the grain gives a work of fracture which is 200 times higher. In a material with few or no fibres, such as potato parenchyma, which essentially shows brittle fracture, the instrumented microtome gives the same result as other tests. This can be very useful if the test specimen is very small. Additionally, since the knife

blade registers the force to fracture only in that part of the specimen which is just ahead of the cutting edge, variations in texture of the specimen give rise to variations in the force recorded. Thus the instrumented microtome acts as a mechanical microscope and can give extra information about mechanical inhomogeneities (Aubert *et al.*, 1984; Willis, 1989). In theory, records of cutting force taken in different directions across the same face would allow micro-determination of fracture properties of cells and structures. To date this has not been attempted.

3. Compressing and Bending

Tests in compression are more complex than tensile tests in that the structure of the material becomes more important. In parenchymatous tissues, compression can result in tensile, compressive or shear failure of the tissue and tensile or shear failure of the cell walls depending on orientation and cell volume–fraction effects. In compressive fracture in plant materials, failure can be initiated by imperfections, so that failures can occur remote from the site of eventual fracture and give a much higher value of toughness ("apparent fracture toughness") than a tensile test does, where it is simpler to control the site of fracture by putting large notches into the test piece. For this reason the simple compression test is useful for estimates of strength but will give unreliable and high estimates of the work of fracture (which is a parameter of the material and should therefore be independent of the method of its measurement). The specimen must be no more than about eight times longer than wide. At aspect ratios of 20 and above the specimen starts (in engineering terms) to behave more as a strut than a column. Compressive failure occurs normal to the direction of the force, commonly appearing as a line of cells whose walls have fractured (allowing the cell contents to escape) and buckled (Fig. 14). The force–deflection curve clearly shows a drop in force as successive layers of cells collapse across the specimen catastrophically. Failure, apparently in shear, occurs at an angle of about 45° to the direction of applied force and is much more controlled. The force–deflection curve shows no features associated with specific failure events. Theories for the behaviour of cellular materials in compression have been developed and tested (Gibson and Ashby, 1988).

Various types of beam test (Fig. 15) are exceedingly useful with parenchymatous plant material (including non-fibrous storage tissues), but less so with inhomogeneous material such as fibrous stems and petioles, at least where fracture properties are to be measured. The beam presents few problems with mounting in the test rig. There are, as usual, some problems. With point loads, a beam will experience both shear and tensile/compressive stresses when it is bent. As the span of the beam increases with respect to its depth (i.e. as its aspect ratio increases) the shear stresses become relatively less important. It is difficult to say, for any particular material or structure, at what point the shear forces cease to be significant. The only way is to experiment.

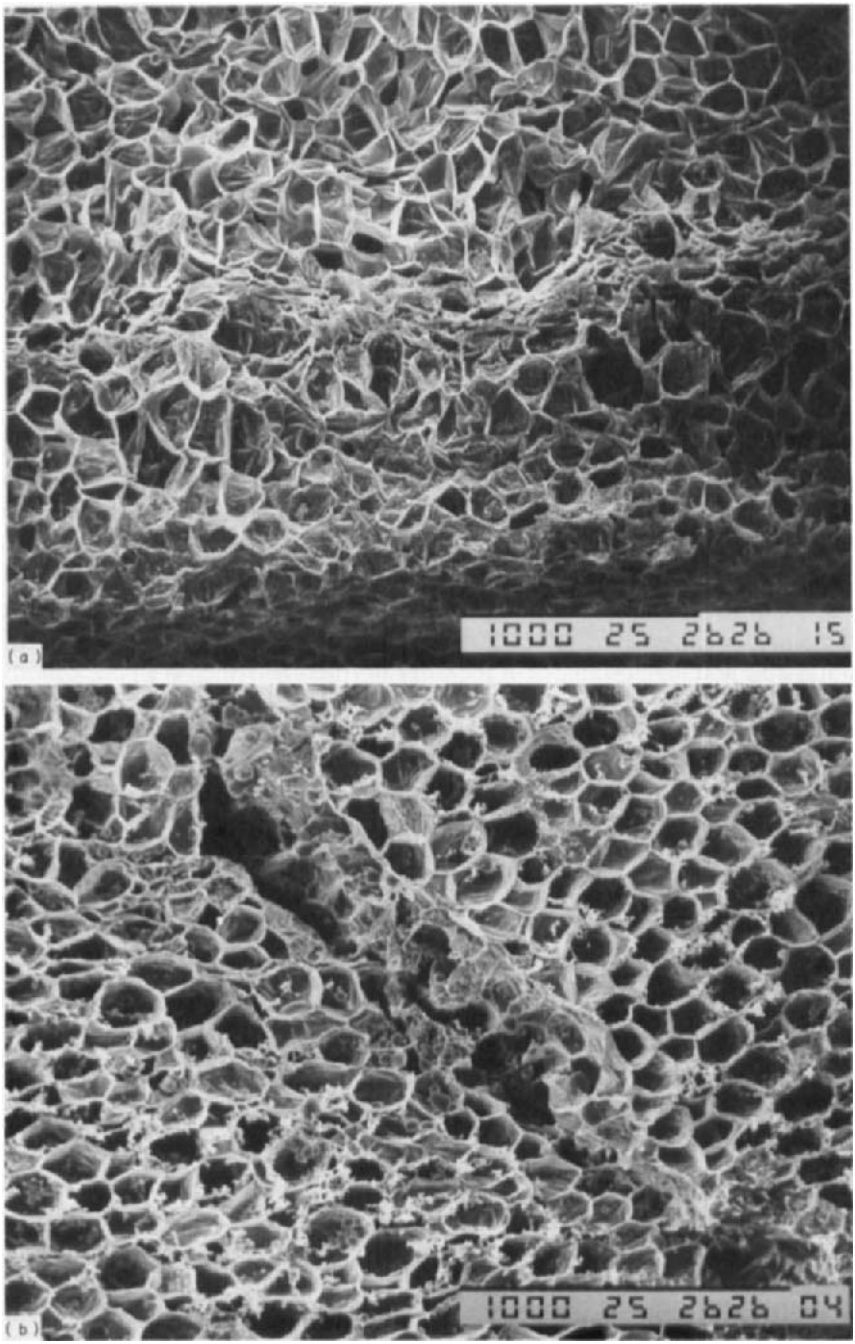

Fig. 14. Failure in parenchyma due to compression as seen in SEM. (a) A plane of cells fails across the specimen (an apple) or (b) the cells fail in shear (a potato). Compression was applied vertically. Scale line = 1 mm. From Khan (1989).

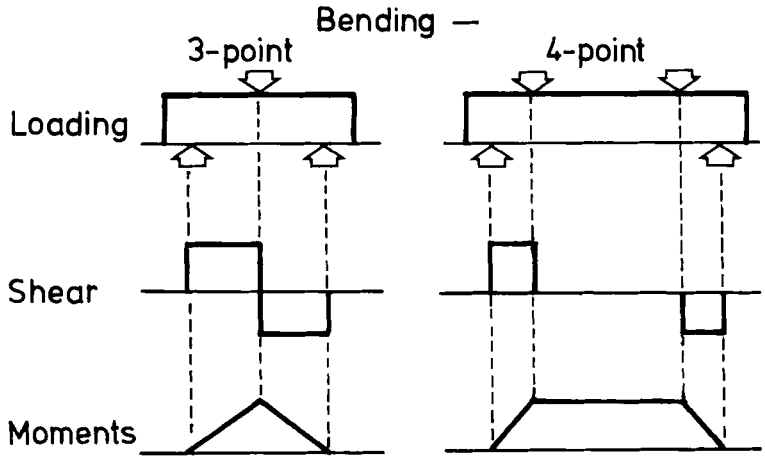

Fig. 15. Two types of beam test showing morphology, shear and bending moment. The advantage of the four-point test is that the part of the specimen between the inner pair of supports is uniformly stressed.

A span-to-depth ratio of up to 20 is not unknown. The beam may then be notched in order to direct the fracture (Atkins and Mai, 1985) and the toughness estimated from mathematical manipulation of the recorded forces and deflections or by direct measurement of the force–deflection curve. As usual, the best estimate for use with the latter is obtained by loading the specimen slowly so that there is no stored elastic energy remaining when fracture is complete, or unloading the specimen before it has fractured fully and estimating the energy stored elastically. If the notch is too small, or absent, it may be impossible to control the rate of fracture, which will inevitably lead to an overestimate of the toughness. To some extent this can be overcome by using a triangular notch, which is especially developed to introduce some controllability into brittle materials (Tattersall and Tappin, 1966). Another factor is "remote yielding" such as happens when the specimen is bruised by the bars which support it. If this is a significant factor (determined by noting the area enclosed by a hysteresis curve obtained by loading and unloading the specimen, but not breaking it) then the test may not be appropriate. This is a matter for judgement.

Many tests on plant materials are conducted at high rates of loading in order to simulate impact. This is of especial importance to horticultural and agricultural business where products can be damaged during handling. The general technique is to drop a load, either freely falling or on the end of a pendulum, onto the test specimen and to calculate energy input from the movement of the load before and after impact. This is then equated with the damage inflicted.

III. FRACTURE PROPERTIES OF PLANT MATERIALS AND STRUCTURES

A. CELLULOSE

Cellulose is very strong. Its theoretical strength (calculated from the crystalline structure) is 25 GPa. The strength of real fibres depends on the winding angle of the cellulose in the cell walls (Jeronimidis, 1980), but in general terms the strength of bast fibres, which contain 35–40% water (Cohn, 1892) is about 0.9 GPa (Frey-Wyssling, 1952) and of wood cells, which contain about 25% water (Cohn, 1892) is about 0.5 GPa (Jeronimidis, 1980). Sclerenchyma from leaves of *Lolium perenne* breaks at about 0.5 GPa; bundles (including some vascular tissue) from the same plant break at about 30 MPa (J. F. V. Vincent, unpublished). Ambronn (1881) reported a strength of 0.1 GPa for collenchyma from a variety of plants. Esau (1936) quoted figures between 10 and 50 MPa for strength of collenchyma from the petiole of celery and about 5 MPa for the strength of the vascular bundle, bundle cap and xylem. She noted a tendency for collenchyma from older petioles to be stronger by a factor of two. Collenchyma contains about 60–75% water (Cohn, 1892) and is associated much more with parts of the plant which are growing (Esau, 1936). Many plants have relatively little fibrous tissue. The strength of the plant then arises entirely from the cellulose in the walls of the fluid-filled cells. The pressure within these cells can range from zero (in the wilted plant) to 50 atm (5 MPa). In the wall of a turgid cell in the outer part of the stem of a dandelion (*Taraxacum officinale*) the maximum tensile stress is about 10 MPa (Vincent and Jeronimidis, 1990). When the stem bends due to side (wind) loading, this turgor pre-stress is reduced to zero on the compression side of the stem and the stress on the tension side will be of the order of 20 MPa (Fig. 16). This is well within the strength range for load-bearing cellulose fibres. Thus the stem fails by buckling of cell walls rather than tensile failure of the cellulose, and stability turns out to be a problem of structures rather than a problem of materials.

B. PARENCHYMATOUS TISSUES

Fracture of plant tissues is most easily studied on simple cellular arrays such as are found in the parenchyma of apples and potatoes. Even in such apparently simple cases the modes of failure can be varied and complex—compression, tension and shear modes of failure can all be found in a bruise or compression test. What dictates the mode of failure is not always clear, since anisotropic arrangements of the cells, the presence of air spaces and the ratio of thickness of the cell wall to the diameter of the cell can all affect the type of failure. In addition, the degree to which the cells adhere can have a profound

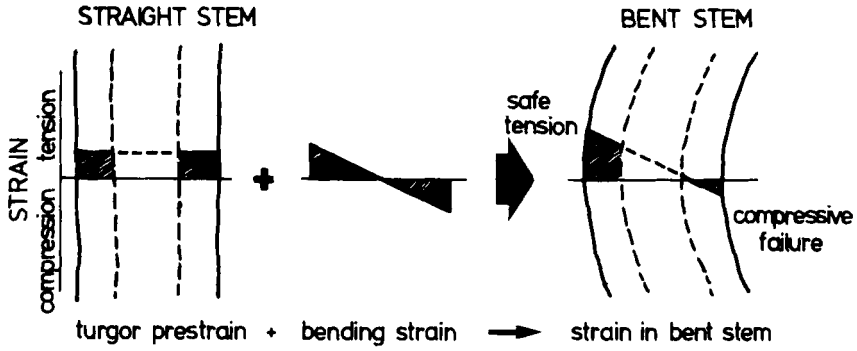

Fig. 16. Strain distribution in a stem stiffened by turgor, then bent. From Vincent and Jeronimidis (1990).

effect, since it controls the shear stiffness and therefore the transfer of strain energy within the material; the degree of adhesion can also control whether the fracture path goes between cells (as seems usually to happen in most varieties of apple) or through cells (in the potato, which has smaller, thicker-walled, cells which adhere more tightly). The fracture toughness measured in different modes of failure also varies. Since cellular materials are also of great interest to materials engineers, they have been well analysed and modelled (Gibson and Ashby, 1988).

Toughness measured by cutting gives the lowest work of fracture (Table I) since the toughening mechanisms inherent in the material are either inhibited or circumvented. For comparison, a tensile fracture test on potato (which has more or less isotropic parenchyma) gives a similar result to cutting (Table I). The inference that cell walls break rather than come apart down the middle lamella is confirmed by direct observation. This mode of fracture in tension occurs in potato probably because the cells are very cohesive. Thus tensile fracture of uniform, naturally turgid, parenchyma is essentially brittle. This brittleness must be due to two main factors: intrinsic brittleness of the cell wall and unimpeded transfer of strain energy.

Fracture in tension of notched strips of apple parenchyma is more complex due to the radial arrangement of air spaces in this tissue which introduces anisotropy which is morphological (Reeve, 1953) and therefore mechanical (Vincent, 1989; Khan, 1989). These spaces represent 15–40% (depending on the variety of apple) of the entire volume of the apple and appear to exert complete control over the path of a fracture. Not only is a higher stress required to enable the crack to propagate across the air spaces, but the fracture toughness in this orientation is significantly greater than that measured with the crack running radially along the air spaces. This is probably partly due to a Cook–Gordon type of mechanism which blunts the crack tip when it runs into an air space and thus disperses the stress concentration (Gordon, 1976), but it must also represent the difference between driving a crack along

TABLE I
Toughness of parenchyma from apple and potato
(all values in $J\,m^{-2}$)

Variety	Compression	Wedge opening	Tension	Cutting
Apples				
Cox's Orange Pippin	62.13	72.4	68.6[a]	
Bramley	60.94	50.5	46.3[a]	
Gloster	269.4	164.07		
Rock Pippin	762.5			
Norfolk Beefing	972.0			
Granny Smith			350[b]	
Delicious		211.6	250[b]	
Jonathan			250[b]	
Potatoes				
Record	376.0[a]	389.0		200
Bintje	541.0[a]			
Maris Piper	473.0[a]	332.5		
Pentland Dell	539.0[a]			
Sebago	1350.0[c]			
Pontiac	1230.0[c]			

[a] From Kahn (1989).
[b] From Holt and Schoorl (1984).
[c] From Schoorl and Holt (1983).

the air spaces which involves only separating rows of cells, and through the middle lamella between cells (Fig. 17a) or through the cell walls (Fig. 17b). Unfortunately other authors have been unaware of this anisotropy, although they frequently take care to control the orientation of their specimens. Since

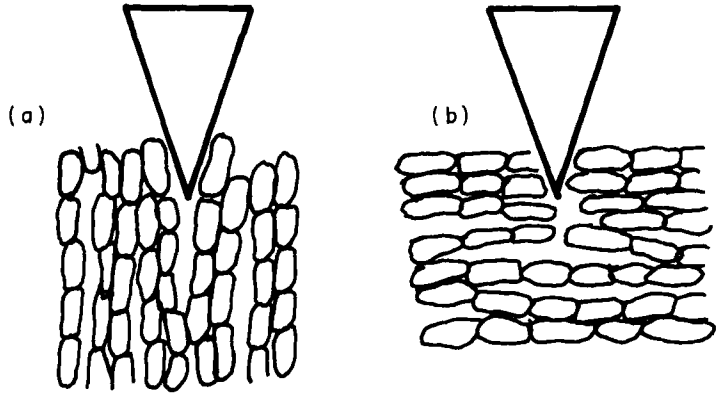

Fig. 17. Pushing a wedge through apple tissue is found to be easier along the air passages (a) than across them (b). From Khan (1989).

the major orientations in apple are radial, samples for mechanical testing which are taken tangentially (the commonest direction) therefore contain a variety of cellular orientations. Results are frequently highly variable due to this fact alone (Vincent, 1989), but this has not prevented the application of statistical models of failure (McLaughlin, 1987) although the failure criterion used in this study was stress rather than energy which is questionable both on theoretical grounds (see above) and experimental grounds (Holt and Schoorl, 1977). Of course, a statistical approach skates over the mechanisms involved.

A series of force–penetration curves for a wedge penetrating blocks of parenchyma of two varieties of apple is shown in Fig. 18 and the fracture toughness of three varieties of apple using a wedge with an included angle of 30° in this test are shown in Table II (Khan, 1989). The values are almost identical to those measured in the tensile test, emphasizing the equivalence between the wedge and tensile tests: both involve simple crack-opening. Once again there is highly significant anisotropy. This seems to be due to the air spaces continually controlling the course of the crack; a crack travelling across an air space will be at least blunted and frequently totally deflected (Fig. 11). This orientation effect can easily be detected when you eat an apple.

In compression, parenchyma cells can fail compressively in a single layer across the specimen (Fig. 14) or in shear at an angle (usually 45°). In both instances cells are rupturing since the failure plane turns brown on exposure to

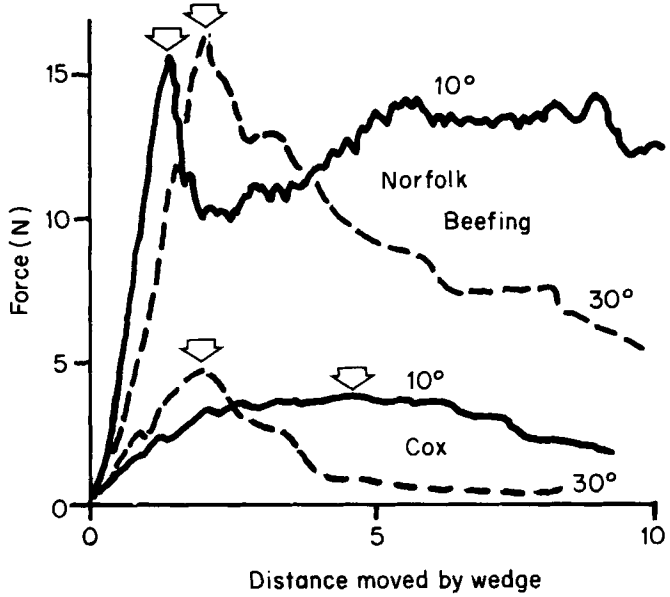

Fig. 18. A wedge (Fig. 11) with a large included angle (here, 30° or 10°) can store more strain energy in the specimen leading to a reduction in force needed to propagate the crack once it has started. Cox is a brittle apple, Norfolk Beefing is a tough one. From Khan (1989).

TABLE II
The wedge test on apple

Variety	Fracture toughness ($J\ m^{-2}$)		
	Radial	Tangential	Significance test
Cox	69.0	103.0	$0.05 > P > 0.02$
Gloster	194.0	341.0	$0.05 > P > 0.02$
Norfolk Beefing	668.0	1044.0	$0.001 > P$

air, due to the oxidation of phenols. It is therefore not true to say that shear failure represents debonding of cells (Lin and Pitt, 1986). In apple parenchyma, the mode of failure is entirely dependent on orientation (Khan, 1989). Radial compression (normal to the orientation of the air spaces; Fig. 19) produces compressive failure, tangential compression produces shear failure. As the specimens (cylinders 20 mm diameter × 12 mm high) are compressed they expand laterally, both at the bulk and the cellular level. If there

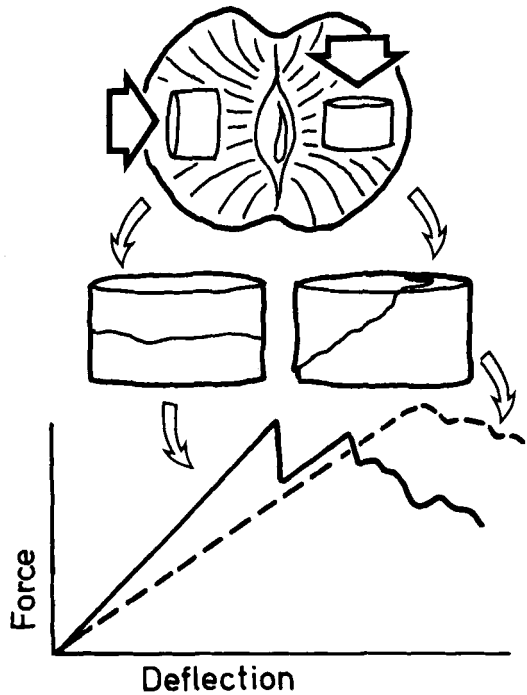

Fig. 19. Compressed apple tissue can show both "simple" compressive and shear failure depending on orientation. When a layer of cells breaks in compression (solid curve) there is a sudden drop in force. Shear failure (dashed curve) gives more progressive failure. The damage at the cellular level is shown in Fig. 14. From Khan (1989).

are lateral air spaces into which the cells can deflect, they can reach failure strain more readily and show the compressive type of failure. If the air spaces are normal to the direction of applied force the stiffness will be lower, but the cells will have no lateral spaces into which they can expand and the specimen will fail in shear, a mode of failure typical of a constant volume material. Thus potato parenchyma, which has hardly any internal air spaces, fails in shear unless the sample is below a certain (undetermined) size.

Energy absorption to failure has been studied in whole apples and potatoes at both high (impact) and low strain rates. There is an exceedingly good correlation between energy absorption and the area of the resultant cracks on potato, once the energy lost due to hysteresis has been discounted (Schoorl and Holt, 1983), leading to a reliable estimate of fracture toughness (Table I). A similar approach has been used with apples (Holt and Schoorl 1977, 1984), although in the latter study the observed reduction in work to fracture with storage was ascribed to an increase in the number of previously ruptured cells, a factor which leads, at least initially, to an *increase* in toughness. Both strength and toughness of apples reduce linearly with time—to more or less zero if the published graphs (which contain no data points) are correct (Holt and Schoorl, 1984). For a given energy absorption, impact of relatively long duration (i.e. relatively low loading rate) results in bruising, whereas a higher loading rate leads to more internal shattering (Noble, 1985). This is a result of the viscoelasticity of potato parenchyma. It is highly likely that there are other ways in which parenchymatous tissue can absorb damage, since it always shows a high hysteresis, at least on the first loading–unloading cycle (Pitt, 1984), and then shows signs of fatigue, suffering a reduction in stiffness and failing at lower loads after a number of cycles. None of these studies used the approach of fracture mechanics, leading Pitt (1982, 1984) to complain that fracture is an apparently random process rather then an accumulative one. This is to be expected with brittle failure which is characterized by low fracture energy and therefore a small length of critical crack. A short crack is more likely to occur by chance. Brittle fracture is none the less open to analysis given the proper tools developed in materials science.

Bruising can, or indeed should, be analysed as a fracture process, since it is due to cell walls breaking. The only difference is that the fractured cells are not confined to a single plane. The volume of bruised tissue in the parenchyma of apple cv. Granny Smith correlates strongly with the total mechanical energy absorbed but is totally unrelated to any elasticity parameters (Holt and Schoorl, 1977). The shape of the bruise is probably related to the distribution of strain, since a compressed Perspex sphere, viewed between crossed polars, shows a distribution of shear strain similar to the outline of a bruise obtained under similar loading conditions (Holt and Schoorl, 1982). The mode of failure is rate-dependent since the cell walls and the intercellular adhesives are viscoelastic (Pitt, 1982; Pitt and Chen, 1983). Thus static loading produces a smaller bruise for a given energy input than does a dynamic

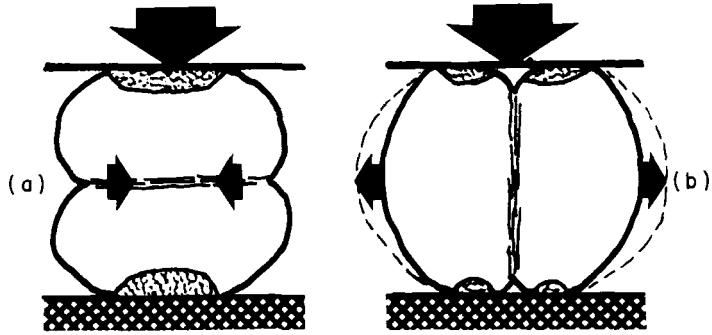

Fig. 20. The effect of compressing whole apples in different directions. Compression with the apple (a) horizontal, and (b) vertical. From Khan (1989).

load, suggesting that, given time, energy can be absorbed at sites remote from the bruise by viscous mechanisms. In addition, a bruise produced at high deformation rates is softer than that produced quasi-statically, suggesting that the strain energy cannot dissipate itself throughout the parenchyma if there is insufficient time available. In whole apples, the anisotropy due to the cellular orientations and to the presence of a core introduces further complications. Since the core is relatively stiff in tension it will resist deformation when the apple is compressed laterally and the tissue will bruise. When the apple is compressed along the line of the core it can expand laterally and absorb the deformation elastically. Thus loading in this direction produces relatively little bruising (Figs 20 and 21; Khan, 1989).

Effects of turgor are also apparent in fracture. Measuring toughness by cutting, Atkins and Vincent (1984) found that carrot is much less tough when turgid (210 J m^{-2}) than it is when flaccid (about 300 J m^{-2}). This difference is also easily appreciated without the need of a testing machine—turgid plant tissues are more brittle or "crisper". Crispness can be attained by putting the plant tissue into tap water (a mechanism which is understood) or by chilling it in a refrigerator (a mechanism which is not understood, but which may be related to the viscoelastic properties of the middle lamella; it seems likely that, at reduced temperature, the shear stiffness of the cellular array is increased simply by making the "glue" stiffer but this is speculation). When the turgor is reduced the tissue shows a lower modulus (i. e. becomes flaccid) but the strength remains constant (since the same number of cell walls remains to be broken; Pitt and Chen, 1983) or even increases (possibly indicating orientation effects in cell walls taken to a higher strain; Lin and Pitt, 1986). It follows from simple geometry (Fig. 22) that toughness increases as turgor decreases. Other, less satisfactory, ways of measuring toughness (e.g. with a penetrometer) also show this change with turgor (Lewis, 1982). Turgor pressure has been associated with mode of failure in apples in compression (Lin and Pitt, 1986) where, at around normal turgor pressures, failure is in shear, but at lower turgor it occurs by failure of a single layer of cells in a plane

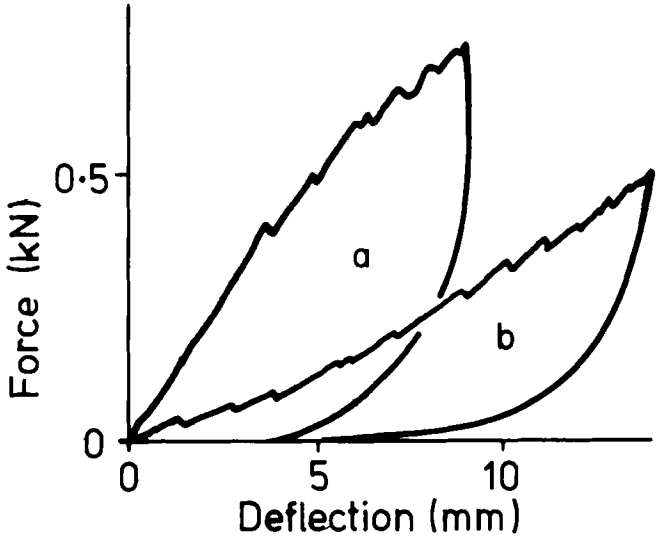

Fig. 21. Force-deflection traces from the experiments shown in Fig. 20. Compression with the apple (a) horizontal and (b) vertical. From Khan (1989).

normal to the direction of compression. Both modes of failure are apparent in fresh apple tissue dependent on orientation (Fig. 21; Khan, 1989). Unfortunately Lin and Pitt (1986) did not report the orientation of their samples. Failure zones in both modes of failure turn brown on exposure to air indicating that cells have fractured. Local deformation of the cell walls under these loading conditions can be very complex showing compression, tension and shear (Gibson and Ashby, 1988) and it is not really possible to say, as do Lin

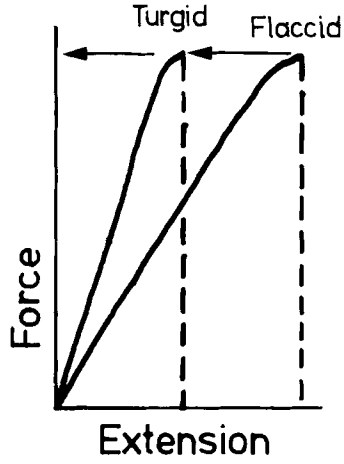

Fig. 22. When the turgidity of parenchyma is reduced the stiffness drops but the strength (horizontal arrows) does not, leading to greater strain to failure and greater work to fracture.

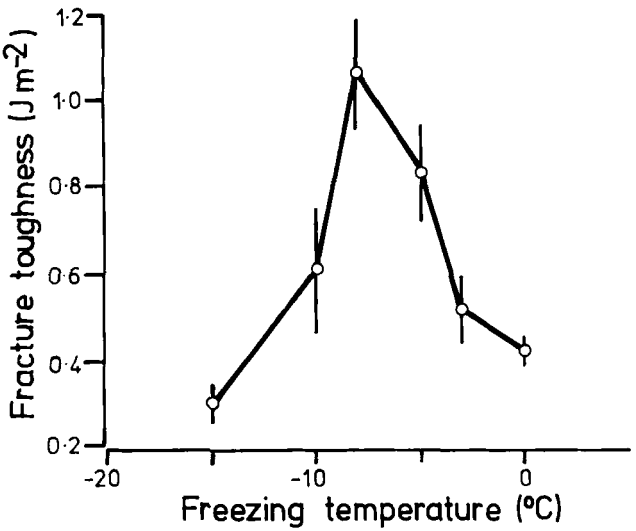

Fig. 23. A limited amount of damage caused by freezing can increase the toughness of parenchyma (here it is from the potato cv. Maris Piper). As the damage increases the parenchyma is eventually weakened totally. The sample was cooled to the temperature indicated at 1°C min $^{-1}$ then brought back to room temperature for testing. From Khan (1989).

and Pitt (1986), that specific types of macro-failure (e.g. shear, compression) are associated with specific types of micro-failure.

An additional factor is damage to the cells. Parenchyma from apple cv. Ida Red soaked in tap water yields at a lower maximum stress than that soaked in isotonic or in hypertonic mannitol (which makes the cells flaccid). This was shown to be due to massive rupture of the cells at the very high turgor pressures induced by tap water (Pitt and Chen, 1983), leaving large spaces, although an elasticity model (Pitt, 1982) predicted that an increase in initial turgor pressure, by prestressing the cell walls, reduces the amount of overstress required to rupture the cell walls. The two mechanisms can be differentiated by the strain to failure which will be higher in the damaged tissue. Such damage can have the surprising effect of making the tissue tougher. Similar effects are found with damage due to freezing (Fig. 23) or cooking (Khan, 1989). In each instance the tissue was taken to a temperature above or below ambient, held at that temperature for 10 min, brought back to room temperature and then tested, both in tension (single-edge notch) and with the penetrating wedge. These tests are both "crack opening". The likely mechanism of toughening is that the presence of a space within the cellular array allows greater local displacement of the tissue. The stress concentration at the crack tip can then be dissipated, partly due to blunting of the crack tip as it enters the hole and partly due to absorption of energy by plastic deformation around the hole, even when the hole is remote from the crack (Fig. 24). Basically, the holes allow spatial rearrangement in all parts of the specimen

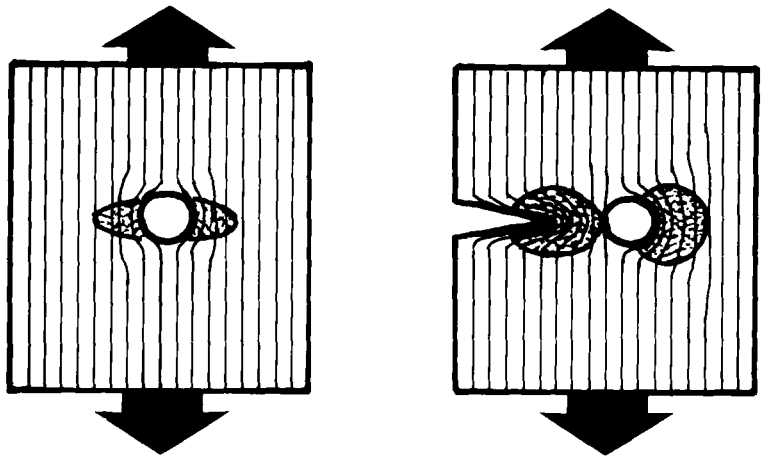

Fig. 24. The effect of damage on enlarging the volume of deformation remote from the damaged area or advancing fracture (shaded), thus increasing the work to fracture. The closeness of the lines indicates the relative stress intensity.

giving plastic absorption of energy. This effect continues with an increasing amount of damage, but the damage itself weakens the tissue, reducing energy absorption. Thus the toughness shows a peak at an intermediate level of damage. Model experiments using paper with holes punched in it showed that the effect was very marked with tracing paper, a brittle type of paper with no intrinsic yield mechanisms (Fig. 25a) but not apparent with "Kleenex" tissue paper which yields readily (Fig. 25b). Thus one would expect limited damage initially to increase the toughness of a brittle parenchyma but to decrease the toughness of a more yielding parenchyma such as that of ripe peach.

C. SKINS

The fracture properties of skins surrounding fruit are obviously important both for the fruit (since it provides protection from mechanical damage of various kinds during growth and also during harvesting, transport, packaging, etc.) and for the consumer wanting to get at the flesh beneath the skin. It is simple to show that the skin of a fruit is prestressed by cutting it, thereby releasing the strain: the cut gapes open. The magnitude and direction of prestress varies with growth in apples (Skene, 1980) and with the degree of insolation in plums (Mrozek and Burkhardt, 1973), where prestrain can reach 0.02. Small fruits of a particular variety tend to be more strained than larger ones (Skene, 1980). The pattern of fractures in the skin of a sphere will be random, since the strain in the skin will be uniform. However, once the

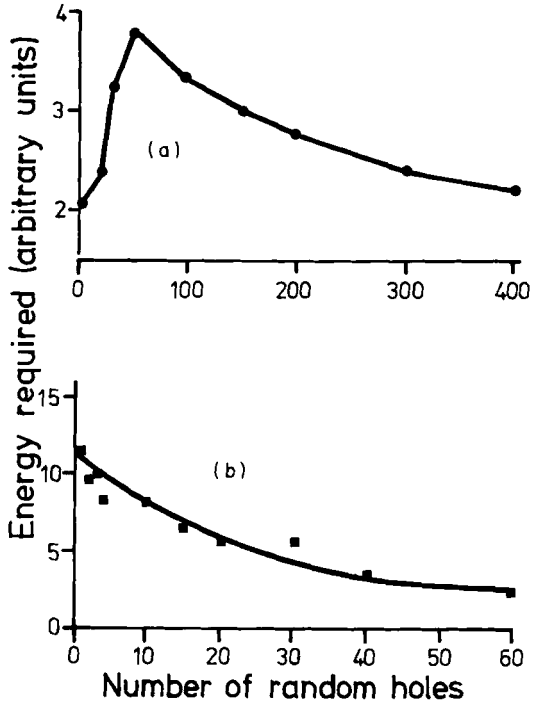

Fig. 25. Effect of holes on toughness in a brittle (a) and a ductile (b) paper, showing how holes can increase ductility. From Khan (1989).

shape becomes non-spherical, as in most fruits, fractures will tend to occur in directions normal to that of the principal strains. These strains are due to the shape of the fruit rather than the inability of the skin to grow as fast as the underlying tissues. Thus the skin of a prolate spheroid such as a plum will tend to fracture longitudinally, starting at the equator, whereas that of an oblate spheroid such as an apple or grape fractures circumferentially in the polar regions (Considine *et al.*, 1974; Considine and Brown, 1981; Considine, 1982). There is no evidence that the skin is anisotropic in a such a way as to resist fracture. The lenticels in the skin could act as starter cracks (Brown and Considine, 1982), in which case they would be expected to be orientated normal to the most probable direction of fracture and to be shorter than the critical (Griffith) length. There is some evidence for this in the plum where the shape of the fruit and the strains observed in the intact fruit indicate that a vertical split is much more likely. However, more splits are horizontal at an angle which is within a few degrees of the mean orientation of the long axis of the lenticels. The lenticels are reported to give a stress concentrating factor of 3 (Mrozek and Burkhardt, 1973). In the grape this effect has been avoided by reinforcement around the lenticels, although the reinforcement itself serves to concentrate stress, leading to the formation of micro-ring fractures around

TABLE III
Fracture properties of fruit skins

Fruit	Strength (MPa)	Strain at fracture	Fracture toughness (J m^{-2})
Tomato[a]	78.3	0.114	
cv(R) Scout[b]	15.4	0.28	
cv Rideau[b]	11.2	0.265	
cv(S) Moreton Hybrid[b]	9.5	0.235	
Beefsteak[f]	2.5		7.5
Grape			
cv(R) Dattier 1[e]	3.7	0.142	
cv(R) Muscat de Hamburg[e]	1.9	0.113	
cv(S) Cardinal[e]	1.9	0.055	
"Black" (undefined)[f]	1.3		1.57
Apple			
Winesap[d]	1.06	0.139	
Golden Delicious[d]	0.923	0.086	
Red Delicious[d]	1.41	0.151	
Red Delicious[f]	4		142.2
Granny Smith[f]	5		100–450
Merton Russet[f]	3		169
Christmas Pearmain[f]	4.5		236
Kings Collee[f]	3		46.0
Plum[c]	1.8	0.44	

All tests performed in one dimension except [c] which were performed in two dimensions. cv, cultivar; (R), resistant, or (S) susceptible to splitting.

[a] From Voisey and Lyall (1965).

[b] From Voisey *et. al.* (1970). Note—the strains at fracture quoted from this paper are not significantly different.

[c] From Lustig and Bernstein (1985).

[d] From Clevenger and Hamann (1968).

[e] From Mrozek and Burkhardt (1973). Note—the value for strain seems excessive.

[f] From S. Sealey and J. F. V. Vincent (unpublished data).

them (Considine, 1982). There are no published observations on the fracture mechanics of fruit skin although there are a number of reports of related properties such as strength (Table III) and strain to break.

Kate Young, a descendant of the Young who invented the Young modulus, measured the notch sensitivity of apple skin in a project with the author's group. Her data have recently been confirmed by Sarah Sealey, also in the author's group. The skin of apple, tomato and grape is moderately notch sensitive (Fig. 26) but the work to fracture is very different for the different fruits (Table III). The skin of grape and tomato (and maybe other brittle-skinned fruits) has a high modulus of 400 MPa (black grape) to 600 MPa ("Beefsteak" tomato), about ten times the stiffness of apple skin. Since the fracture strain of the skin of apples and plums (and presumably

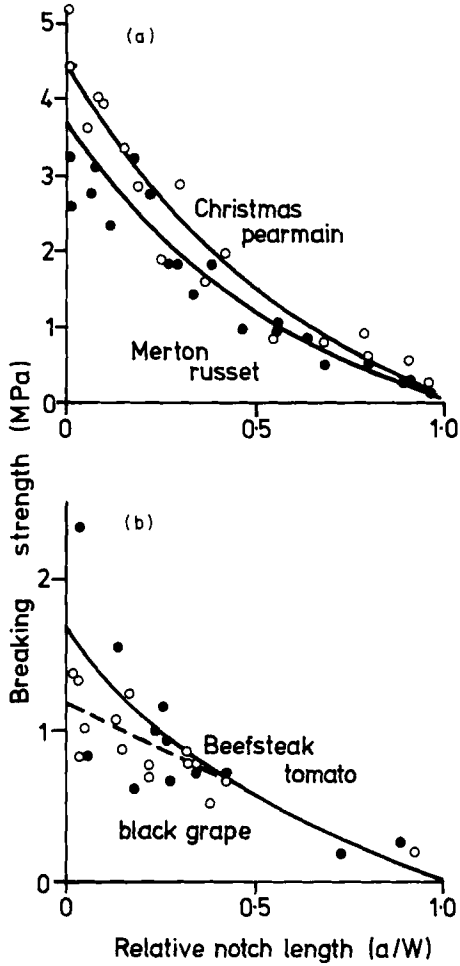

Fig. 26. Notch sensitivity of skins of two varieties of apple (a) and a tomato and grape (b) (cf. Fig. 7). From S. Sealey and J. F. V. Vincent (unpublished).

other fruits) is about an order of magnitude greater than the maximum strains observed in the intact fruit, the cells beneath the skin must be providing very significant support. When the skin on a fruit *does* break, the internal structure must be providing a significant amount of the fracture energy. Conversely, the skin protects the inner tissues from damage by cracking. When a New Rock Pippin apple has a vertical crack of longer than 5 mm (a sixth to an eighth of the height of the apple) in the skin, force and deformation at failure (by cracking rather than bruising) decrease very rapidly. When the crack is more than 10 mm long, the apple cracks as if it had no skin at all (Fig. 27; Khan, 1989). Although not measured it is suspected that the important factor in fruit skin is not its resistance to crack propagation but rather its

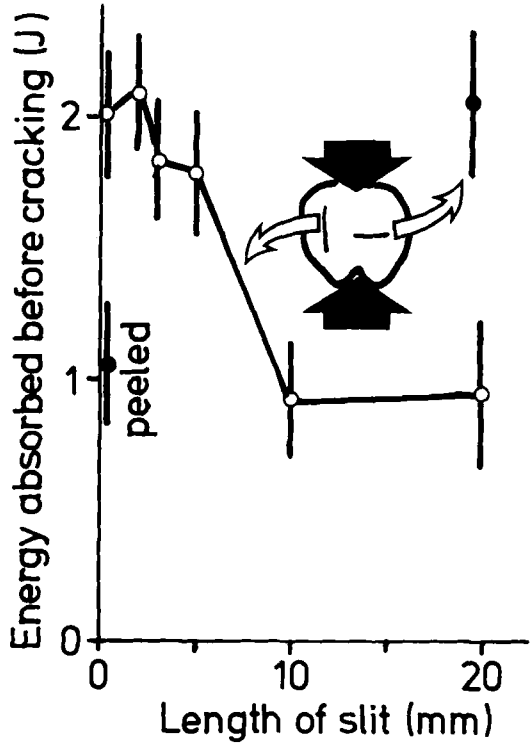

Fig. 27. The effect of skin on energy absorption by an apple (Rock Pippin) compressed vertically to the core. The skin increases total energy absorbed until it contains a vertical split longer than a certain length (here, 10 cm), when it ceases to protect the apple from further damage. A horizontal split does not reduce energy absorption. From Khan (1989).

resistance to crack initiation. An analogy may be made with many stiff commercial packaging plastic films.

The problem remains—what factors impart resistance to damage by splitting of the intact fruit? A common mistake is to assume that the strongest skin will be the most resistant (e.g. Considine *et al.*, 1974). But fracture mechanics tell us that strength is not the main factor; the crack has to be initiated (which will be governed by the presence and orientation of stress-concentrating defects) and propagated (which will be governed by the transmission of energy to the advancing crack tip). The best protection will be gained from a tough skin which is therefore likely to be relatively extensible (Lustig and Bernstein, 1985). However, the strains observed in isolated skins, of the order of 15–25% (Table III), are never achieved when the skin is on the fruit. This is partly because the skin is being supported by the cells beneath and partly because the skin on the fruit is being stretched in two directions at once and the Poisson ratio effects (i.e. the narrowing of the sample observed when it is stretched in a uniaxial test) are not available. The skin is then less deformable

and its stiffness increases. The analysis of fracture in two-dimensional strain is very difficult or even insoluble. Very often the best approach is experimental. True two-dimensional strain can be achieved in a number of ways such as mounting the skin over a hole as a diaphragm, then pressurizing it and measuring pressure and deflection of the centre of the diaphragm (Voisey and Lyall, 1965). Unfortunately these authors did not include sufficient information in their paper to allow calculation of any fracture parameters and did not measure the deflection of the diaphragm. They *did* quote the pressure to cause fracture of the diaphragm and showed that this is not well correlated with susceptibility to cracking. This is not surprising, since the deflection of the diaphragm is also needed in order to calculate strength and would, if incorporated into the calculations, probably improve the correlation with cracking. Another technique is to pressurize the whole fruit and measure how much the skin stretches (Lustig and Bernstein, 1985). This is much more representative of the conditions which the skin of a fruit has to cope with and results in fracture at lower strains averaging 7%.

D. LEAVES

The leaf is often a relatively delicate membrane across which tears can be propagated easily. Conducting tissue and the associated sclerenchyma fibres provide crack stoppers in a number of different ways. In many water plants (e.g. *Elodea*, *Potamageton*) there is a thread of sclerenchyma around the edge of the leaf which resists cracks being initiated. Where the edge of the leaf has notches in it, there may be a vein running across the tip of the notch (Fig. 28), or specialized cells at the tip strengthening it (Haberlandt, 1914). In leaves of dicotyledonous plants the fracture path is continually being deflected by the network of veins. This makes it very difficult to measure fracture properties of these leaves since it is almost impossible to measure the area cleaved. Several studies have attempted to overcome this problem by using penetrometers or punches (e.g. Cherrett, 1968; Coley, 1983). Although such measurements may be quoted in "absolute" units such as newtons, they can-

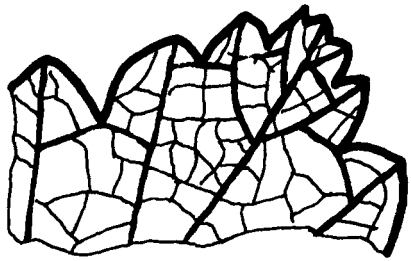

Fig. 28. Crack-stoppers in a leaf of *Ribes rubrum*. A short vein extends across the base of each notch in the edge of the leaf.

not produce proper estimates of fracture toughness comparable with those from other tests. Even so, the results can be correlated with feeding preferences which can be shown, by experimental elimination of variables, to be based on a mechanical property related to toughness (Waller, 1982).

In a similar way it is possible to correlate the tendency of plants to cause bloat in ruminants (due to the cells breaking open too easily, releasing their contents) with the mechanical strength of the cells and tissues (Lees *et al.*, 1981). Leaf tissue of a number of forage legumes, some causing bloat and some not, was disrupted by crushing with glass beads, grinding in a homogenizer or sonication. Isolated cells were sonicated. In each instance the number of cells broken open was estimated from the amount of chorophyll released. Legumes which cause bloat have weak tissue and the cells are more readily broken open; those which do not cause bloat have stronger cell walls, but the tissue can be strong or weak. The stronger cells have thicker walls (Lees, 1984) and the stronger tissues have more complex venation (Lees *et al.*, 1982). Although this particular example does not reveal any measures of fracture toughness which are comparable with other systems, it does show clearly the necessity of considering fracture at a number of levels of organizational hierarchy, a topic to be returned to.

Some of the problems in studying the fracture of the leaves of dicotyledonous plants are overcome in a study of breaking cabbage leaves which is elegant in its simplicity (Holt and Schoorl, 1983). Cabbages were dropped from different heights and showed a very strong relationship between height and the total length of cracks in the leaves. Unfortunately neither strength nor work of fracture was quoted, so the results cannot be readily compared with other tests and tissues. The technique does not preclude this, however.

In many species of plants, notably monocotyledons such as grasses, the veins are parallel and the leaf essentially linear. This makes experimentation much easier. There have been several studies on the strength of grass leaves, but mostly they quote simple force to break (Kneebone, 1960), force per unit width of leaf (Martens and Booysen, 1968) or correlate force with fibre content (Evans, 1967). There is partial concensus that fibre, probably sclerenchyma, is an important factor. However, it is possible to be much more precise using the tools of materials science. In tension, the leaves of grasses can be modelled as a composite material with preferentially orientated fibres (Vincent, 1982a). The simple Voigt (parallel-element) model for such materials can be applied, when it appears that the sclerenchyma accounts for about 95% of the longitudinal stiffness. Using specimens with centre or edge notches the work to fracture of leaves of *Lolium perenne* was found to be about $400\,J\,m^{-2}$ (Vincent, 1982a) although in grasses with higher fibre content such as *Stipa*, which has about three times the volume fraction of sclerenchyma, the work of fracture is about two orders of magnitude greater. There is some discussion as to whether it is appropriate to use normal fracture mechanics to estimate the toughness of grasses since the mathematics are

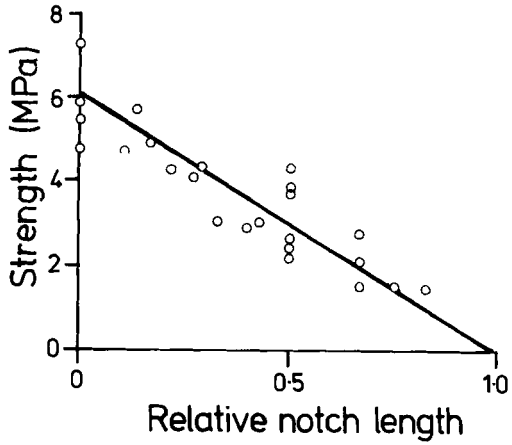

Fig. 29. Notch-sensitivity of a grass, *Dactylis glomerata* (see Fig. 7).

based on concepts of crack propagation. But, most grass leaves are almost completely notch-insensitive (Fig. 29). This is most probably because the shear stiffness of the cells between the fibres is relatively low. Thus even if several fibres have been broken, stress is not sufficiently transmitted laterally to cause a stress concentration in the remaining fibre(s) and a notch will not weaken the leaf significantly. Therefore one cannot use the concepts of stress concentration at the crack tip in a grass with separated fibres. This is not so with *Stipa* which has a sclerenchymatous sheath around the leaf. Thus a crack can propagate through this layer and the *Stipa* leaf breaks in a brittle manner (J. F. V. Vincent, unpublished).

The notch-insensitivity of the softer grasses has a number of important consequences for animals feeding on it since it means that teeth are of little use other than for gripping the grass. Like meat it must be torn by brute strength or cut with a shearing action. Large animals such as cows and sheep hold the grass (respectively with tongue and teeth) and pull. They thus have a limitation on the number of leaves which they can break, illustrated by considering the effects of "enrichment" of pastures. Longer grasses will be harvested at least as easily as shorter ones, so it is worth encouraging grasses to grow tall. But if the grass tillers grow more densely the cow or sheep, having finite strength, will find the size of its bite reduced, even though it might be taking in the same number of grass leaves. This is because the strength of grass and its apparent stiffness (as measured from the total cross-section area of the leaf) are directly proportional to the amount of fibre present. Thus increasing tillering will increase intake only up to a point (A. Antuna, personal communication). A number of different studies have shown inverse correlation between the strength of grass and "palatability" (e.g. Theron and Booysen, 1966) so that the weaker grass is, the more the animal will eat. It has also been pointed out that when deer age their teeth tend to rot. If their back teeth remain, food

intake is not impaired. However if their back teeth decay they cannot survive, even if their front teeth are in good condition. Clearly deer do not need their front teeth for gathering grass, but need their back teeth for comminution. Small animals (rabbits, locusts, etc.) have to cut through the individual fibres of the leaf since they are not strong enough to break the grass in tension.

There is also some indication that some grasses with more fibre fail at a lower strain and that they are rather less notch sensitive. Thus although the leaves of such grasses are stronger, the individual fibres act as if they were more brittle (J. F. V. Vincent, unpublished). Since such behaviour is noted more in xerophytes it seems possible that they are more lignified and contain less water. With less plasticizer there may also be better shear coupling between adjacent fibres which will increase notch sensitivity.

As grass dries out the stiffness increases but the work to fracture hardly changes at all (Vincent, 1983). At a water content of about 0.2 g per g dry weight of grass, the fracture properties of the cells between the fibres go through a transition and these cells become very brittle and weak. The grass now tends much more to fracture between the fibres, and to fracture in a very brittle fashion. This seems to be the condition for hay-shatter; hay containing 25% or so of water is considered to be suitable for bringing in from the field. Hay with a lower water content is considered to be very susceptible to shatter with consequent high losses of the crop. Water content has been shown to be the major factor in hay-shatter in non-grass hay species (Shepherd, 1961).

Leaves can also fail in bending, when the load-bearing walls of the cells on the underside of the curve buckle and break. This is more easily understood if the leaf is envisaged as a beam with relatively low-density foam core and stiff outer (upper and lower) faces. In engineering terms this is a sandwich beam (Gibson *et al.*, 1988). G. Jeronimidis and J. F. V. Vincent (unpublished) have studied the leaves of two species of reed: *Iris pseudocoras* growing in clumps on the bank of a lake, and *Acoras calanus* growing half-submerged in the same lake. In bending, leaves of *I. pseudocoras* failed both in compression (on the inside of the bend) and tension (on the outside of the bend) but the epidermis remained intact, folding away from the parenchyma cells on the inside of the bend. The large cellulose bundles failed in tension with spiral thickenings of the cells becoming "unwound". Leaves of *A. calanus* (which contain much less fibre and are almost entirely filled with loose parenchyma) were almost completely disrupted at the site of failure and the epidermis broke in tension (Fig. 30). The fracture (failure) stress at the base of a leaf of *I. pseudocoras*, length 0.6 m, was calculated from experiment as about 70 MPa and the average stress due to wind loading in the field was measured as about 17.3 MPa, giving a safety factor of 4. By contrast, similar measurements for the 0.9 m leaf of *A. calanus* gave 74 MPa and 112.6 MPa, giving it a safety factor of 0.66, i.e. less than unity! This anomaly is more apparent than real, since *A. calanus* is supported by surrounding water, and so the leaves are supported for much of their length. The leaves also grow much more densely

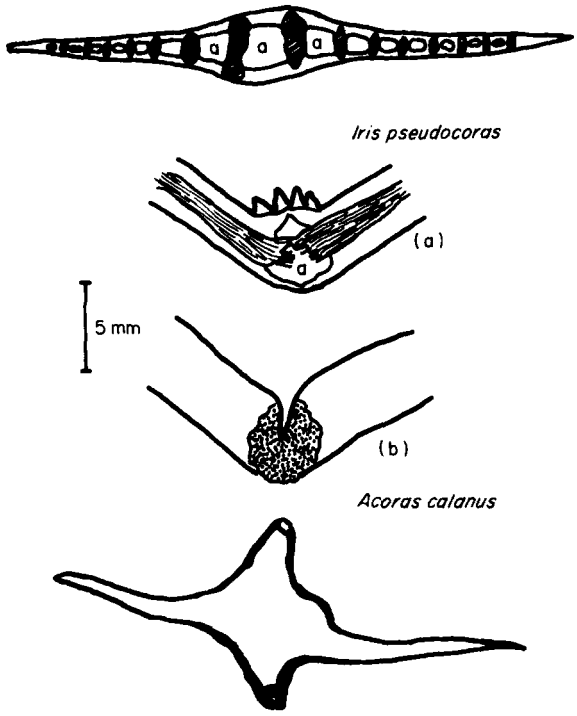

Fig. 30. Failure of leaves of Yellow Flag (a) (shaded areas represent fibres; air spaces indicated by 'a') and Sweet Rush (b) (damaged area stippled) in bending. Transverse sections are shown of the entire leaf and longitudinal sections of the failed areas. See text for full explanation. From G. Jeronimidis and J. F. V. Vincent (unpublished).

than those of *I. pseudocoras*, so each leaf gains support from its neighbours. This approach to the mechanical design of plants has been applied to the metabolic cost of supporting leaves (Chazdon, 1986). Three species of understorey palms were found to have a safety factor of 3.3 to 4 for the bending strength of the petiole of the largest leaves, suggesting that mechanical constraints, and in particular constraints of strength rather than stiffness, limit the maximum size of leaf.

E. STEMS

There is very little information on the fracture properties of plant stems. As with grass leaves, tensile strength is a direct function of fibre content (Prince, 1961; Halyk and Hurlbut, 1968; Balhovec, 1988). Most herbaceous stems have bundles of fibres running longitudinally. There are usually several bundles arranged around the periphery of the stem and it seems likely that these dictate the tensile and bending strength of the stem: in the culm of rice (*Oryza sativa*) the cortical fibre and epidermis support about 90% of the load

(Matsuda *et al.*, 1983). By further analogy with grass leaves, it seems very likely that this arrangement will either deflect cracks or resist the development of stress concentrations. Preliminary experiments have shown that the culms of rice plants which are more resistant (WAR 77-3-2-2 and Roden Jawa) to damage by crabs (*Sesarma huzardi*) are neither stiffer nor significantly stronger than the susceptible ones (WAR 71-1-1-4 and IR 29725-22-3-3-3), but break with a more complex fracture suggesting that the deflection of the crack is important in resistance to crushing (J. F. V. Vincent, unpublished). Water content is also important; as with simple parenchyma and leaves, wilting increases toughness by increasing strain to failure. For instance the stalks of sorghum fail in a brittle fashion when turgid but in a much less "well-defined" manner at lower moisture (Bashford *et al.*, 1976). The common problem of lodging has not been satisfactorily related to the mechanical properties of the stem.

Recent work on thigmomorphogenesis has produced some interesting results. It seems that the Young modulus of plant stems is not significantly affected by mechanical stimuli such as brushing or touching (Jaffe *et al.*, 1984: these authors maintain that the stiffness of the stem is reduced, but their graphical data do not support this interpretation) or the modulus may be increased (Heuchert *et al.*, 1983: these authors confuse strength and stiffness). In addition, an important parameter of the cross-sectional shape of the stem, the second moment of area, I (Wainwright *et al.*, 1976), seems to increase. If the stem can change its cross-sectional characteristics then it is quite possible that the arrangement of fibres within the stem has also changed, which would in turn affect the estimate of the Young modulus, which was measured in bending in both these studies. The only way to overcome this problem is to measure stiffness in tension, which is independent of structure. In both these studies, the stems became able to withstand a far greater degree of bending with no change, or even an increase, in flexural stiffness. Concomitantly total fibre increased and water content decreased. These results suggest that the stem develops greater toughness as a result of mechanical stimulation, but the specific experiments remain to be done.

F. SEAWEEDS

Seaweeds are of interest since they contain about 10% dry matter, similar to herbaceous land plants. Yet they withstand far greater environmental forces (a plant on a stormy shore experiences drag forces equivalent to a wind of $2000\,km\,h^{-1}$). One way in which they do this is by allowing the stipe to extend to relatively high extensions which takes a finite time to achieve. Before the seaweed has been stretched to its breaking strain, the wave which was stretching it has passed and the stipe is still intact. The high strains also allow storage of a large amount of strain energy before fracture occurs. Des-

TABLE IV
Some mechanical properties of seaweeds

	Ultimate strain	Strength (MPa)	Young modulus (MPa)	Work to fracture ($J\,m^{-2}$)
Fucus serratus	0.1[a] 4.2[b]	0.42[a]	3.9[a]	
F. vesiculosus		0.5[a]		
Ascophyllum nodosum	0.3[a]	0.39[a] 1.5[b]	1.6[a]	
Laminaria digitata	0.16[a]	0.4[a] 0.9[b] 1.7[c]	1.7[a]	 500[e] 1.0[f]
Durvillaea antarctica		0.7[d]		4000[d]
Lessonia nigrescens		1.2[d]		4000[d]

[a] Recalculated from Delf (1932).
[b] From Wheeler, W. N. and Neushul, M. (1981).
[c] From Vincent and Gravell (1986).
[d] From Koehl (1987).
[e] Fracture perpendicular to long axis of frond.
[f] Fracture parallel to long axis of frond.

pite their low strength, seaweeds can be exceedingly durable (Koehl, 1987), although unfortunately the fracture toughness of the stipe has never been properly measured. Some relevant figures are shown in Table IV. The extreme variability of strength may be intrinsic or it may be due to failure to define carefully which part of the weed is being tested. When seaweeds finally fracture, the mode of fracture varies among species. The stipe of *Laminaria digitata*, which is mostly parenchymatous, breaks cleanly; the stipe of *Fucus* spp. which has a central core of filaments, breaks irregularly with the filaments breaking last. *Ascophyllum nodosum*, despite its morphological similarity to *Fucus*, breaks in a much more brittle fashion (Delf, 1932). This is correlated with the degree of exposure to wave action which these plants commonly experience by their position on the shore.

The fronds of *Laminaria digitata* are highly anisotropic due to the orientation of the cells within them. In addition the frond provides remarkably large and uniform specimens, making it possible to compare three different approaches to fracture measurement (Vincent and Gravell, 1986). The fronds are notch-insensitive in the same way as grass; tensile tests on notched samples give a work to fracture of about $550\,J\,m^{-2}$. One of the assumptions made in the analysis of testing notched samples is that the sample is isotropic. This is clearly not the case. However, since the lamina of *L. digitata* is so

uniform it is possible to check this answer with a test which relies on purely graphical estimation of work. This is complicated by the viscoelastic nature of the frond. The experimental procedure finally adopted was to extend the frond up to a force estimated to be just below its strength and then to take it back to zero extension. The resulting hysteresis loop represents viscous losses; when the loop was of constant area (after about five cycles of extension and retraction) the specimen was broken and the area of hysteresis subtracted from the total area under the curve. This gave a somewhat lower work to fracture of about 430 $J\,m^{-2}$ which was, however, within the range of the analytical estimate. Finally, a trouser-tear test showed that fracture between the fibres of the lamina proceeds with the remarkably low work to fracture of 1 $J\,m^{-2}$. At the base of the frond the orientation of the cells on each surface (front and back) tends to move round to the right such that the stipe has a spiral winding. Thus the parallel architecture of the frond, which encourages fracture along the frond and leads to the formation of branching fronds, gives way to a crack-stopping type of cross-ply just above the stipe.

The anisotropy of *Laminaria* frond also probably affects the way large animals feed on it: the classic example is the sheep of North Ronaldsay in the Orkney Islands. Although *Laminaria* is the commonest plant on the shore it is rarely eaten and even then the sheep take a long time to eat it. However, if it is presented to the sheep in small pieces it is readily taken, showing that it is not noxious (Patterson, 1985). It seems likely that the sheep cannot break the fronds easily, because even if they put notches into the frond with their teeth they will not be able to introduce a stress concentration. In this respect, *Laminaria* is very much like grass.

G. WOOD

The fracture properties of wood have received much attention, since wood is such an important structural material. Its usefulness is largely due to its 'safeness', i.e. its failure is usually predictable since cracks (at least those across the grain) travel relatively slowly. Such cracks are usually presaged by clicks and cracking sounds due to small scale failure of individual fibres breaking in tension within the bulk of the material, providing a warning before any major failure occurs. Additionally, although a piece of wood may be fractured sufficiently for it not to carry a load in bending, it frequently happens that fibres extend across the fracture plane enabling a load to be carried in tension. This is much more apparent in fracture of green wood.

Wood is different from the materials so far discussed in that it takes compressive loads in the cell wall rather than the cell contents; it does not support compression by means of turgor (Jeronimidis, 1980, on which the rest of this section is based). The hollow tubes which make up wood can fail in compres-

sion by Euler buckling or by local buckling, but the support given by the surrounding cells will limit deformation to the latter mode. Failure of wood in compression is initiated by localized damage in the S2 wall (Dinwoodie, 1968) followed at higher loads by a macroscopic "compression crease". Local buckling in a tube wall occurs when the compressive stress is of the order of Et/d, where E is the compressive Young modulus, t is the thickness of the cell wall and d is the diameter of the tube. If the wood is to be stronger, stiffness or wall thickness should be increased, or diameter reduced. The response of trees, as with non-woody plants under turgor, seems to be to prestress in tension those parts most liable to compressive failure. This tensile prestress must be overcome by compressive forces before the cells are exposed to compressive stress.

Like grass, wood is highly anisotropic. When split longitudinally it is brittle with a work of fracture of the order of 100–200 Jm^{-2} (Atack *et al.*, 1961). This split passes mainly through the middle lamella and the interface between the primary and secondary cell walls. The only bonds which are broken are those immediately across the local crack plane. However, the main energy-absorbing process is more subtle. The individual cells of the xylem have cellulose fibrils winding around them in the same direction throughout the entire tree. The strength of wood cells pulled along the grain is about 0.5 GPa. The work of fracture across the grain is about 10 $kJ\,m^{-2}$, this is about 100 times the value along the grain (Jeronimidis, 1976). This is due to cells pulling away from each other laterally at some distance away from the main fracture site. The stress is transferred along the spirally wound cellulose fibrils, which then causes the cells to buckle inward on themselves ("tension buckling"; Pagano *et al.*, 1968) and away from each other, and also for the walls to fail in shear (Fig. 31). This causes a huge increase in the area of material cleaved, thus accounting for the high fracture energy. It does not involve fracture of individual cellulose fibrils, so the material can still carry a tensile load, making wood a "safe" material. This mechanism has been successfully modelled with glass fibres and epoxy resin (Gordon and Jeronimidis, 1980).

H. BARK

The importance of bark to wood is much the same as that of skin to fruit in that it is providing mechanical and physiological protection. Table V shows some directional strength properties of fruit tree bark (Diener *et al.*, 1968). The anisotropy is due to the orientation of the bark cells along the length of the trunk or branch, but is by no means as marked as that of the underlying wood.

Hampshire (1985) studied the ease with which squirrels can remove bark from trees ("bark-stripping"). The bark tends to fracture at the cambium.

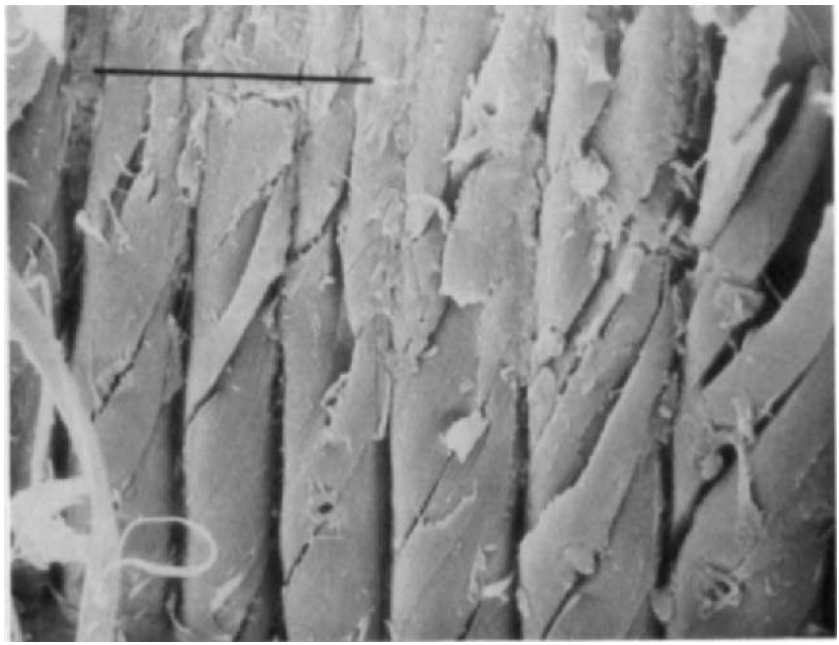

Fig. 31. Tensile failure of wood (*Picea sitchensis*) showing cells pulling away from each other laterally and shear failure in the cell walls. Scale line = 100 μm. From Jeronimidis (1980).

TABLE V
Strength and stiffness of tree bark

	Young modulus (MPa)	Strength (MPa)	Ultimate strain
Apple			
Parallel[a]	335	5.94	0.05
Perpendicular[b]	6.89	—	0.06
Cherry			
Parallel	132	4.15	0.08
Perpendicular	6.89	7.72	0.44
Peach			
Parallel	496	4.11	0.05
Perpendicular	6.89	8.37	0.44

[a] Parallel to the length of the tree trunk or branch.
[b] Perpendicular to this direction.
[c] From Diener *et al.* (1968).

TABLE VI
Work to peel bark

	Energy of peeling (J m^{-2})	
	Early May	mid-June
Silver birch	500	100
Hazel	650	95
Sweet Chestnut	500	90
Oak	500	140
Sycamore	400	140
Horse Chestnut	320	120
Beech	300	55

From Hampshire (1985).

Since the phloem is external to the cambium, the bark will contain significant amounts of sugar and will therefore be a useful food source. The work required to peel a 10-mm wide strip of bark was measured over a period of months (Table VI) using a simple adaptation of a standard tensile test machine (Fig. 32). The test morphology is thus the same as a tensile test. What is remarkable is just how weak the cambium becomes. It normally has a toughness of the same order as parenchyma from apple or potato, but this is reduced by a factor of five or more when the cells are actively dividing. As far as the squirrels are concerned, the frequency with which they remove bark from trees is largely dictated by the ease with which they can remove it—an apparently trivial result but one which had not been considered by those studying the behaviour of these animals.

I. NUTS

The shell of the macadamia nut, which comes from the Australian evergreen *Macadamia ternifolia*, is legendary for its toughness. It has been likened to an an "isotropic wood" (Jennings and Macmillan, 1986) but is still an order of magnitude weaker and less tough than wood. The techniques used for measuring the material parameters of such a small and oddly-shaped specimen are worthy of note. A circular section was cut from the centre of the shell and a further section cut from this leaving a specimen in the shape of a letter "C". This can then be notched and squashed or pulled (Jennings and Macmillan, 1986). When its specific gravity (about 1.3, indicating that the structure is really very solid with very small air spaces) is taken into account the nutshell emerges as a remarkably efficient and damage-tolerant structure compared with man-made materials (Table VII), though wood is again much better since its specific gravity is around 0.6.

The shell of the coconut has similar fracture properties. In a study in

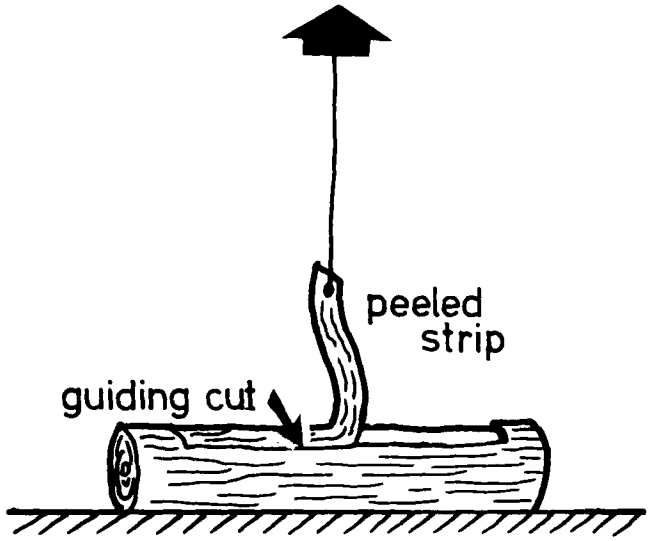

Fig. 32. Method of pulling bark from a branch in a tensile test machine. The strip to be removed is delineated by a vertical cut through the bark, the specimen clamped to the base of the machine (clamps not shown) and a long wire attached to the bark and the machine cross head. The use of the long wire ensures that the geometry of the test does not vary significantly from beginning to end.

TABLE VII
Stiffness, strength and toughness of nutshells

	Young modulus (GPa)	Fracture strength (MPa)	Work of fracture ($kJ\,m^{-2}$)
Macadamia nut			
Polar, tension	2.7[a]	40[c] . 65[d]	0.9
Polar, compression	5.2[a]	51[c] . 83[d]	0.9
Equatorial	4[a]	50	0.4
Coconut			
Polar	2.9		1.7[b]
Equatorial	4.9		1.9[b]

[a] and [b] Two sets of results which are not significantly different.
[c] Green.
[d] Dry.
Macadamia nut data adapted from Jennings and Macmillan (1986).
Coconut data from J. F. V. Vincent and G. Jeronimidis (unpublished).

Reading (J. F. V. Vincent and G. Jeronimidis, unpublished), samples cut from the outer shell were tested in impact using a weight swinging on a pendulum and at low loading rates in three-point bending. Whole nuts were tested in compression. The results (Table VII) show that coconut is much more anisotropic in stiffness due to the predominantly circumferential (= equatorial) orientation of fibres making up the shell, and is much tougher (though strangely the toughness is isotropic) than the macadamia nut shell. Since intrinsic toughness is inversely proportional to the size of the structure being tested (see discussion) it would be very interesting to see how material properties of the shells of nuts of different sizes compare, but it appears that the difficulty of breaking into a macadamia nut may be more due to its size than to any intrinsic properties of the shell material. It would be exceedingly interesting to examine a range of nuts since, by and large, the ecology of these structures is based on their mechanical properties.

J. ENDOSPERM

The endosperm in the kernels of corn (*Zea mays*) has a reported work of fracture, measured on notched beams in bending, 1–2 kJm^{-2} which is not only surprisingly high but independent of temperature (over a range of 30–60°C) and moisture content (11.5–18.6%) (Balastreire *et al.*, 1982a). The work of fracture of the un-notched beam is reported to be twice as high as in the notched beam, which is suspicious, since the work of fracture is a material constant. The suspicion is, therefore, that the tests on the un-notched samples were performed such that the crack propagated unstably which will inevitably lead to an overestimate of the work of fracture. The high work of fracture for the endosperm is confirmed by impact fracture experiments using a swinging pendulum (Mensah *et al.*, 1981). The mode of fracture, as judged from microscopic analysis of the fracture surfaces, appeared to be brittle at lower temperatures, ductile at higher temperatures. This suggests some element of viscoelasticity, so it is surprising that the mode of fracture shows no sign of changing with strain rate (Balastreire *et al.*, 1982b) although the range of strain rates covered only one order of magnitude, while the range of temperature (30°C) is equivalent to nearly four orders of magnitude of strain rate. At the higher temperature one might also expect that H-bonds will start to be disrupted allowing more flow. However, it seems likely that at impact rates of fracture, the mode of fracture changes with water content since the rate of breakage in a hammer mill shows a minimum at a moisture content of about 22%, representing a peak in toughness (Fig. 33; Jindal *et al.*, 1979). It may be difficult to compare these results with those from the notched beam tests, since the latter were performed on material removed from the centre of the kernel, whereas the impact tests were performed on intact kernels. It

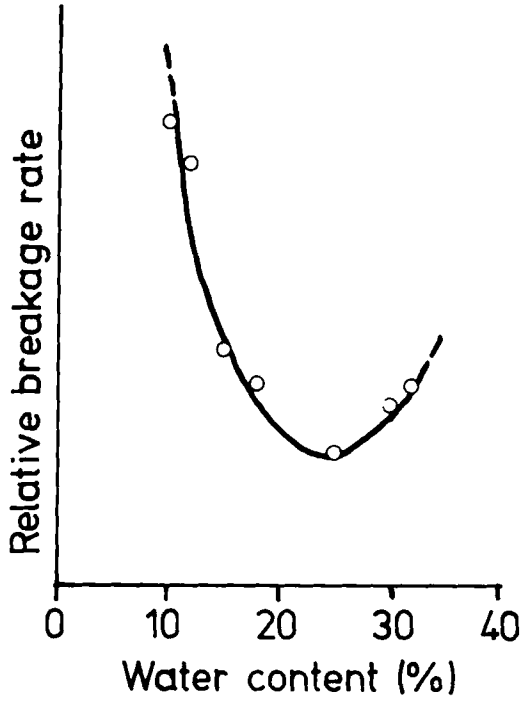

Fig. 33. Effect of moisture content on the rate at which kernels of corn break in a hammer mill rotating at 3000 r.p.m. The "relative breakage rate" is a pragmatic parameter based on how much of a standard sample will pass through a seive with mesh openings of 5.6 mm. From Jindal *et al.* (1979).

seems very likely that the outer covering of the kernel will have some protective effect.

IV. DEHISCENCE AND ABSCISSION MECHANISMS

Deciduous plants shed their leaves by changing the fracture properties at the base of the petiole. The fracture mechanics of abscission of leaves have not been investigated, though some attention has been paid to fruit (Fluck, 1970). Most of the forces involved have not been measured, with the exception of the physiological mechanism involved in weakening the attachment sufficiently for the fruit to be harvested mechanically. However, a variety of raspberry which the author once examined, and which was shedding fruit into the mechanical harvester before it was ripe, showed a sharp notch at the point where the fruit stem met the main stem which was obviously acting as a stress concentrator (Fig. 34). Unfortunately nothing has been reported of the development of this strain of fruit. The interaction of cellular changes and gross morphology should not be overlooked when considering abscission.

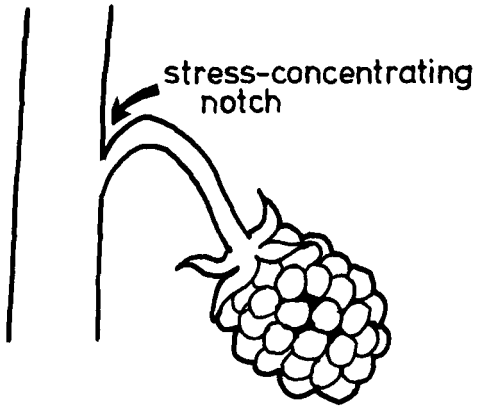

Fig. 34. Showing how the way a lateral structure branches off a main stem can give a notch capable of stress-concentrating effects.

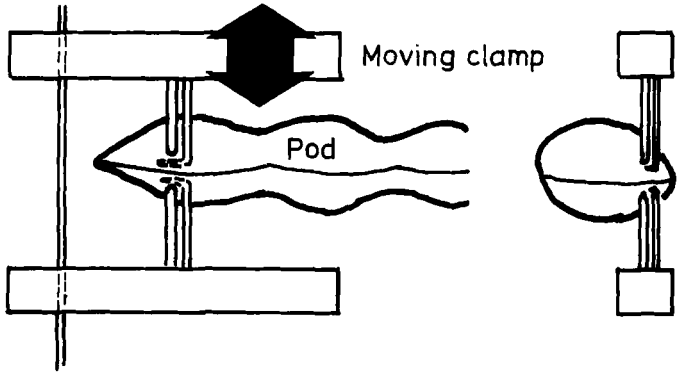

Fig. 35. Apparatus for measuring dehiscence properties of bean pods. The two mounting blocks are held parallel with an alignment rod (left). Each half of the pod is pulled apart by a hook or pushed together by a prod as necessary. From Weeks *et al.* (1975).

Dehiscence in fruits is, mechanically, of two kinds. The capsule may simply split open to expose the seeds, or it may dehisce explosively to broadcast the seeds. In the former case, although it has not been measured, the expectation is that the line of dehiscence will be a line of weakness. This is not true of the latter case. The mechanics of explosive dehiscence have been investigated in the soybean (Weeks *et al.*, 1975). The strength of the dehiscence layer and the force available from the pod were measured by pulling the pod open and then forcing the pod valves together again using a simple system for grasping the pod valves in both tension and compression (Fig. 35). They assumed that the force needed to close the pod is that available for dehiscence and is directly related to the thickness of the pod (in other words the elastic prestrain is

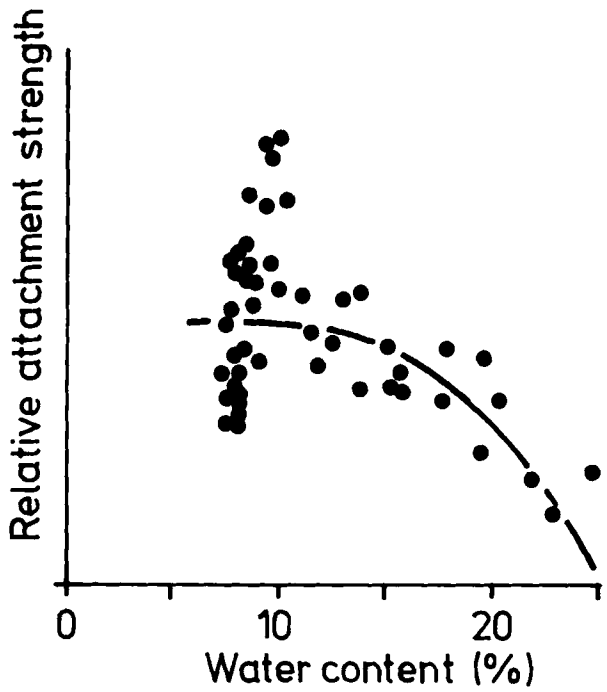

Fig. 36. Attachment strength along the line of dehiscence of soybean pods versus moisture content. Note how, at a moisture content of about 10%, the values for strength become highly variable indicating the onset of brittle fracture. From Weeks *et al.* (1975).

probably constant and independent of thickness) and increases greatly as the pod dries out (i.e. as the stiffness increases with reduced water content, a constant prestrain will result in higher prestress). However, the actual tensile stresses in the dehiscence layer are going to be much greater: the arguments leading to this statement are beyond the scope of this review. The strength of the dehiscence layer increases as water content falls, then becomes more or less constant but very variable (Fig. 36). This may be interpreted as follows: as the water content drops the material stiffens and the strength increases. At about 13% moisture content there is insufficient water to act as a plasticizer and the mode of fracture changes to become brittle and very sensitive to cracks and imperfections. However, the dehiscence layer must not become weaker as it dries out since that will reduce the amount of prestrain which the pod walls will be able to store before the pod breaks open. Ideally the dehiscence layer will be as strong as possible but also as brittle and with as low a work of fracture as possible, because then the maximum amount of energy from the pod halves will be available to throw the seeds and the fracture will travel quickest. The quicker the energy can be released, the more power is available to disperse the seed. Additionally, if one wanted to breed a pod which would not dehisce before harvesting, it might be easier to select for a

thinner pod wall for a given size of pod, rather than a stronger dehiscence layer (Weeks *et al.*, 1975).

It is to be expected, then, that the fracture properties of different types of dehiscence and abscission layers, when they are investigated properly, will be found to be different. Abscission and "slow" dehiscence layers will be found to be weak and brittle (more like dessert jelly) and may well contain more water. "Explosive" dehiscence layers will be found to be strong and brittle (more like biscuit) and will be very dry. These mechanisms must be associated with morphological and biochemical differences.

V. FRACTURE AND THE PLANT

Fracture in a plant can occur at one or more of three levels: organ, tissue or cell. Ultimately, of course, fracture can be traced to failure on the molecular scale, but the way in which the cells, tissues or organs are arranged can direct the fracture in a manner which would be impossible without that particular morphology. Additionally, it can be difficult to say whether fracture is being controlled by a tissue or an organ, since it can be difficult to make the differentiation. It is none the less a useful distinction if it can be made, since it can change the perspective and manner of analysis of the particular fracture problem. Examples of organs would be stem or leaf or fruit, where stresses are distributed in a complex manner which may not be apparent in tissues isolated from the organ. An example has been given with fruit skins, where the strains in the skin when it is on the fruit may be very different from those achieved in simple mechanical tests: it is likely that the two-dimensional strains in tomato skin when it is intact and on the fruit could be reproduced accurately in the isolated tissue since it is loosely bonded to the inner fruit tissues, whereas apple skin is so dependent on the mechanical properties of the underlying parenchyma, to which it is very firmly attached, that any test on the isolated tissue will be misleading if taken at face value. Another series of examples concerns the parallel arrangements of sclerenchyma and other fibres in a leaf or stem which can control the fracture properties and hence control damage which may be done to the plant by external physical or biotic agents.

Most mechanical tests on plant tissue have been performed at the tissue level (e.g. plant fibres, wood, storage parenchyma), but few tests have differentiated between fracture at the tissue and cell levels. The only satisfactory study (unfortunately unquantified) quoted in this review is Lees' work on forage legumes (Lees *et al.*, 1981, 1982; Lees, 1984). This differentiation is a very important one for any animal feeding on plants, since it is the contents of the cells which provide a large part of the nutrition. Something is known, in an anecdotal manner, of the way in which bacterial action can weaken cell walls in the rumen, but the attempts made to put numbers to the mechanical properties involved have been less than satisfactory. For instance Evans *et al.*

(1974) measured the mechanical properties of the tissues when they should have measured the properties of the cells. However, it is possible to get some idea about fracture at the cellular level from a simple calculation. The toughness of parenchyma is of the order of $300\,J\,m^{-2}$. If the average diameter of the cells is 0.1 mm then there will be about 10^8 cells in a square metre, giving a work to fracture of a single cell of 3×10^{-6} J. This figure is a minimum for tissues, but can be used in calculations which partition the work to fracture amongst different tissues. If one could estimate the work to fracture of different cell types, then it would be possible to calculate work to fracture of different tissues and organs and compare these values with those measured and hence discover some of the structural mechanisms in fracture.

The interaction of these properties with the "natural environment" is the interest of the ecologist. Very few studies have properly made this connection at the mechanical level, and even fewer in the more specific area of fracture properties. Koehl (1987) shows how the general connections can be made and Chazdon (1986) shows how useful the more specific approach can be. The failure and fracture properties of plant organs and tissues can be very closely and specifically related to environmental factors and strategies using concepts such as the safety factor. Yet for various reasons, mostly to do with problems of comprehension of the problem and its analysis, these relationships have not been explored adequately.

Fracture mechanics are dependent not only on material and structure but also on size. In general, smaller objects are tougher since there is relatively less volume (a term in length cubed) for the storage of strain energy to feed to the advancing fracture (whose area is a term in length squared). Also, since the length of a critical crack remains the same, being a property of the material, a smaller object will be less likely to contain a crack of this length and therefore be safer at the same loads. Thus smaller plants have inherently greater toughness and structural integrity. The evolutionary increase in the size of plants must be related not only to the development of supportive structures but also to the development of suitable toughening mechanisms. This principle will also apply to parts of a plant, for instance seeds. A small seed will be more difficult to break open and can therefore more readily survive being eaten. Thus a fruit has either a few large seeds protected by a hard shell or a large number of small seeds which need not have such extreme protection and may even have large amounts of edible material associated with them which will encourage the attentions of an animal. This implies that the seeds are as well protected from the trituration mechanisms of the animal as they are from the more commonly assessed digestive chemical mechanisms. What is the degree of mechanical damage to seeds which have been eaten and how many survive? Remarkably, there is some information in this area. Paulsen (1978) measured some mechanical properties of soya beans in compression. He recorded the strain energy density (which he mistakenly called "toughness") at the load when the seedcoat ruptured. There is a very marked

effect of size such that the smaller (5.95–6.35 mm long) soya beans store up to twice the energy per unit volume before fracture than do the larger (6.75–7.14 mm long) soya beans.

Similar considerations of scaling apply to dehiscence mechanisms. The energy available to break the tissues on the dehiscence line is proportional to the volume of the pod tissue whereas the area to be cleaved along the dehiscence line will vary as a square function of size. Thus pods of different species will not scale linearly, suggesting that specific differences among pods may be related to their final size.

Another set of examples is provided by horticultural "improvement" of plants, where the relative or absolute sizes of organs (e.g. fruits) may be changed in the interests of productivity. As a fruit increases in size through selection it will become more likely to fracture due to scaling effects. Unless the precise mechanisms of fracture are known, it will be impossible to breed for resistance to fracture other than pragmatically. The embarrassment is that a larger fruit has to be tougher to survive and so will be less "palatable". In each of these examples (and no doubt there are many others; it may be that the shells of nuts come under this heading) the properties of the materials will be independent of size and may be determined on conveniently large samples. The scaling effect will be less easy to determine; perhaps all that is important for the plant is the limiting smallness where, for a particular structure made of a particular material, fracture becomes so difficult or rare that a particular size confers a significant selective advantage.

Fracture mechanics has revolutionized the design of aircraft and ships. Its importance has been recognized in food science. The fact that animals and plants do not fall apart unintentionally shows us that not only do living organisms manage to avoid the worst excesses of stress; the chances are that they have some very interesting ways of going about it.

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