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Alternative modes of leaf dissection in monocotyledons

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Although a majority of monocotyledons have simple leaves, pinnately or palmately dissected blades are found in four orders, the Alismatales, Pandanales, Dioscoreales and Arecales. Independent evolutionary origins of leaf dissection are indicated by phylogenetic analyses and are reflected in the diversity of mechanisms employed during leaf development. The mechanism of blastozone fractionation through localized enhancement and suppression of growth of the free margin of the leaf primordium occurs in the Araceae and Dioscoreaceae. By contrast, the corrugated, dissected leaves of palms (Arecaceae) develop through a two-step process: first, plications are formed through intercalary growth in a submarginal position and, second, the initially simple leaf blade is dissected through an abscission-like process of leaflet separation. A third mechanism, perforation formation, is employed in *Monstera* and five related genera of the Araceae. In this mode, discrete patches of cells undergo programmed cell death during lamina development, resulting in formation of open perforations. When perforations are positioned near the leaf margin, mechanical disruption of the thin bridges of marginal tissue results in a deeply pinnatisect blade. Whereas blastozone fractionation defines the early primary morphogenesis phase of leaf development, the other two modes occur later, during the secondary morphogenesis/histogenesis phase. Evolution of these mechanisms presumably has involved recruitment of other developmental programmes into the development of dissected leaves. *Botanical Journal of the Linnean Society*, 2006, **150**, 25–44.

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INTRODUCTION

The leaves of monocotyledons are typically simple, with striate venation and sheathing leaf bases, but also display a diversity of forms ranging from unifacial to bifacial, petiolate to non-petiolate, and linear to expanded blades (Rudall & Buzgo, 2002). The striate, convergent pattern of major veins and the parallel, closed pattern of minor veins are often regarded as hallmarks of monocotyledonous leaves and distinguish them from the pinnate/palmate major veins and open reticulate minor venation of the broad, petiolate leaves of dicotyledons (Troll, 1939; Kaplan, 1973; Dahlgren, Clifford & Yeo, 1985). Exceptions to these broad generalizations occur, of course. A correlation among petiolate leaves, a broad, expanded lamina, and reticulate, open minor venation is found in numerous groups (Ertl, 1932; Troll, 1939; Inamdar, Shenoy & Rao, 1983; Triplett & Kirchoff, 1991; Chase *et al*., 2000; Cameron & Dickison, 1998; Rudall & Buzgo, 2002). In many of these reticulate veined monocotyledons the major venation appears to be pinnate but, with the exception of some members of the Araceae and the Taccaceae, this pattern has been shown to be a modification of a striate system in which individual strands of a multistranded midrib form the lateral veins (Ertl, 1932; Troll, 1939; Kaplan, 1973). Another striking exception to generalized monocotyledonous leaf morphology is the occurrence of dissected leaves, in which the lamina is represented by multiple leaflets, in at least four orders (Dahlgren & Clifford,

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1982; Dahlgren *et al*., 1985; Kubitzki, 1998a). About one-quarter of the Araceae (order Alismatales) have pinnately, palmately or pedately dissected leaves (Mayo, Bogner & Boyce, 1997, 1998), and a handful of genera of the Taccaceae and Dioscoreaceae (Taccales) also have palmately dissected (or sometimes bifid, pinnatifid or palmatisect) leaves (Huber, 1998; Kubitzki, 1998). The leaves of palms (Arecaceae; Arecales) may be simple or only bifid at the apex, but are more often palmately or pinnately dissected into one- or several-ribbed leaflets (Uhl & Dransfield, 1987; Tomlinson, 1990; Dransfield & Uhl, 1998). Leaves of the Cyclanthaceae (Pandanales) closely resemble those of the palmately dissected palms, although dissection of adjacent leaflets is usually incomplete (Harling, Wilder & Eriksson, 1998).

Reconstructions of angiosperm phylogeny indicate that ancestral angiosperms had simple leaves (Taylor & Hickey, 1996) and that, within the dicotyledons, complex leaf shapes and/or fully dissected leaves have arisen at least 29 times, with multiple reversions to an ancestral simple leaf shape (Bharathan *et al*., 2002). Despite this pattern of convergence in dissected leaf morphology, the major features of leaf development, including morphological aspects of the mode of dissection, appear to be shared in all dicotyledonous groups that have been examined in detail (e.g. Troll, 1939; Hagemann, 1970; Kaplan, 1973; Hagemann & Gleissberg, 1996). Leaves arise as dorsiventral primordia on the flanks of the shoot apical meristem. Even at early developmental stages, it is possible to recognize a radially thickened upper leaf zone and a flattened lower leaf zone (Eichler, 1861; Troll, 1939; Hageman, 1970; Kaplan, 1973). In dicotyledons, the lower leaf zone gives rise to the leaf base, while the upper leaf zone gives rise to the blade and petiole. The primordial blade retains the potential for organogenic activity in a strip-like zone along its lateral margins, the marginal blastozone (Hagemann, 1970; Hagemann & Gleissberg, 1996). In simple leaves, morphogenetic potential of the marginal blastozone is not expressed; by contrast, in dissected and deeply lobed leaves, activity of the marginal blastozone is prolonged during development. The marginal blastozone undergoes fractionation, forming distinct regions of growth enhancement and suppression that result in separate leaflet primordia borne on the axis of the main leaf primordium. In species with more complex leaf shapes, activity of the blastozone is further extended, allowing higher order branching to occur through fractionation (Hagemann & Gleissberg, 1996; Gleissberg, 2004). Duration of organogenic activity of the marginal blastozone defines the process of primary morphogenesis, which is brought to a close by the onset of histological differentiation (Hagemann & Gleissberg, 1996). Later differential elaboration of already formed parts during the leaf expansion and histogenesis phase of development results in secondary morphogenesis. For instance, the amount of elongation growth along the petiole–rachis axis determines whether a dissected leaf is pinnate or palmate: lack of extension of the rachis results in a palmate leaf, whereas extension results in a pinnate leaf (Hagemann & Gleissberg, 1996; Gleissberg & Kadereit, 1999; Kaplan, 2001).

Recent reconstructions of monocotyledonous phylogeny using molecular sequence data indicate that the four orders having dissected leaves lack a common dissected-leaved ancestor, indicating that this trait has evolved through convergence in monocotyledons as well as in dicotyledons (Chase *et al*., 2000; Soltis *et al*., 2000; Stevenson *et al*., 2000). The earliest stages of leaf development in all monocotyledons that have been studied resemble those of the dicotyledons: initiation produces a lateral dorsiventral organ primordium characterized by upper and lower leaf zones (Troll, 1939; Kaplan, 1973; Rudall & Buzgo, 2002). In a majority of monocotyledons, the leaf blade is derived from the lower leaf zone, while development of the upper leaf zone is suppressed (Troll, 1939; Knoll, 1948; Kaplan, 1973; Bharathan, 1996; Rudall & Buzgo, 2002). Considering diversity, leaf blades are derived from the upper leaf zone in broad-leaved species of the Alismatales (*Sagittaria*, Bloedel & Hirsch, 1979; *Arisaema*, Periasamy & Muruganathan, 1986), Dioscoreales (*Dioscorea*, Periasamy & Muruganathan, 1985; Bharathan, 1996), Pandanales (*Carludovica*, Wilder, 1976), Liliales (*Smilax*, Martin & Tucker, 1985; Bharathan, 1996) and Arecales (*Chamaedorea*, *Chrysalidocarpus*, *Rhapis*, Kaplan, Dengler & Dengler, 1982a). In addition, variation in the relative proportions of elongation and thickening growth may enhance the initial dorsiventrality of the lower leaf zone, resulting in a bifacial leaf, or may obscure it, resulting in a unifacial leaf (Kaplan, 1973, 1975; Bharathan, 1996; Rudall & Buzgo, 2002). The occurrence of dissected leaves is restricted to monocotyledons with broad leaf blades, but a rigorous examination of a correlation between derivation of the blade from the upper leaf zone and blade dissection has not been undertaken. The striking differences in the developmental mechanisms that give rise to dissected leaves have been recognized for well over 100 years, however. The large plicate leaves of palms aroused the curiosity of early developmental morphologists who recognized that the plications arose early in leaf morphogenesis and that adjacent folds had to be separated to form the individual leaflets (von Mohl, 1845; Trecul, 1853). Similarly, early botanists were aware that the fenestrations and deep sinuses in the leaves of *Monstera* and other aroid genera arose through a pattern of cell death (Trecul, 1854; Schwarz, 1878) and that these processes were fundamentally quite different from the

reiterative branching process that results from blastozone fractionation. Thus, it is clear that the independent evolutionary origins of leaf dissection have left a substantial signal in the developmental patterns that give rise to these dissected leaf shapes in monocotyledons.

Our primary goal in this paper is to review what is currently known about three different modes of leaf morphogenesis in monocotyledons having dissected leaves: (1) marginal blastozone fractionation, (2) leaflet separation by abscission and (3) perforation formation by cell death. This literature was last reviewed over 20 years ago by Donald R. Kaplan, who has contributed substantially to our understanding of this and other aspects of monocotyledonous leaf development, including critical evaluation of the phyllode and sympodial theories of monocotyledonous leaf construction and demonstration of the developmental basis of heteroblastic variation in leaf form and the homologies of unifacial leaves (Kaplan, 1970, 1973, 1975, 1983, 1984). In this paper, we review the specific examples of leaf dissection in monocotyledons used by Kaplan (1984) and provide new information on the cellular mechanisms of leaf dissection, particularly those related to the least investigated of these, perforation formation through programmed cell death. Although the comparative knowledge of leaf development in this large and diverse group of flowering plants is still fragmentary (Rudall & Buzgo, 2002), new information on developmental mechanisms in the monocotyledons can contribute to the understanding of the phylogeny of the group, character state evolution and the evolution of developmental pathways themselves.

DESCRIPTIONS OF THREE MODES OF DISSECTED LEAF DEVELOPMENT IN REPRESENTATIVE TAXA

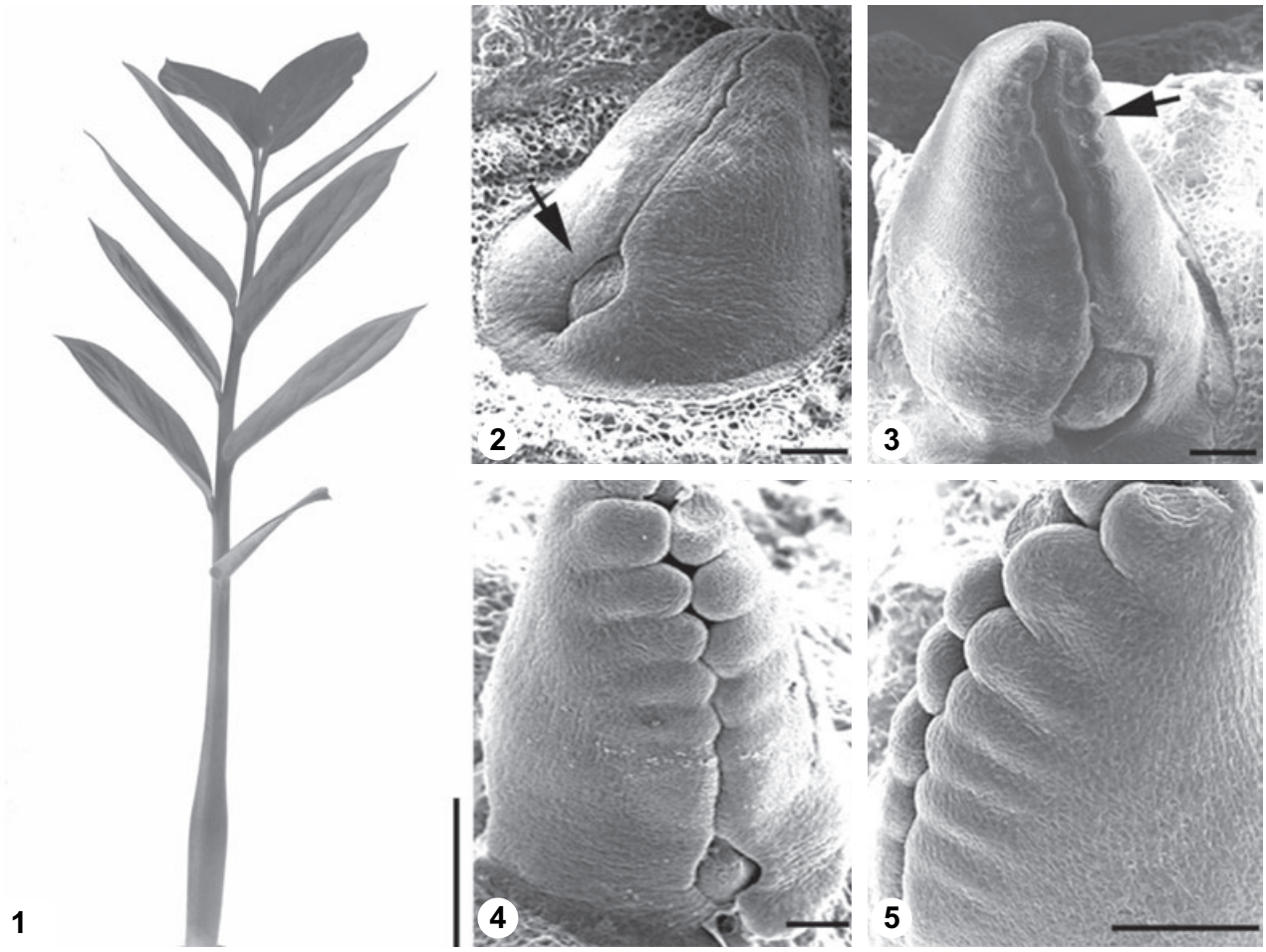
Blastozone fractionation in the Araceae

The family Araceae provides numerous examples of dissected or deeply lobed leaves that arise by the first of these developmental mechanisms, blastozone fractionation. Developmental studies of representative species of the genera *Zamioculcas* and *Anthurium* (subfamily Lasioideae), *Syngonium* (Colocasioideae), and *Dranunculus* and *Arisaema* (Aroideae) show that leaflets and lobes arise by the common mechanism of blastozone fractionation (accompanied by lamina folding in *Arisaema*; Troll, 1939; Kaplan, 1984; Periasamy & Muruganathan, 1986). The once-pinnately dissected leaves of *Zamioculcas zamiifolia* (Loddiges) Engler illustrate this widespread mechanism of leaf development. Adult foliage leaves of *Zamioculcas* consist of a short sheathing leaf base, a long succulent petiole and 4–8 pairs of elliptic, coriaceous leaflets borne on an elongate rachis (Fig. 1). The petiole and proximal portion of the rachis are unifacial in cross-sectional shape and anatomy, while the distal region of the rachis is bifacial (Kaplan, 1984). Individual leaflets have a pinnate primary vein pattern and reticulate higher order venation. Interestingly, the leaflets and rachis are deciduous from the persistent petiole during dormancy and leaflets are capable of rooting and forming new plants (Mayo *et al.*, 1997).

During primary morphogenesis, leaf primordia are hood-shaped and encircle the shoot apical meristem (Fig. 2; Kaplan, 1984). Leaflets arise as bump-like protuberances along the free marginal blastozone, and a gradient in leaflet primordium size indicates that leaflets are initiated in a basipetal sequence (Figs 3–5; Kaplan, 1984). During secondary morphogenesis, the leaflets expand in size and adopt a vertical orientation. At the same time the rachis region undergoes thickening growth, resulting in a broad, transversely orientated zone of insertion on the rachis axis (Kaplan, 1984). The petiolar region becomes intercalated between the sheathing leaf base and the distal leaflet-bearing part of the leaf axis. Rachis segments between the bases of individual leaflets expand late, separating the leaflets; finally pulvinar regions at the base of each leaflet reorientate the leaflet blades to a horizontal or oblique plane in mature leaves (Fig. 1). Thus, *Zamioculcas zamiifolia* illustrates leaflet formation through the mechanism of blastozone fractionation, a mode that is found across the dicotyledons as well as in this small subset of monocotyledons (Troll, 1939; Hagemann, 1970; Kaplan, 1984; Periasamy & Muruganathan, 1985, 1986; Hagemann & Gleissberg, 1996).

Leaflet separation in the palms (Arecaceae)

The conspicuous pleated leaves of palms are a distinctive feature of the family and possess a mode of development that differs fundamentally from that described above. Palm leaves share the common features of a sheathing leaf base, distinct petiole region and corrugated blade (Figs 6, 8), but also display a great diversity in size and form, including the largest leaves known, the 25 m-long leaves of *Raphia* (Hallé, 1977; Uhl & Dransfield, 1987; Tomlinson, 1990; Dransfield & Uhl, 1998). Leaf blades may be simple, palmate, pinnate, bipinnate or an intermediate condition, costapalmate, but always are pleated like the bellows of an accordion. The corrugated leaf blades show variable degrees of separation between the folds: for instance, in many pinnately dissected leaves, the separation extends to the rachis and leaflets are fully separated as the rachis elongates, but in most palmate palms, leaflet separation does not extend completely to the base of the folds and the rachis does not extend



Figures 1–5. Leaf development in *Zamioculcas zamiifolia* (Araceae) illustrating marginal blastozone fractionation. Fig. 1. Mature leaf. Scale bar = 5 cm. Fig. 2. Scanning electron micrograph (SEM) of young leaf primordium. Arrow, approximate boundary between lower leaf zone and upper leaf zone. SEM scale bar = 200 μm. Fig. 3. SEM showing fractionation of marginal blastozone (arrow). SEM scale bar = 200 μm. Fig. 4. SEM showing later stage of leaflet growth. SEM scale bar = 200 μm. Fig. 5. SEM of same leaf at higher magnification showing basipetal gradient in leaflet size. SEM scale bar = 200 μm.

(Fig. 6A, B). The position of the lines of separation between folds also varies among taxa: in palms with reduplicate leaf segmentation, splitting occurs along the abaxial ridges, forming inverted V-shaped leaflets (Fig. 6C). In leaves with induplicate segmentation, splitting occurs along the adaxial ridges, forming V-shaped leaflets (Fig. 6D). In certain other palms, separation occurs in the intercostal panels of tissue between the adaxial and abaxial ridges in a pattern that results in individual leaflets with several pleats (Fig. 6E). The phylogenetic distribution of reduplicate and induplicate separation of leaflets is not correlated with that of palmate, costapalmate or pinnate blade shape, but combinations of these characters are important for the circumscription of subfamilies and genera (Uhl & Dransfield, 1987; Tomlinson, 1990; Dransfield & Uhl, 1998).

The distinctive morphology of palm leaves has long been known to develop through a two-step process. First, an initially simple, smooth blade develops corrugations (plications) at a submarginal position, and second, separation occurs to separate adjacent plications as leaflets and to free them from the non-plicate strip of tissue at the leaf margin. The independence of these two stages is generally recognized, as the juvenile leaves of many species (as well as the adult leaves of some of these) are corrugated, but remain simple or bifid in shape (Goebel, 1926; Tomlinson, 1960; Kaplan *et al.*, 1982a). The unique features of palm leaf development were recognized and described by early botanists such as von Mohl (1845) and Trecul (1853). von Mohl and Trecul illustrated young palm leaves undergoing plication formation and noted the slit-like appearance of the folds. This slit-like appearance of

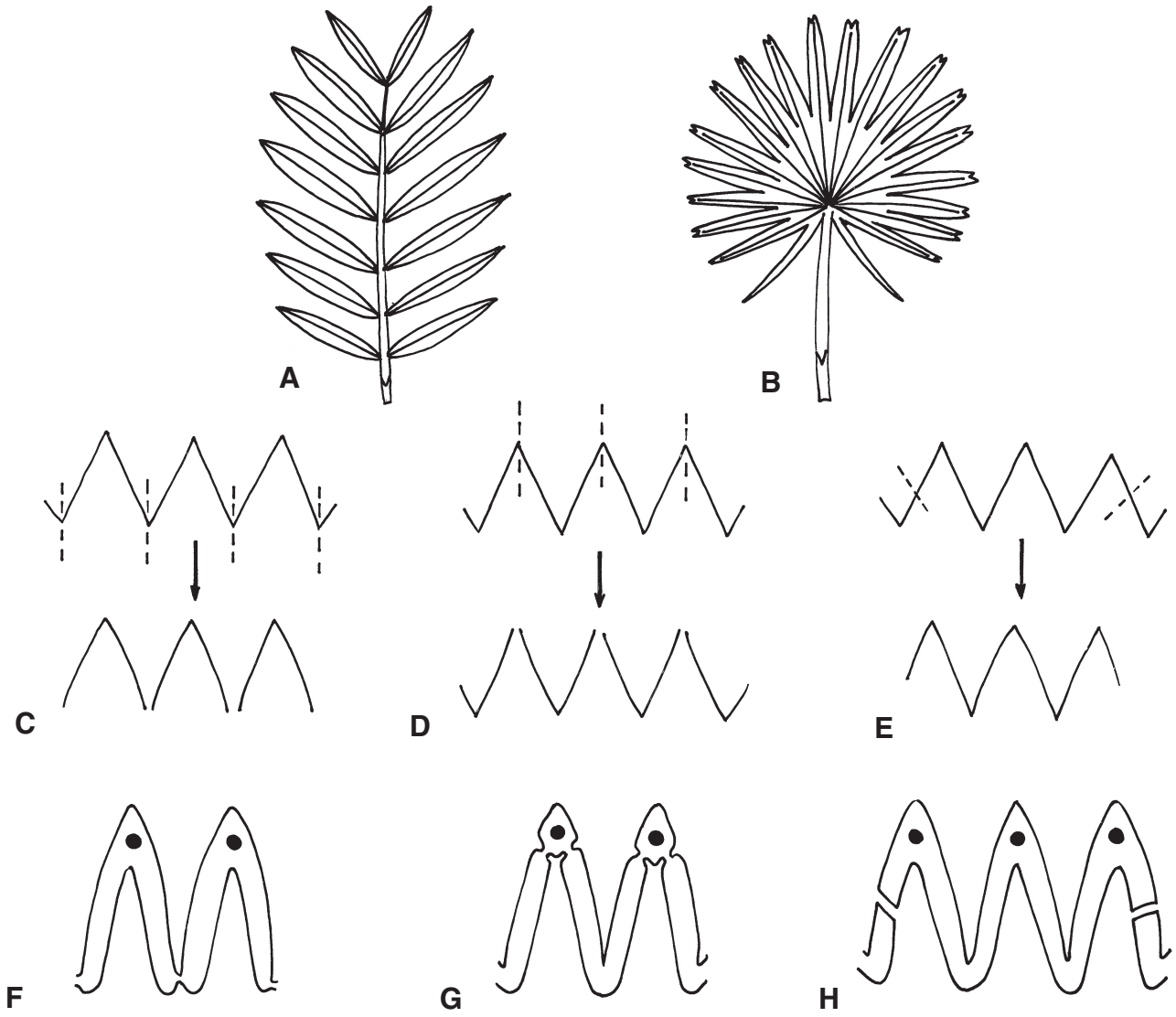


Figure 6. Diagram illustrating dissection of simple plicate blade into separate leaflets in the palms (Arecaeae). A. Pinnate leaf. B. Palmate leaf. C. Separation through abaxial folds gives reduplicate leaflets. D. Separation through adaxial folds gives induplicate leaflets. E. Separation between folds gives multiribbed leaflets. F–H. Separation by schizogeny may be complete (H) or may be incomplete, leaving a narrow bridge of tissue which must be mechanically disrupted (F, G).

the young plications can be deceptive and led to a long-term controversy about the mechanism of plication formation that lasted for well over a century (reviewed in detail by Kaplan *et al*., 1982a). On the one hand, proponents of the tissue splitting model favoured a tissue splitting mechanism in which schizogenous slits either were initiated internally and extended outward or were penetrated inward from the leaf surface in a regular pattern (illustrated in Fig. 7B), giving rise to the alternating ridges and furrows on the adaxial and abaxial sides of the leaf (Fig. 7A–C). As pointed out by Deinega (1898), this mechanism would disrupt the dermal layer and require that internal tissues reform the protoderm layer. On the other hand, others favoured a differential growth model in which an intercalary region of the expanding leaf blade, constrained by the leaf base, rachis, apex and non-plicate margin, is deformed into a regular pattern of pleats (Fig. 7D–F). According to this hypothesis, the protoderm layer is always continuous over the ridges and folds. This controversy probably persisted as long as it did because of the challenges of analysing the three-dimensional shape of a minute complex structure with the limited resolution of light microscopy and of orientating the plane of sections so that it is orthogonal to the developing plications (Kaplan *et al*., 1982a).

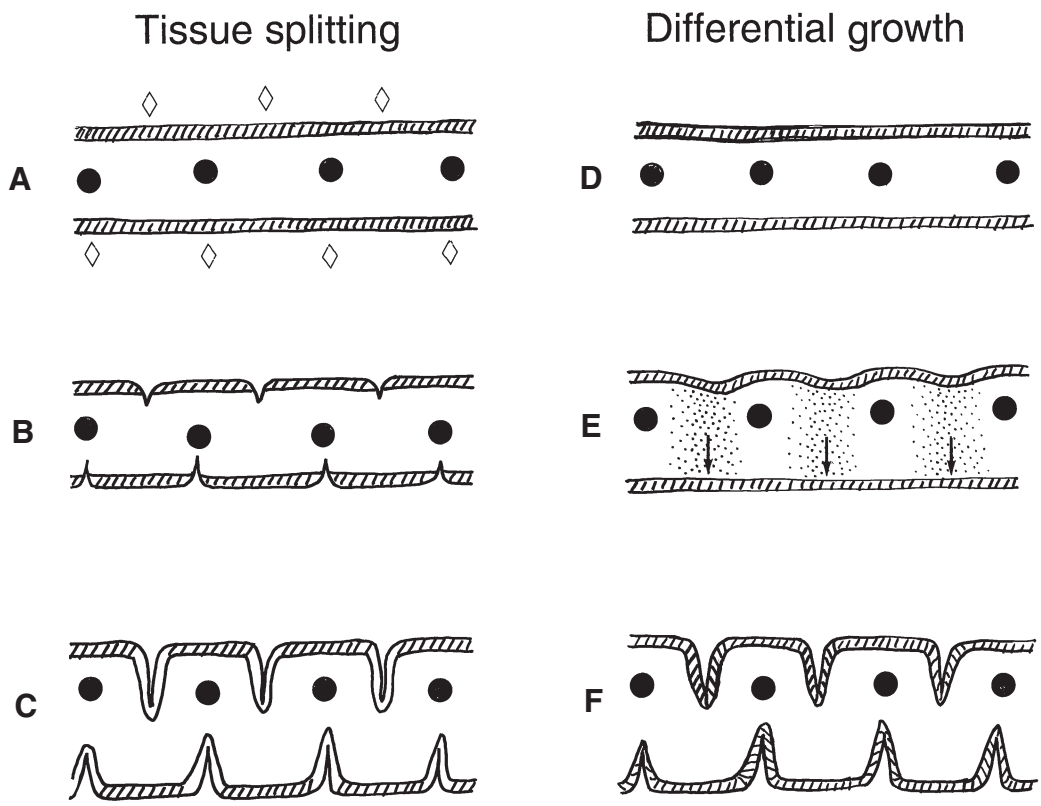
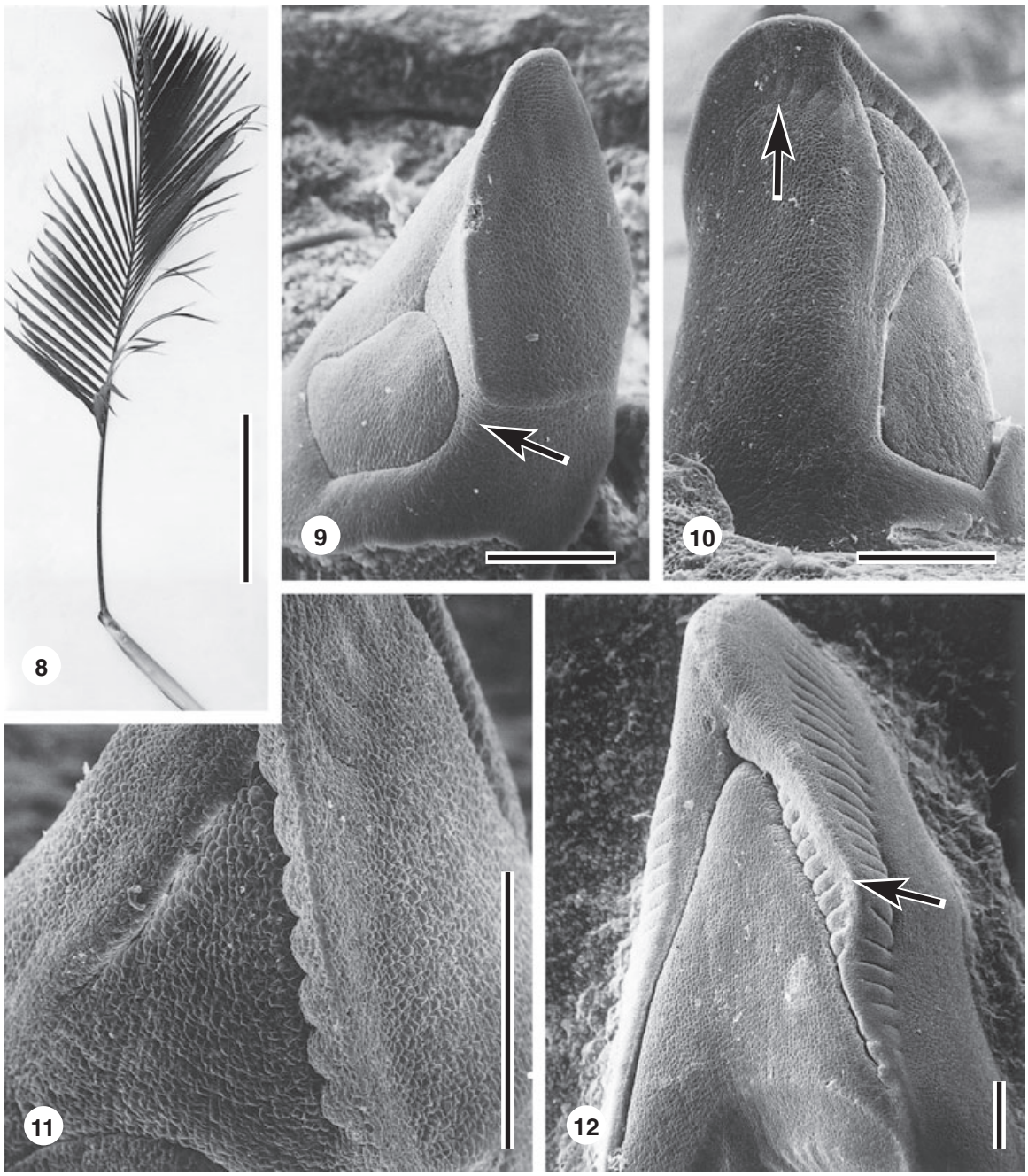


Figure 7. Diagram illustrating alternative hypotheses for plication formation in palms (Arecaeae). A–C. Tissue splitting hypothesis. After formation of lateral vein procambium (A), schizogenous slits develop in a regular pattern (B) that results in the plicate appearance of the leaf blade (C). Schizogeny breaches the dermal layer so that new protoderm must differentiate from ground tissue. D–F. Differential growth hypothesis. After formation of lateral vein procambium (D), localized growth in the intercostal panels between the procambial strands results in deformation of the blade toward the abaxial side (E). Continued deformation resulting from intercalary growth results in formation of plications (F). White, ground meristem; black, procambium; hatched, protoderm; diamonds, location of slits; stippling, intercalary growth; arrows, direction of growth.

Resolution between the tissue splitting and differential growth models of plication formation depended on careful attention to the plane of section, analysis of serial sections and, when available, use of the scanning electron microscope to resolve the fine details of topography of primordia and young leaves. In a series of papers published in the 1960s, Periasamy (1962, 1965, 1966a, b, 1967) provided a detailed description of the formation of plications in four palms: the pinnate reduplicate *Cocos nucifera* L. (Subfamily Arecoideae), pinnate, induplicate *Phoenix sylvestris* Roxb. (Coryphoideae), costapalmate induplicate *Borassus flabellifer* L. (Coryphoideae) and bipinnate induplicate *Caryota nitis* Lour. (Arecoideae; Periasamy, 1962, 1965, 1966a, b, 1967; classification of Dransfield & Uhl, 1998). Despite the considerable diversity in mature leaf morphology, Periasamy (1962) demonstrated that the plication formation process occurs through differential growth alone and that this process was essentially identical in all four species. Later, Kaplan and co-workers (Dengler, Dengler & Kaplan, 1982; Kaplan *et al*., 1982a, b) compared plication formation in the pinnate reduplicate palms *Chrysalidocarpus lutescens* Wendl. (Arecoideae) and *Chamaedorea seifritzii* Burret. (Ceroxyloideae) and in the palmate induplicate *Rhapis excelsa* (Thunb.) Henry (Coryphoideae). Their results corroborated those of Periasamy, and also provided strong additional support for plication formation through differential growth. As illustrated by *Chrysalidocarpus lutescens* (Figs 8–12), plications are first visible externally as a series of regularly spaced parallel ridges and narrow grooves on the abaxial side of the leaf (Figs 10, 11). The first plications to be formed are near the leaf apex and are orientated obliquely, whereas those formed later toward the base of the leaf are horizontal in orientation; as the leaf extends in length, all plications come to lie orthogonal to the axis of the leaf



Figures 8–12. Leaf development in *Chrysalidocarpus lutescens* (Arecaeae) illustrating plication formation in the palms. Fig. 8. Mature leaf. Scale bar = 1 m. Fig. 9. Scanning electron micrograph (SEM) of leaf primordium prior to plication formation. Arrow, approximate boundary between upper leaf zone and lower leaf zone. SEM scale bar = 200 μm. Fig. 10. SEM of leaf showing early stage of plication formation. Note adaxial ridges visible on right leaf margin and slit-like appearance of abaxial grooves (arrow) on left leaf margin. SEM scale bar = 200 μm. Fig. 11. SEM of same primordium at higher magnification. SEM scale bar = 200 μm. Fig. 12. SEM of primordium showing adaxial and abaxial ridges and non-plicate marginal strip (arrow). SEM scale bar = 200 μm. Reproduced by permission from Dengler NG, Dengler RE, Kaplan DR. 1982. *Canadian Journal of Botany* **60:** 82–95.

(Fig. 12). The apical-most plications are submarginal to the hood-shaped tissue of the leaf apex and the more basal plications are delimited by the marginal strip of non-plicate tissue (Fig. 12). Longitudinal serial sections of the blade reveal that, as observed by Periasamy (1962) for other palm species, the first indications of plication formation are slight ridges on the adaxial side of the leaf that are associated with the lateral vein procambial strand positions (compare Fig. 13B with A). The tissue between these ridges extends by intercalary growth and becomes folded into a series of abaxial ridges that alternate with the first-formed adaxial ridges (Fig. 13C, D). Whether abaxial ridge folding results from growth that is actively directed toward the abaxial side or simply from buckling of a growing sheet that is constrained on all sides is unknown. Kaplan and co-workers (Dengler *et al*., 1982; Kaplan *et al*., 1982a, b) also provided two additional lines of evidence that strongly supported the hypothesis of differential growth. First, electron microscopy of plications at a range of developmental stages showed that a continuous cuticle, a marker of protoderm identity and continuity, was present at all stages (Dengler *et al*., 1982). Second, counts of the number of cell layers present at the adaxial ridges, the abaxial ridges and the intercostal sectors indicated that the numbers of cell layers increased or remained the same; there was no indication of a reduction in cell layer number, as would be predicted if tissue splitting occurred (Dengler *et al*., 1982; Kaplan *et al*., 1982b). Thus, evidence strongly supports the differential growth model for plication formation in a broad phylogenetic sample of palms.

The second step in palm leaf morphogenesis, the separation of the plications into individual leaflets – and of the marginal strip from the leaflet tips, is much less studied. The separation of the corrugations along precise lines to form reduplicate, induplicate or multiribbed segments has been well known for over 150 years, however. Observations made during the 19th century by Eichler (1885), Naumann (1887) and Deinega (1898) described two different modes of leaflet separation. In the first, the process of separation appears to result from a simple tissue schizogeny (a 'mucilaginous disintegration', *Verschleimung*) at relatively early stages of plication development. Schizogenous separation appears to occur either progressively, from the outside in across the lamina (*Rhapis flabelliformis*, Periasamy, 1967), or simultaneously (*Chamaedorea seifritzii*, Kaplan *et al*., 1982b). As blade tissue is still meristematic, the ground meristem is able to re-establish the continuity of a protoderm layer and therefore the epidermis in mature leaves (Fig. 6H; Periasamy, 1967). In the second mode, schizogeny occurs, but does not extend completely across the blade, leaving a narrow isthmus of tissue

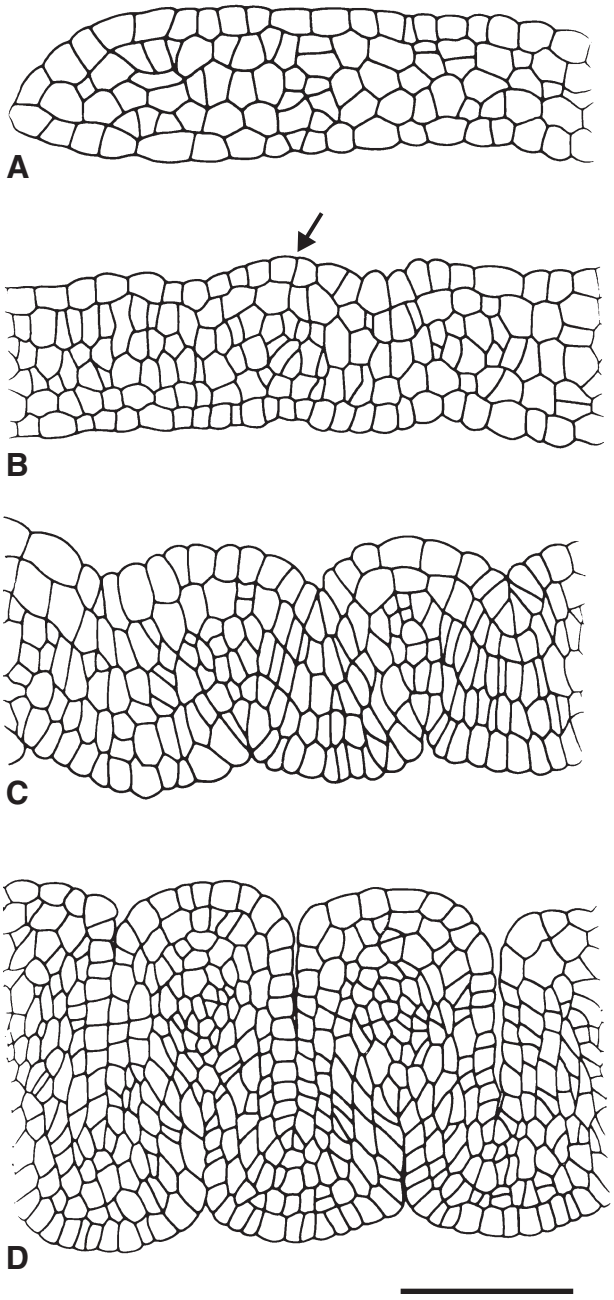


Figure 13. Outline drawings taken from serial longitudinal sections of developing leaf blades illustrating plication formation in *Chrysalidocarpus lutescens* (Arecaeae). A. Unplicate lamina of leaf 0.5 mm in length. B. Plication inception (arrow) in 0.75-mm leaf. C. Intercalary growth of intercostal sector in 1.0-mm leaf. D. Plications in a 1.5-mm leaf. Scale bar = 50 μm. Reproduced by permission from Dengler NG, Dengler RE, Kaplan DR. 1982. *Canadian Journal of Botany* **60:** 82–95.

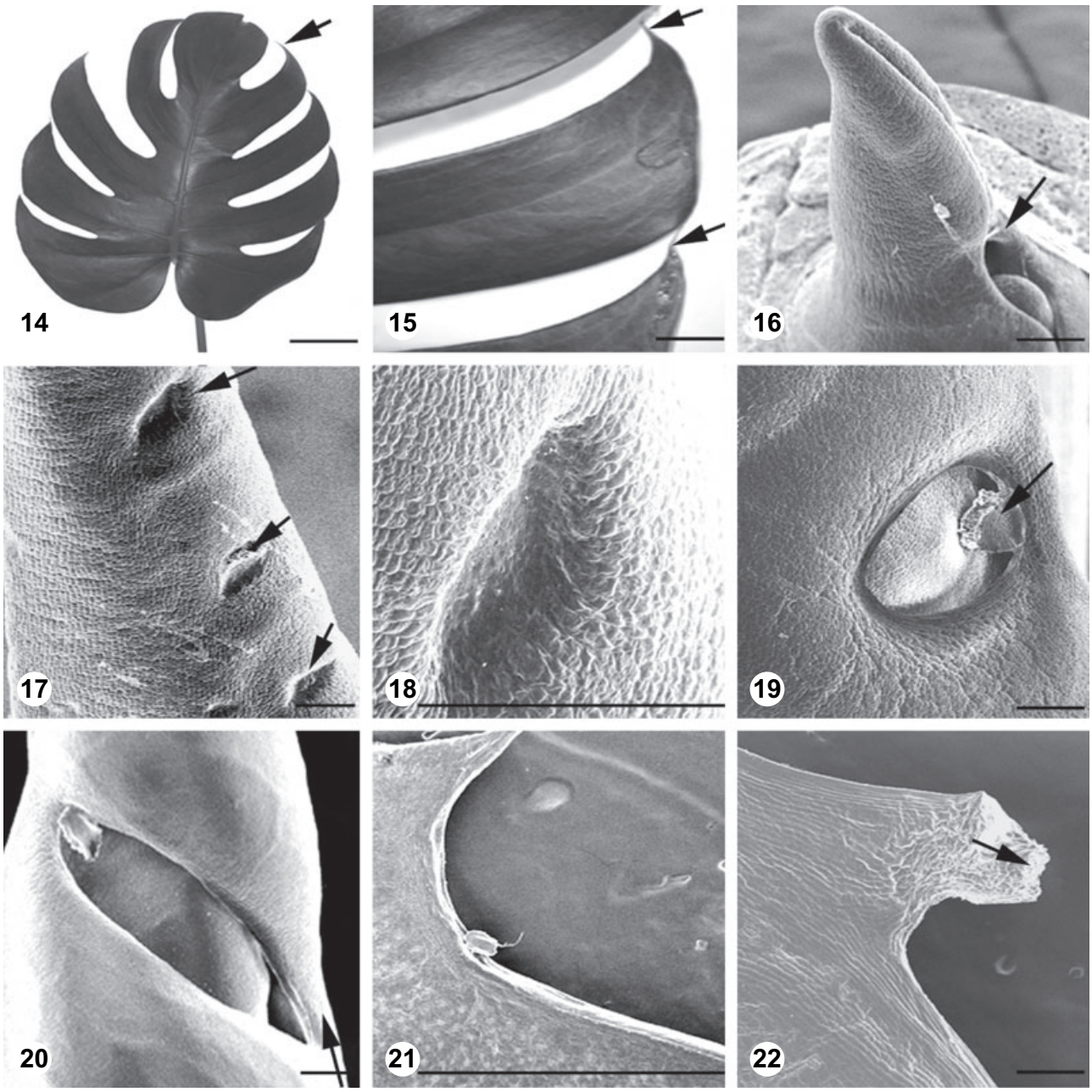
that holds adjacent leaflets together (Fig. 6F, G; Dengler *et al*., 1982). This narrow bridge of tissue is disrupted mechanically, usually late as the blade unfolds from the crown, and may be identifiable on the margin of the leaflets as brown membraneous tissue (Eichler, 1885). This mode has been described for the palmate induplicate leaves of *Pritchardia filifera* Sudw. (Coryphoideae, Naumann, 1887) and the pinnate reduplicate leaves of *Cocos nucifera* (Arecoideae, Periasamy, 1965). The presence of dense epidermal trichomes in many palms obscures the actual process of separation and often makes it difficult to determine the specific mode of separation without detailed observations of young stages.

The location of leaflet separation in relation to vascular architecture also influences whether simple schizogeny or wholesale cell death is involved. When leaflet separation occurs in an intercostal panel between the adaxial and abaxial ridges, the zone of schizogeny develops between major vascular bundles and involves dermal and ground tissues only (Fig. 6H; Kaplan *et al*., 1982b). When leaflet separation occurs in the vascular bundle-free abaxial ridges of reduplicate palms, schizogeny also affects dermal and ground tissues only (Fig. 6F; Dengler *et al*., 1982). By contrast, because the earliest-formed vascular bundles within the leaflets occur in the adaxial ridges (Periasamy, 1962, 1966b; Dengler *et al*., 1982), the zone of schizogeny in induplicate palms must accommodate the position of these bundles. Separation typically occurs on both sides of the vascular bundle, forming constricted bridges that cut off a strip of tissue containing the bundle from the adjoining intercostal panels (Fig. 6G; Eichler, 1885; Naumann, 1887; Deinega, 1898). These strips may persist as 'interfold filaments', as seen for *Pritchardia filifera*, or as fibrous leaflet margins as in *Livistona australis* (Naumann, 1887; Uhl & Dransfield, 1987). Separation of the tissue containing the adaxial vascular bundles is taken a step further in the genus *Phoenix* in which a thin, membraneous sheet of tissue (the *Haut*) is separated from the adaxial side of the plications along with the non-plicate marginal strip (Goebel, 1926; Periasamy, 1966a; Padmanabhan, 1969). Periasamy (1966a) showed conclusively that this membraneous sheet is actually a composite tissue that develops from proliferations of the adaxial ridges of the plications. The *Haut* grows in surface area along with the plications and becomes vascularized; finally it is separated from the plications, in a process involving the gradual constriction of the separation zone and ultimately a mechanical disruption (Periasamy, 1966a).

Schizogenous separation of the non-plicate marginal strip from the distal tips of the leaflets is a conspicuous feature in many palms and was also noted by early observers (von Mohl, 1845; Trecul, 1853; Eichler, 1885). In some palms such as *Chrysalidocarpus lutescens*, the tissues making up the marginal strip are ephemeral and difficult to detect in leaves expanding from the crown (Eames, 1953), whereas in others the marginal strip forms a prominent band that connects the tips of all the leaflets in newly expanded leaves (illustrated in Eames, 1953; Tomlinson, 1990). In other genera, the marginal strip can either be highly persistent, vascularized and similar to the rest of the blade in texture, or it can persist as dry fibrous strips, or have a delicate, cobweb-like texture (Eames, 1953). The strip is usually separated into two pieces by an oblique separation zone near the leaf apex (Fig. 36E); one piece is a simple band, but the other carries the apical portion of the non-plicate strip, forming a hook-like structure (Eames, 1953). When the strip is persistent, each half remains attached to the basal-most leaflets of pinnately dissected leaves, forming a pair of rein-like structures that hang below the palm crown (Eames, 1953). Although a persistent marginal strip is prominent in many species with pinnately dissected leaves, it is ephemeral and inconspicuous in palmately dissected palm leaves; in some palmate genera, the marginal tissue disintegrates without being freed as a unitary structure (Eames, 1953). While separation of leaflets and the marginal strip is usually described as an abscission-like process (Kaplan *et al*., 1982a; Tomlinson, 1990), virtually nothing is known about the cell biology of the process. Cell death of the abscised tissues is clearly involved, but whether this precedes schizogenous separation or follows it and whether an abscission layer is formed before mechanical disruption occurs are unknown.

Perforation formation in the Araceae and *Aponogeton madagascariensis* (Alismataceae)

The presence of perforations in the leaves of *Monstera deliciosa* Liebm. and other aroids has also attracted the interest of plant morphologists for more than 100 years (Figs 14, 15; DeCandolle, 1827; Trecul, 1854). Species of the genus *Monstera* and the related *Rhaphidophora*, *Amydrium* and *Epiprennum* (Monsteroideae) are often conspicuously and elaborately perforated, or more rarely, deeply pinnatifid (Mayo *et al.*, 1997, 1998). Perforations also occur in the genera *Dracontium* and *Cercestis* of the Lasioideae (Mayo *et al.*, 1997, 1998). DeCandolle (1827; as cited in Trecul, 1854) appears to be the first to write about this unusual phenomenon and speculated that the holes were indicative of plant degeneration and a 'lack of vigour'. DeCandolle thought that the holes represented a failure of the individual segments of the leaf blade to weld themselves into a whole lamina and that they therefore revealed the process of compound leaf development (Trecul, 1854). Trecul (1854), who had



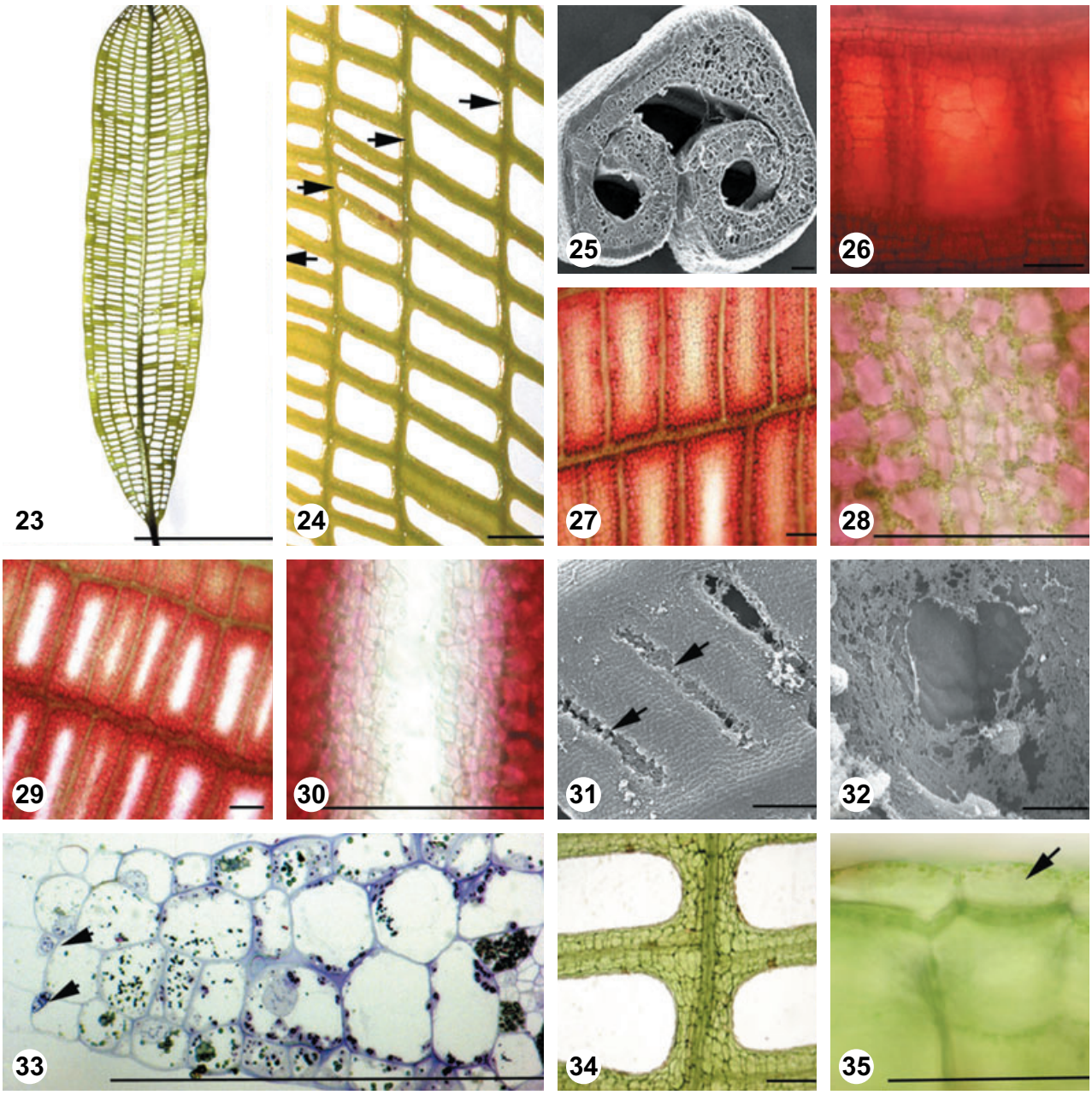
Figures 14–22. Leaf development in *Monstera deliciosa* (Araceae) illustrating perforation formation. Fig. 14. Mature leaf showing perforations that extend to the leaf margin, resulting in a pinnatisect leaf. Arrow indicates region illustrated in Fig. 22. Scale bar = 5 cm. Fig. 15. Higher magnification showing thin bridges of marginal tissue (arrows) that must be mechanically disrupted. Scale bar = 2 cm. Figures 16–22. Scanning electron micrographs (SEMs). Scale bars = 200 μm. Fig. 16. Young leaf primordium prior to perforation. Arrow demarcates upper and lower leaf zone. Fig. 17. Leaf blade from 5-mm leaf showing three perforations (arrows). Fig. 18. Perforation from same leaf. Fig. 19. Disc of dead tissue remains attached to margin of perforation (arrow) in expanding leaf. Fig. 20. Expanding perforation. Marginal tissue (arrow) is intact. Fig. 21. Portion of perforation near midrib in mature leaf. Fig. 22. Margin of mature leaf. Note mechanical disruption of marginal tissue adjacent to perforation (arrow), resulting in pinnatifid leaf shape.

just completed a lengthy treatise of the development of simple, lobed and dissected leaves (Trecul, 1853), recognized that the leaves of *Monstera* developed through a process that was diametrically opposed to that described by DeCandolle (1827), but was also completely different from the other dissected leaves that he himself had studied (Trecul, 1853). Trecul (1854) emphasized that *Monstera* leaves first form a complete simple leaf blade, and then tissues at the site of each perforation 'destroy themselves' to form the hole. He did not describe the behaviour of tissues involved in perforation formation in *Monstera*, but argued that it would be similar to the processes that he had observed in other plant material [referred to as *Pothos repens* (Lours.) Druce, an imperforate species] where discrete patches of leaf mesophyll lose chlorophyll and die, forming the mottling seen on the leaf surface (Trecul, 1854).

The process of perforation in *Monstera deliciosa* has been studied in more detail by Schwarz (1878), Melville & Wrigley (1969) and Kaplan (1984). The leaves of *Monstera* arise as conical structures that shortly develop a sheathing leaf base encircling the meristem (Fig. 16; Melville & Wrigley, 1969; Kaplan, 1984). Primary morphogenesis through blastozone activity does not occur, and the leaf enters the secondary morphogenesis/histogenesis stage almost immediately. Intercalary growth results in a convolutely rolled leaf blade, with the narrow half to the outside and the position of the narrow half alternating between nodes on the distichous shoots. The first-formed perforations arise in the panels of tissue demarcated by the lateral veins and are positioned more or less equidistantly between them (Fig. 17). The perforations are recognizable as elliptical patches of brown, necrotic tissue that are depressed in contrast to adjacent regions of the leaf blade (Fig. 18; Melville & Wrigley, 1969; Kaplan, 1984). In sectional view, the patch of necrotic tissue first appears stretched (Schwarz, 1878; Melville & Wrigley, 1969), and in scanning electron micrographs, it can be seen to detach from surrounding tissues along part of its circumference (Fig. 19; Kaplan, 1984). As the leaf blade expands and the perforation extends in area, the patch of necrotic tissue is retained on one side of the perforation (Figs 19–21; Kaplan, 1984). The mechanical disruption of the thin bridges of tissue between the perforation and margin converts the blade outline from simple and entire to deeply pinnatifid (Figs 14, 22). The disrupted tissues lose chlorophyll before breakage, and so it is possible (but unstudied) that cell death and/or schizogeny are involved in this late stage of leaf morphogenesis.

The centripetal sequence of formation of successive perforations was described in detail for the large-leaved cultivar of *M. deliciosa* by Melville & Wrigley (1969): the first-formed perforations arise near the blade margin, whereas the second-formed perforations arise equidistantly between the first and the midrib in each intercostal panel of tissue, presumably reflecting the greater amount of intercalary expansion near the midrib. Late-formed perforations arise equidistantly between the first two and each lateral vein and then between the second perforation and the midvein. Melville & Wrigley (1969) found that a more or less constant distance of 0.13–0.15 mm separated sequentially formed perforations (or perforations and lateral veins) at the time of perforation initiation, suggesting to them that a positionally dependent signalling system was at play.

A striking example of leaf shape development through perforation formation at the secondary morphogenesis stage also occurs in a single species of the Aponogetonaceae, *Aponogeton madagascariensis* (Mirbel) H. Bruggen. Unlike many *Monstera* species in which perforations break through the leaf margin (Madison, 1977), the entire margin of *A. madagascariensis* leaves is not disrupted; nevertheless, perforation formation results in a highly complex leaf shape, at least in terms of perimeter to area ratio (Serguéeff, 1907; Gunawardena, Greenwood & Dengler, 2004). *A. madagascariensis* is a Madagascar endemic belonging to the monogeneric Aponogetonaceae, a family of about 40 species of submerged aquatics from the Old World tropics and subtropics (Tomlinson, 1982; van Bruggen, 1985, 1998). In nature, its submerged leaves are variable in size and degree of fenestration (van Bruggen, 1985), but under stable aquarium conditions, leaves reach lengths of 20–25 cm and have a short, open sheathing leaf base, a long petiole and an oblong blade (Fig. 23). A conspicuous midvein and at least eight lateral veins diverge from the midvein near the base of the lamina and converge near the apex; in addition, frequent commissural veins extend perpendicularly to the longitudinally orientated lateral veins (Fig. 24; Gunawardena *et al*., 2004). The higher order reticulate veins described for the floating leaves of other *Aponogeton* species are lacking in the submerged, fenestrate leaves of *A. madagascariensis* (Tomlinson, 1982). Seedlings produce small, simple non-fenestrate leaves, whereas the leaves of juvenile plants typically have a few perforations near the midrib (Serguéeff, 1907). Under stable growth conditions, adult plants produce leaves with large rectangular perforations in 95% or more of the panels lying between the longitudinal and cross veins (Figs 23, 24; Serguéeff, 1907; Gunawardena *et al*., 2004). Because the perforations are wider than the bars of tissue that include the veins, the blade has a grid-like or lattice-like appearance, suggesting the common names 'lace plant' and 'lattice leaf'.



Figures 23–35. Leaf development in *Aponogeton madagascariensis* (Aponogetonaceae) illustrating perforation formation. Fig. 23. Mature leaf. Scale bar = 3 cm. Fig. 24. Higher magnification of mature leaf showing rectangular perforations between four longitudinal veins (arrows) and transverse commissural veins. Scale bar = 2 mm. Figs 25–29. Scale bars = 200 μm. Fig. 25. Scanning electron micrograph (SEM) of sectioned young leaf prior to perforation formation showing involute rolling of leaf blade. Fig. 26. Light micrograph of abaxial surface of leaf showing vein pattern and anthocyanin accumulation. Fig. 27. Light micrograph of leaf at the 'window' stage. Loss of anthocyanin colour is one of first indications of initiation of programmed cell death. Fig. 28. Higher magnification of same leaf showing loss of anthocyanin in transparent window. Fig. 29. Light micrograph of leaf at stage when perforation first breaks through the blade. Fig. 30. Higher magnification of same leaf. Scale bar = 50 μm. Fig. 31. SEM of leaf at same stage showing new perforation (arrow). Scale bar = 200 μm. Fig. 32. SEM of same leaf showing degradation of cell walls. Scale bar = 5 μm. Figs 33–35. Scale bars = 200 μm. Fig. 33. Light micrograph of cross-section of embedded leaf at perforation formation stage. Arrows, mesophyll cells that will transdifferentiate as epidermal cells. Fig. 34. Light micrograph of fully expanded leaf showing mature perforations. Fig. 35. Higher magnification of same leaf showing transdifferentiated mesophyll cells (arrow) at periphery of perforation. Reproduced by permission from Gunawardena AHLAN, Greenwood JS, Dengler NG. 2004. *Plant Cell* **16:** 60–73.

It is perhaps surprising that the process of perforation formation in this well-known species, prized by aquarists, has been so little studied. In her doctoral dissertation on the morphology of *A. madagascariensis* at the University of Geneva, Serguéeff (1907) described seed germination and seedling growth, the morphology of the adult plant, and the anatomy of the corms, roots, leaves, inflorescence and flower. She also described some intriguing details of perforation formation. Serguéeff (1907) recognized that perforations are not formed when the leaf is enfolded within the apical bud, as occurs for *Monstera* species, but rather appear late, when the leaf is over 2 cm in length and already unfurled. Serguéeff (1907) reported that perforation formation was preceded by deposition of a brownish substance in subepidermal cell walls, forming an elliptical or rectangular pattern in surface view. Resistance of walls with the brownish deposits to sulphuric acid treatment suggested that the deposits were suberin in nature, and Serguéeff (1907) hypothesized that the suberized layer isolates the enclosed cells so that they wither and die. She also reported that cells lining the perforation become tangentially stretched as the perforation expands and that the perforations of mature leaves resemble the necrotic spots produced by some pathogens.

The cell death process during leaf development in *A. madagascariensis* has recently been characterized (Gunawardena *et al*., 2004). Not only does this represent an intriguing and highly unusual mode of leaf morphogenesis, but it also provides a potentially useful system for studying the cell biology and developmental regulation of cell death in intact living plants. Cell death is not initiated until after the leaves, consisting of a sheathing base, short petiole and involutely rolled blade, have extended from the apical bud. At this stage, the pattern of longitudinal major veins and transverse minor veins is fully formed and cells of both the dermal and the ground tissue layers accumulate vacuolar anthocyanin and conspicuous chloroplasts (Figs 25, 26). As the blade unrolls and flattens, distinct transparent regions appear in the rectangular panels of tissue between the veins as a result of loss of anthocyanin and chlorophyll (Figs 27, 28). These transparent 'windows' appear near the midvein first and progress toward the margin, following the order in which tissue is exposed as the leaf unrolls. Cytoplasmic streaming is altered as the window cells become transparent: movement of organelles and the nucleus becomes more rapid and erratic, followed by cessation of streaming and cytoplasmic collapse (Gunawardena *et al*., 2004). At the same time, nuclei of cells within the transparent area become TUNEL-positive, indicating that nuclear DNA is being degraded (Gunawardena *et al*., 2004). These early indicators of cell death begin in a discrete subpopulation of cells near the centre of the window and then progress toward the periphery, stopping short within 5 ± 1 cells of the vein (A. H. L. A. N. Gunawardena, unpubl. data). In contrast to the cells undergoing cell death, adjacent cells retain their anthocyanin and chlorophyll, cytoplasmic streaming is unaltered and nuclei are TUNEL-negative. Following these initial events, cell wall and cytoplasmic degradation allow rupture of the blade in the window areas (Figs 29–32; Gunawardena *et al*., 2004). These changes appear to occur simultaneously in all four cell layers, so that an opening that extends right through the leaf is formed as the blade begins to expand (Fig. 33). In fully expanded leaves, living mesophyll cells at the periphery of the perforation acquire an elongate shape and reform the epidermal layer (Figs 34, 35). As reported by Serguéeff (1907), brown deposits in cell walls at the rim of the perforation were observed, but these appeared to be a late developmental event, similar to a wounding response, occurring after cell death had formed the perforation (Gunawardena *et al*., 2004).

Although the details of the cell biology of programmed cell death differ between *Aponogeton* and *Monstera* (Gunawardena *et al.*, 2005), many aspects of this process are directly comparable. Perforations are placed at regular, predictable distances from veins (and from earlier-formed perforations in *M. deliciosa*). The size of perforations reflects both the timing of initiation and the distribution of leaf expansion: in *A. madagascariensis* the marginal part of the blade expands less, resulting in smaller, square perforations in this region, whereas in *Monstera*, the marginal part of the blade expands more, resulting in very large early-formed perforations near the margin. In *A. madagascariensis*, the zone of dying cells spreads outward from the locus of initiation, but always appears to stop about five cells from the veins. In *Monstera*, the boundary between dying and living cells is sharp, with simultaneous cell death across the perforation site. In both cases, however, mesophyll cells exposed at the surface by perforation formation undergo transdifferentiation as epidermal cells (Schwartz, 1878; Serguéeff, 1907; Melville & Wrigley, 1969; Kaplan, 1984; Gunawardena *et al*., 2004). Such a transformation is subtle in the aquatic *A. madagascariensis*, in which the epidermis lacks a detectable cuticle and possesses numerous chloroplasts (Sculthorpe, 1967); however, the shape of these transformed cells is more epidermal than mesophyll-like (Fig. 36; Gunawardena *et al*., 2004). In *Monstera*, mesophyll cells exposed at the periphery of the perforation elongate dramatically, in contrast to other ground tissues, and secrete a cuticle, maintaining the continuity and distinct features of the dermal layer (Gunawardena *et al.*, 2005).

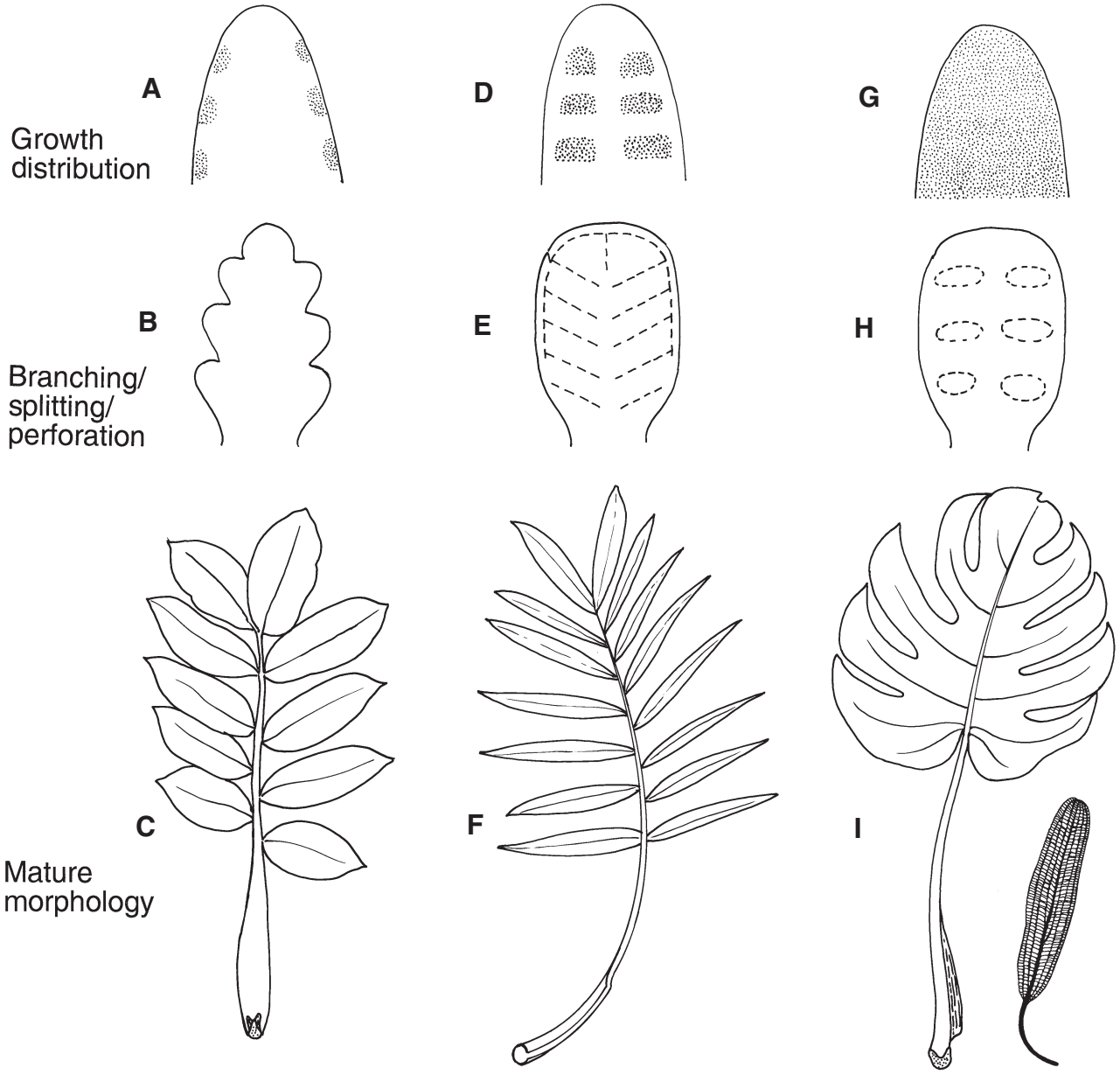


Figure 36. Diagram summarizing three alternative modes of development of dissected leaves in monocotyledons. A–C. Leaf development in *Zamioculcas zamiifolia* (Araceae) illustrating marginal blastozone fractionation. A. Localized growth (stippling) in marginal blastozone of leaf primordium. B. Leaflet primordia at end of primary morphogenesis stage. C. Mature pinnately dissected leaf. D–F. Leaf development in *Chrysalidocarpus lutescens* (Arecaceae) illustrating plication formation and leaflet separation. D. Localized growth (stippling) in submarginal strips of tissue form plications. E. An abscission-like process (dashed lines) separates leaflets from each other and from the non-plicate marginal strip and leaf apex. F. Mature pinnately dissected leaf. G–I. Leaf development in *Monstera delicosa* (Araceae) illustrating perforation formation. G. Diffuse intercalary growth (stippling) in leaf primordium. H. Perforation formation through programmed cell death of discrete patches of cells (dashed lines). I. Mature pinnately dissected leaf of *M. deliciosa* (left) and fenestrate leaf of *Aponogeton madagascariensis* (Aponogetonaceae, right).

GENERAL DISCUSSION

Modes of dissected leaf development

These three strikingly different mechanisms of leaf morphogenesis reflect the multiple independent origins of dissected leaves in monocotyledons. In each mode, more widely used developmental processes, such as localized enhancement and suppression of growth, abscission or programmed cell death, are differentially regulated in space and time to produce complex leaf shape. In the first, blastozone fractionation, the morphogenetic potential of the leaf primordium blastozone is expressed only at sites of leaflet formation and is completely suppressed in intervening regions during the primary morphogenesis stage (Fig. 36A, B), giving rise to a pinnately dissected leaf with free leaflets borne on an elongate rachis (Fig. 36C). In the second, leaflet separation, morphogenetic potential of the blastozone is not expressed; instead the locus for growth is shifted to discrete submarginal strips of tissue (Fig. 36D). Intercalary growth of these strips is confined by the primordium base, margin, apex and rachis, so that expanding tissues are deformed into regular folds or pleats. Following this stage, individual (or several) pleats are separated from each other and from the non-plicate marginal tissue through an abscission-like process (Fig. 36E), literally dissecting a simple leaf blade into individual leaflets that may later be separated by rachis extension (Fig. 36F). In the third mode, perforation formation, the blastozone is inactive, and early lamina growth is intercalary and diffuse (Fig. 36G). At a relatively late stage of secondary morphogenesis, discrete and regularly spaced patches of tissue within the simple blade undergo programmed cell death. As the blade continues to expand, tissues are ripped apart at the periphery of the dead cells, forming an open perforation (Fig. 36H). Placement of the perforation near the margin, coupled with mechanical disruption of the thin strip of tissue lying between the perforation and margin, results in a deeply pinnatifid leaf in many *Monstera* species (Fig. 36I, left). In the highly unusual *Aponogeton madagascariensis*, the leaf outline remains simple, but perforation formation through programmed cell death results in a complex lattice-like leaf shape (Fig. 36I, right),

Despite these different modes of leaf dissection, all of these representative species share fundamental aspects of leaf development common to the monocotyledons and to the flowering plants in general. Leaves are formed through fractionation of the shoot apical meristem, accompanied by a shift in growth direction of the fractionated region (Hagemann, 1970; Hagemann & Gleissberg, 1996). Shortly after initiation, the leaf primordium is differentiated into an upper leaf zone which is thick in the dorsiventral plane and a lower leaf zone which is flattened in the dorsiventral plane (Troll, 1939; Knoll, 1948; Kaplan, 1973; Rudall & Buzgo, 2002). In most monocotyledons, the leaf initiation process extends from the initial site around the circumference of the shoot apical meristem, giving rise to a lower leaf zone that encircles the meristem. During early growth, distinct regions of leaf base, blade and petiole (if present) are delimited by differing proportions of growth in the dorsiventral and mediolateral planes (Kaplan, 1973; Bharathan, 1996; Rudall & Buzgo, 2002). A distinct phase of primary morphogenesis in the strict sense (Hagemann, 1970) does not occur in most monocotyledons, however, because growth of the upper leaf zone is suppressed and blade formation from the lower leaf zone is intercalary. In sharp contrast, the broad lamina of certain monocotyledons is derived from the upper leaf zone (Wilder, 1976; Bloedel & Hirsch, 1979; Kaplan *et al*., 1982a; Martin & Tucker, 1985; Periasamy & Muruganathan, 1985, 1986; Bharathan, 1996) and, in a small subset of the dissected leaved Araceae, Dioscoreaceae, and (presumably) Taccaceae, the marginal blastozone is activated and undergoes fractionation during primary morphogenesis (Troll, 1939; Kaplan, 1984; Periasamy & Muruganathan, 1985, 1986). By contrast, the dissection events that give rise to the distinctive mature morphologies of the leaves of palms and certain aroids are developmental events that occur much later, after the primary morphogenesis stage that gives rise to essentially simple leaf shapes. Leaflet separation in palms occurs relatively late during the secondary morphogenesis/histogenesis stage; although not studied in detail, actual separation appears to require the final stages of leaf expansion (rachis elongation, leaflet pulvinus expansion) to extricate fully the leaflets from the originally simple blade in at least some species (Eichler, 1885; Eames, 1953; Periasamy, 1966b). In *Monstera* and *Aponogeton*, perforation occurs even later in the course of secondary morphogenesis/histogenesis, as indicated by vein pattern formation that is well underway. Perhaps it is most appropriate to regard perforation formation as a component of the late, histogenetic processes of leaf development, a process that in this case has substantial morphogenetic consequences.

Cellular mechanisms of leaflet separation and perforation formation

Leaflet separation and perforation formation employ cellular mechanisms that have numerous other functions in plant growth and development, and that presumably have been secondarily recruited into leaf developmental programmes. For instance, the process of schizogeny is employed during the formation of most intercellular spaces and secretory cavities (Esau,

1965). Schizogeny is an important component of abscission of leaves and fruits and typically is coupled with the formation of a protective layer, in which cellular properties such as the chemical composition of cell walls are modified on the proximal side of the abscission zone. The subsequent formation of the separation layer on the distal side involves highly localized secretion of wall-degrading enzymes, resulting in detachment of the plant part (reviewed in González-Carranza, Lozoya-Gloria & Roberts, 1998). Although leaf abscission in palms has not been studied at the level of cell biology, it is known to be more complex than formation of a single, planar abscission zone (Tomlinson, 1990). In some palms, a circular abscission zone is formed at the base of the leaf sheath; if the sheath is tubular, an additional vertical abscission zone forms opposite the point of leaf insertion, allowing the leaf to fall cleanly from the trunk (*Veitchia* type; Tomlinson, 1990). In others, two vertical lines of abscission through the leaf sheath flank the basal continuation of the rachis–petiole axis; separation allows the leaf to fall (or at least hang from the central part of its base), while most of the fibrous sheath remains on the trunk (*Cocos* type; Tomlinson, 1990). It is certainly possible that the cellular mechanisms associated with whole leaf abscission are also employed in leaflet separation. In palms for which there is detailed anatomical information about the process (Periasamy, 1967; Dengler *et al*., 1982), separation occurs in tissue that is still undergoing cell division and expansion and occurs when these growing tissues are well protected by the sheathing leaf bases of older leaves. Thus, it is possible that only the separation part of the abscission 'programme' is used, as formation of protection layers would be unnecessary in this developmental environment. The nature of positional signals and how they are translated into specific developmental events is not well understood for any plant developmental process, and it is very unlikely that palms will ever prove to be a tractable system for the study of positional controls in plant development. Nevertheless, they do provide a fascinating example of the precise spatial control of a developmental mechanism that allows it to be used for a novel function.

Similarly, programmed cell death is employed for a wide range of functions in plant development (reviewed in Morgan & Drew, 2004). It is a key event in the differentiation of specialized cell types such as tracheary elements, sclerenchyma fibres and cork cells. Programmed cell death also acts to delete tissues with ephemeral functions such as the endosperm or embryonic suspensor. It is also used in floral shoot morphogenesis, such as in the formation of functionally unisexual flowers from bisexual floral primordia. In addition to internally regulated events, programmed cell death can be environmentally induced, as in the development of lysigenous aerenchyma triggered by hypoxic stress (Gunawardena *et al*., 2001a, b) and the hypersensitive response triggered by pathogen invasion (reviewed by Pontier, del Pozo & Lam, 2004). Thus, an individual organism employs programmed cell death not only for multiple developmental purposes, but also to respond appropriately to environmental perturbations. The extensive literature on the specific cellular and molecular mechanisms of programmed cell death indicates that this is not a unitary process and that many different versions occur. Partly because of the diversity of organisms under study, it is still unclear whether an individual plant uses the same cellular programmed cell death mechanisms for multiple purposes, for example to differentiate tracheary elements and to respond to pathogens. Therefore, it is difficult to predict the scope of preexisting mechanisms that might be available for recruitment into leaf development. In *Aponogeton madagascariensis*, the process of perforation formation involves an early alteration of the cytoplasmic streaming (presumably a reflection of altered tonoplast permeability), degradation of nuclear DNA without detectable laddering into internucleosomal units, thinning and shrinkage of the cytoplasm, chromatin condensation, and late persistence of degraded organelles (Gunawardena *et al*., 2004). This sequence of events is very similar to that observed during the differentiation of tracheary elements from cultured *Zinnia* mesophyll cells (Groover *et al*., 1997; Fukuda, 2000), suggesting that mechanisms used for xylem differentiation might be brought under different spatial and temporal controls in the context of leaf development.

Phylogenetic distribution of dissected leaves in monocotyledons

The phylogenetic distribution of dissected leaves in monocotyledons indicates that this character has had multiple independent origins during their evolutionary diversification. Recent phylogenies also indicate that the morphogenetic mechanisms of leaflet separation and of perforation formation have each arisen more than once. Perhaps the most striking example of such a convergence occurs between the palms (Arecaceae) and the cyclanths (Cyclanthaceae). Several recent molecular phylogenies provide moderate to strong support for placement of the Cyclanthaceae within the Pandanales, and for early branching of the Pandanales, in contrast to later branching of the Arecales (Chase *et al*., 2000; Soltis *et al*., 2000; Stevenson *et al*., 2000). Mature leaves of cyclanths resemble those of palmatisect palms, with large plicate leaf blades, petioles and sheathing bases (Dahlgren *et al*., 1985; Harling *et al*., 1998). Leaf development in mem-

bers of the Cyclanthaceae has long been thought to resemble closely that of the palms (Eichler, 1885; Hirmer, 1919; Eames, 1953), and more recent observations by Wilder (1976) for *Carludovica palmata* indicate that the origin of plications is very similar. Plications are initiated in a submarginal position at the same time as the procambial strands of the major lateral vascular bundles, and first appear as slight adaxial ridges associated with anticlinal expansion and periclinal divisions in several cell layers. Buckling of the blade is thought to result from growth within zones of anticlinal divisions on either side of the adaxial ridges, forming the abaxial ridges (Wilder, 1976). The process of leaflet separation appears to be a late developmental event, however, and resembles that seen for induplicate palmate palms such as *Pritchardia* and *Livistona* (Naumann, 1887). In *Carludovica*, tearing extends only partway from the plication apex to base and appears to be limited by thickened tissue within the adaxial ridge (Wilder, 1976).

Similarly, the Aponogetonaceae and Araceae both belong to the Alismatales clade, but distribution of dissected or fenestrate leaves indicates that leaf morphogenesis through perforation formation is a derived character within each group (Chase *et al*., 2000; Soltis *et al*., 2000; Stevenson *et al*., 2000). The cell biology of programmed cell death differs in detail between *Aponogeton* and *Monstera*: cell walls are degraded in *Aponogeton*, but not *Monstera*, and cells die progressively (from the centre of the perforation site outwards) in *Aponogeton*, but simultaneously in *Monstera* (Gunawardena *et al.*, 2004, 2005). These differing patterns of DNA degradation during perforation formation may reflect separate evolutionary origins and the recruitment of differing programmed cell death pathways into leaf development.

Functional properties of dissected leaves

The convergent evolution of dissected leaves in monocotyledons presumably reflects selection for specific functional properties under the particular environment at the time of origin. For instance, the corrugation of palm and cyclanth leaves permits them to produce very large photosynthetic surfaces and to support these while resisting bending and torsional movements (Tomlinson, 1990; Niklas, 1992). In addition, dissection of these large blades into individual leaflets confers greater wind resistance as leaflets can move independently and therefore reduce drag (Niklas, 1992). Dissected leaves have greater heat transfer conductance than simple leaves and are able to maintain temperatures closer to air temperature, thus avoiding the negative effects of overheating on photosynthetic rate and water use efficiency (Gurevitch, 1988; Gurevitch & Schuepp, 1990). The function(s) of the perforations of *Monstera* and other aroids has not been studied *per se*, but it is likely that they serve to reduce effective leaf size and thus heat transfer properties, much like more conventionally dissected leaves (Madison, 1977). Another intriguing hypothesis is that the perforations serve as camouflage by disrupting leaf outline, much as various forms of leaf coloration and mottling are proposed to do (Givnish, 1990). Mottling and, by extension, perforations are also hypothesized to mimic herbivore damage, which could signal to herbivores that induced chemical and/or physical defences may already be present (Brown & Lawton, 1991). The function of perforations in the leaves of *Aponogeton madagascariensis* is also unexplored. It is possible that perforations reduce resistance to water flow, the explanation favoured by Serguéeff (1907), although other non-fenestrate species also grow in flowing streams (van Bruggen, 1985). Dissection of the leaf blade into a lattice-like structure significantly increases the surface-to-volume ratio and thus would increase the rate of diffusion of dissolved CO_2 and mineral nutrients into the photosynthetic tissues. Most other *Aponogeton* species have very thin leaves, only 4–5 cell layers thick (Tomlinson, 1982), however, so the rate of diffusion might not be limiting for photosynthesis in non-perforate leaves. The perforations might equally serve to provide camouflage against aquatic herbivores, a readily tested hypothesis.

In summary, the dissected leaves of monocotyledons present a fascinating example of evolutionary convergence of form that facilitates one or more functions, a convergence that has employed very different mechanisms of leaf development to the same morphological end.

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