

## Links between early pollen development and aperture pattern in monocots

S. Nadot<sup>1,\*</sup>, A. Forchioni<sup>1</sup>, L. Penet<sup>1</sup>, J. Sannier<sup>1</sup>, and A. Ressayre<sup>2</sup>

<sup>1</sup> Laboratoire Ecologie, Systématique et Evolution, Unité Mixte de Recherche 8079 du Centre National de la Recherche Scientifique, Université Paris XI, Orsay

<sup>2</sup> Station de Génétique Végétale du Moulon, Unité Mixte de Recherche 8120 du Centre National de la Recherche Scientifique, Gif sur Yvette

Received March 22, 2005; accepted June 8, 2005; published online August 31, 2006  
© Springer-Verlag 2006

**Summary.** Although the pollen grains produced in monocots are predominantly monosulcate (or monoporate), other aperture types are also found within this taxonomic group, such as the trichotomosulcate, inaperturate, zonaperturate, di-, or triaperturate types. The aperture pattern is determined during the young-tetrad stage of pollen development and it is known that some features of microsporogenesis can constrain the aperture type. For example, trichotomosulcate pollen is always associated with simultaneous cytokinesis, a condition considered as derived in the monocots. Our observations of the microsporogenesis pathway in a range of monocot species show that this pathway is surprisingly variable. Our results, however preliminary, reveal that variation in microsporogenesis concerns not only cytokinesis but also callose deposition among the microspores and shape of the tetrads. The role played by these features in aperture pattern determination is discussed.

**Keywords:** Microsporogenesis; Pollen; Cytokinesis; Aperture pattern; Callose deposition; Monocot.

### Introduction

The early steps of pollen development consist of the formation of microspores, a process called microsporogenesis that involves the meiotic division of diploid microsporocytes into four haploid microspores enclosed within the callosic wall of the microsporocyte. After the release of the microspores starts the gametogenesis. It consists of the subsequent development of microspores into mature bicellular or tricellular pollen grains, which contain the male gametes of spermatophytes. The pollen grains of angiosperms are surrounded by a complex wall made of sporopollenin: the exine. In most species, pollen grains present apertures, i.e., areas where the exine layer is thin or lacking (Erdtman

1952). The apertures are often fully visible in the late-tetrad stage, indicating that the aperture pattern (aperture structure, number and distribution) is determined during the process of microsporogenesis. It is in fact the first aspect of pollen ornamentation to be established during pollen ontogeny (Blackmore and Barnes 1990). There is now increasing evidence that aperture pattern ontogeny is linked to meiosis (Blackmore and Crane 1998, Ressayre et al. 2002, Harley 2004). Detailed observations of microsporogenesis in species with different aperture patterns, either from the monocots or from the eudicots (Ressayre 2001; Ressayre et al. 2003, 2005), show that the apertures are defined according to the regions where cytokinesis is completed, as already suggested by Wodehouse (1935). Since cytokinesis involves the formation of callosic walls among the microspores (Longly and Waterkeyn 1979a), callose deposition during microsporogenesis is likely to play a key role in aperture pattern determination. Other factors that have been shown to play a crucial role in aperture pattern determination are the presence of endoplasmic-reticulum shields under the plasma membrane in the regions where the apertures are formed (Heslop-Harrison 1963) and the organisation of microtubules (Sheldon and Dickinson 1983, 1986). There is a variety of aperture types in monocots. The monosulcate type (a single distal polar aperture, sometimes reduced to a pore) occurs in most families of the monocots but some families, such as the Araceae (Grayum 1992), Iridaceae (Goldblatt et al. 1998), or Arecaceae (Harley and Baker 2001), display variation in the aperture pattern. Variation concerns different aspects of the aperture pattern: shape (sulci or pores), number (one to several), or position (polar or equatorial). Variation occurs generally among genera, sometimes among species of the same genus (Goldblatt and

\* Correspondence and reprints: Laboratoire Ecologie, Systématique et Evolution, Université Paris-Sud, 91405 Orsay Cedex, France.  
E-mail: sophie.nadot@ese.u-psud.fr

Le Thomas 1993), but there are many records of intraindividual variation (Harley and Baker 2001, Harley 2004), a phenomenon called heteromorphism (Till-Bottraud et al. 1995). Some species produce inaperturate pollen in which the exine layer is either continuous or entirely lacking (Furness and Rudall 1999a, 2000). Data concerning the cellular process of microsporogenesis in monocots are focused essentially on the type of cytokinesis and indicate that some aperture types, such as the trichotomosulcate or triaperturate types, occur only when cytokinesis is simultaneous (Furness and Rudall 1999b, Harley 2004). The widespread monosulcate pollen is commonly associated with successive cytokinesis but also occurs with simultaneous cytokinesis, in which case it is sometimes found together with trichotomosulcate pollen (Rudall et al. 1997, Harley 1999a).

We have undertaken a comprehensive study of microsporogenesis in monocots, with a special focus on cell wall formation during cytokinesis, in order to identify the main features of microsporogenesis in this taxonomic group. In the long run, this study will help us to improve our understanding of aperture pattern determination in monocots and identify the key steps involved in the evolution of this character. In this paper we present observations from selected species belonging to different clades of the monocots. Although the number of species exam-

ined was limited, our results reveal that variation concerns different features of microsporogenesis, such as the type of cytokinesis, intersporal callosic wall formation, and shape of the tetrads. We discuss the role played by these features in aperture pattern determination.

This work was presented as an oral communication at the 11th International Palynological Congress, Granada, Spain, 4–9 July 2004.

## Material and methods

Microsporogenesis was examined in plant material belonging to fourteen species, representing six orders of the monocots, namely, Alismatales, Arecales, Asparagales, Commelinales, Dioscoreales, and Zingiberales. Fresh floral buds, potentially at different stages of microsporogenesis, were collected from plants grown in botanical gardens or found in the wild (Table 1). Floral buds were immediately dissected to extract the anthers. One anther per bud was squashed to extract the sporogenous cells and mounted in aceto-carmine to identify the meiotic stage of the bud. When meiosis was in progress, the remaining anthers were squashed in aniline blue (modified from Arens [1949] by the addition of 15% glycerol), which allowed us to observe callosic wall formation by epifluorescence. When the mature-tetrad stage was observed, half of the remaining anthers were squashed in congo red (Stainier et al. 1967) to visualise the position of apertures within the tetrad. The remaining half was mounted in aniline blue to observe intersporal walls just before the release of pollen grains. In some species it was possible to visualise the apertures with aniline blue staining under epifluorescence. Mature pollen was mounted in congo red. In the case of the species *Dioscorea communis*, the floral

**Table 1.** Species examined in this study

Order and family <sup>a</sup>	Species	Voucher	Origin <sup>b</sup>
Alismatales			
Alismataceae	<i>Alisma canaliculatum</i> A. Braun & Bouché	1983-101	Kew
Araceae	<i>Anthurium hookeri</i> Kunth	4025	MNHN
Dioscoreales			
Dioscoreaceae	<i>Dioscorea communis</i> (L.) Caddick & Wilkin	no voucher	Orsay
Asparagales			
Agavaceae	<i>Beschorneria yuccoides</i>		MNHN
Hemerocallidaceae	<i>Dianella tasmanica</i> Hook. f.	1996-610	Kew
Iridaceae	<i>Dietes grandiflora</i> N.E.Br		Kirstenbosch
	<i>Sisyrinchium striatum</i> Sm.	no voucher	Orsay
Commelinales			
Commelinaceae	<i>Tradescantia × andersoniana</i> W. Ludw. & Rohw.	no voucher	Orsay
	<i>Commelina erecta</i> L.	no voucher	Orsay
Haemodoraceae	<i>Wachendorfia paniculata</i> L.	no voucher	Cape Region
Zingiberales			
Strelitziaceae	<i>Strelitzia juncea</i> Link	no voucher	Cape Region
	<i>S. reginae</i> Aiton	no voucher	Cape Region
Arecales			
Arecaceae	<i>Chamaedorea microspadix</i> Burret	913448	JBVP
	<i>Gaussia attenuata</i> (O. F. Cook) Becc.	569-6656902	Kew

<sup>a</sup> Orders and families are according to Angiosperm Phylogeny Group (2003)

<sup>b</sup> Kirstenbosch, Kirstenbosch National Botanical Garden (Cape Town, South Africa); MNHN, Musée National d'Histoire Naturelle (Paris, France); JBVP, Jardin Botanique de la Ville de Paris (Paris, France); Orsay, Parc Botanique de Launay (Orsay, France); Stellenbosch, Stellenbosch Botanical Garden (South Africa); Kew, Royal Botanic Gardens Kew (U.K.)

buds were squashed directly in the different stains without being dissected, because of their small size. Aceto-carmin and congo red preparations were observed in transmission light with a Zeiss Axiophot microscope. The epifluorescence Zeiss Axiophot microscope was used with filter set 01 (excitation wavelength, 345 nm; emission wavelength, 425 nm long pass) for aniline blue staining.

## Results

### *Alisma, Anthurium, Beschorneria, Commelina, Strelitzia, Tradescantia, and Wachendorfia species*

Eight of the fourteen species examined here presented a developmental sequence of microsporogenesis involving successive cytokinesis (Fig. 1a) and intersporal wall formation achieved by centrifugal cell plates (Fig. 1b, c). In *Anthurium hookeri*, the second meiosis divisions were not exactly synchronous: Fig. 1a shows a dyad in which one cell is at the anaphase II stage while the other cell is at the metaphase II stage. Tetragonal and decussate tetrads (Fig. 1d–f) were observed in all eight species. A few T-shaped tetrads were recorded in *Beschorneria yuccoides* (Fig. 1g). A large variety of shapes were recorded in both *Strelitzia* species such as T-shaped, linear, or Z-shaped (Fig. 1h) in addition to tetragonal and decussate shapes. *Commelina erecta*, *Tradescantia* × *andersoniana*, and *Wachendorfia paniculata* produced monosulcate pollen grains (Fig. 1i, j); *Alisma canaliculatum* and *Anthurium hookeri* produced polyporate pollen (Fig. 1k); whereas the pollen of *Strelitzia juncea* and *S. reginae* was inaperturate (Fig. 1l).

### *Dianella tasmanica*

In *Dianella tasmanica*, cytokinesis was achieved in two different ways. One way involved the formation of three cell plates (Fig. 2a) and resulted in tetragonal or decussate tetrads (Fig. 2b, c). The other way involved the simultaneous formation of six cell plates (Fig. 2d) and resulted in the formation of tetrahedral tetrads (Fig. 2e, f). The pollen grains produced by this species were trichotomosulcate (Fig. 2g), occasionally monosulcate (Fig. 2h) or asymmetric trichotomosulcate (Fig. 2i).

### *Gaussia attenuata*

Cytokinesis in *Gaussia attenuata* was achieved by the simultaneous formation of four to six centrifugal cell plates (Fig. 3a, b) which are covered by extra callose after completion of cytokinesis. The tetrads were tetragonal (Fig. 3c), rhomboidal (not shown), symmetric tetrahedral (Fig. 3d), or asymmetric tetrahedral (Fig. 3e). The pollen

grains were predominantly monosulcate (Fig. 3e), mixed with trichotomosulcate pollen grains (Fig. 3f).

### *Chamaedorea, Dioscorea, and Sisyrrinchium species*

In all three species, cytokinesis was simultaneous and achieved by cell plates growing centripetally from the edges of each of the cleavage planes (Fig. 4a–c). The resulting tetrads ranged from tetragonal to symmetric or asymmetric tetrahedral (Fig. 4d–f), with occasional rhomboidal tetrads in *Chamaedorea microspadix* (Fig. 4g). Pollen grains in *Chamaedorea microspadix* and *Sisyrrinchium striatum* were monosulcate, occasionally trichotomosulcate (Fig. 4h, i). The pollen of *Dioscorea communis* was disulcate (Fig. 4j), i.e., with two apertures in equatorial position.

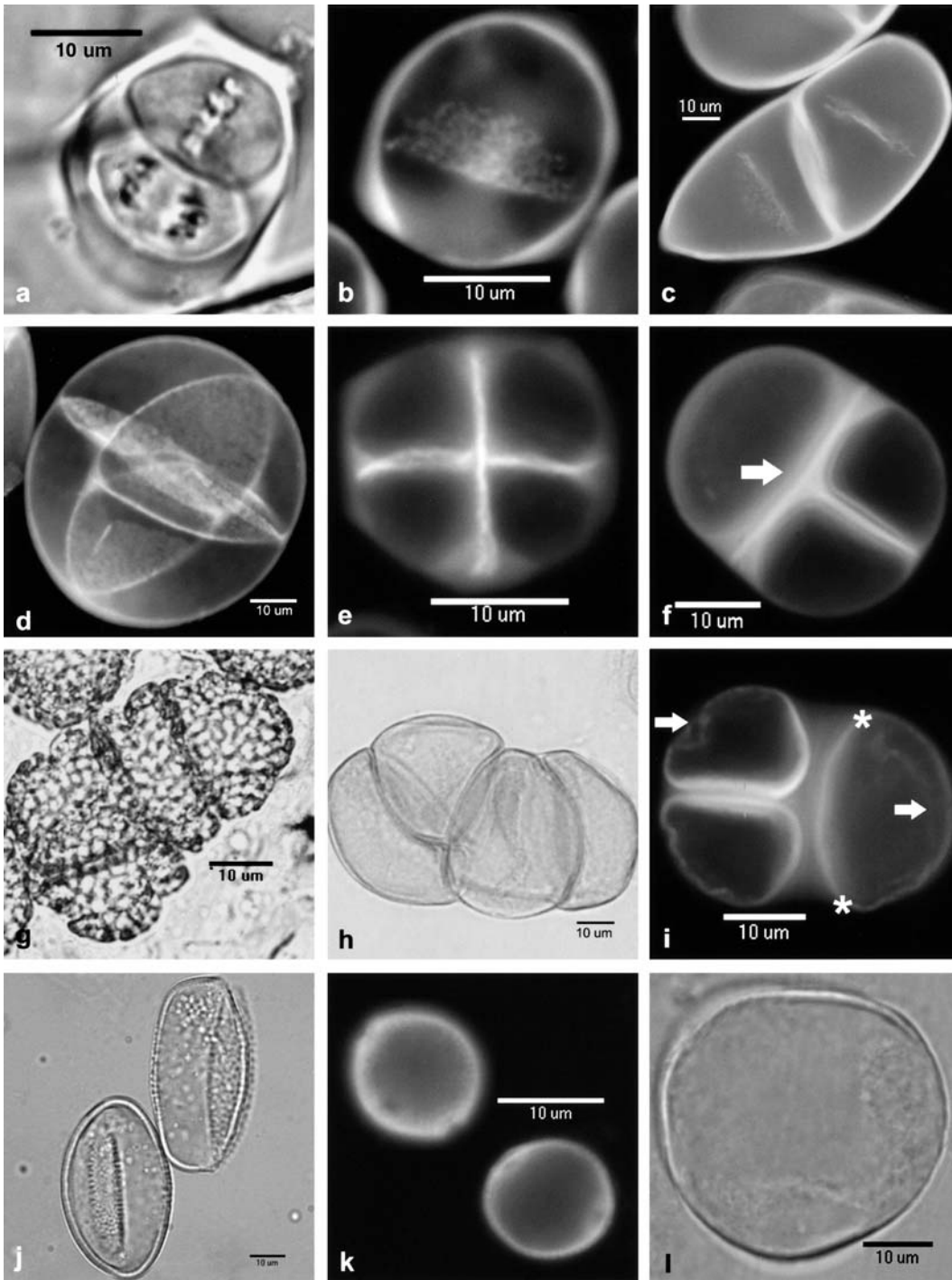
### *Dietes grandiflora*

In *Dietes grandiflora*, intersporal wall formation was achieved by the simultaneous formation of four to six callosic walls starting from the edges of the tetrad and progressing centripetally toward the centre of the tetrad (Fig. 4k, l). The tetrads were tetragonal, rhomboidal, or tetrahedral (not shown) and the pollen grains were zonalsulcate, with a ringlike sulcus running through both poles (Fig. 4m).

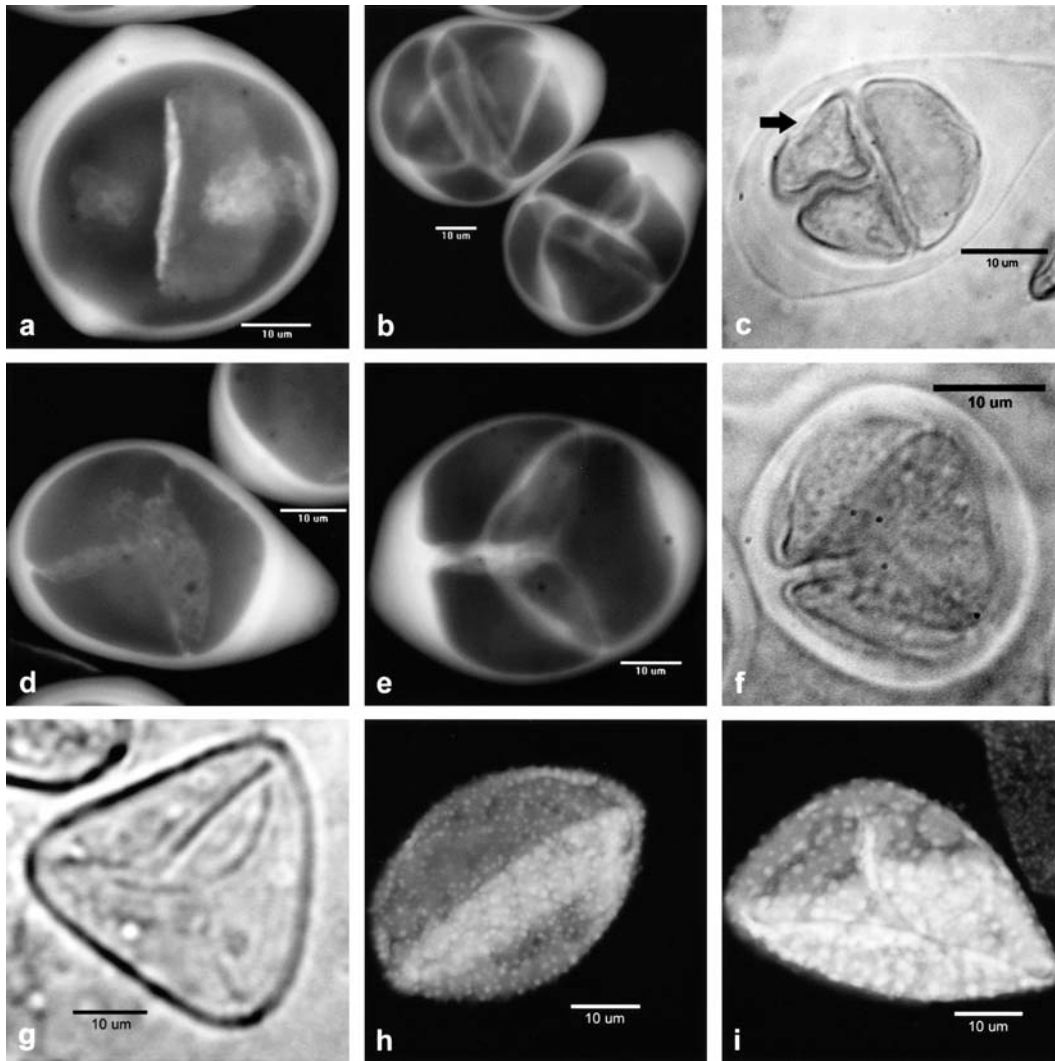
## Discussion

### *Microsporogenesis with successive cytokinesis*

A widespread developmental sequence observed in monocots involves successive cytokinesis and intersporal wall formation achieved by centrifugal cell plates. Such features are found in species belonging to different orders of monocots as shown by the data presented here (Table 2) or by previous works (Longly and Waterkeyn 1979b, Penet et al. 2005). The pollen associated with these features of microsporogenesis is generally boat-shaped and monosulcate, with the sulcus oriented along the longest axis of the pollen grain. In the tetrad, the sulcus of each microspore is oriented in such a way that both ends of the sulcus are located precisely where callose wall formation is completed (Fig. 1i), as predicted by a model of aperture pattern ontogeny (Ressayre et al. 2002). There are exceptions to this association, since species displaying successive cytokinesis and centrifugal cell wall formation were found to produce other pollen types, such as polyporate or inaperturate (Table 2). A low but variable number of pores were recorded in *Anthurium hookeri*, as already noted (Buchner



**Fig. 1 a–l.** Successive cytokinesis. AC, aceto-carmine; AB, aniline blue; CR, congo red. Bars: 10  $\mu\text{m}$ . **a** and **b** *Anthurium hookeri*. **a** Dyad stage with second meiosis division in progress (AC). **b** Formation of the cell plate after meiosis I (AB). **c** Formation of the cell plates after meiosis II in *Strelitzia reginae* (AB). **d** Tetragonal tetrad in *Wachendorfia paniculata* with bare callosic cell plates (AB). **e** Tetragonal tetrad in *Anthurium hookeri* with cell plates covered by additional callose (AB). **f** Decussate tetrad in *Commelina erecta*, callose deposits are particularly important on the cell plate formed after meiosis I, shown by the arrow (AB). **g** T-shaped tetrad in *Beschorneria yuccoides* (CR). **h** Z-shaped tetrad in *Strelitzia reginae* (CR). **i** Decussate tetrad in *Commelina erecta* with apertures indicated by arrows and last points of callose deposition indicated by stars (AB). **j** Monosulcate pollen grains in *Wachendorfia paniculata* (CR). **k** Porate pollen grains in *Anthurium hookeri* (AB). **l** Inaperturate pollen in *Strelitzia reginae* (CR)



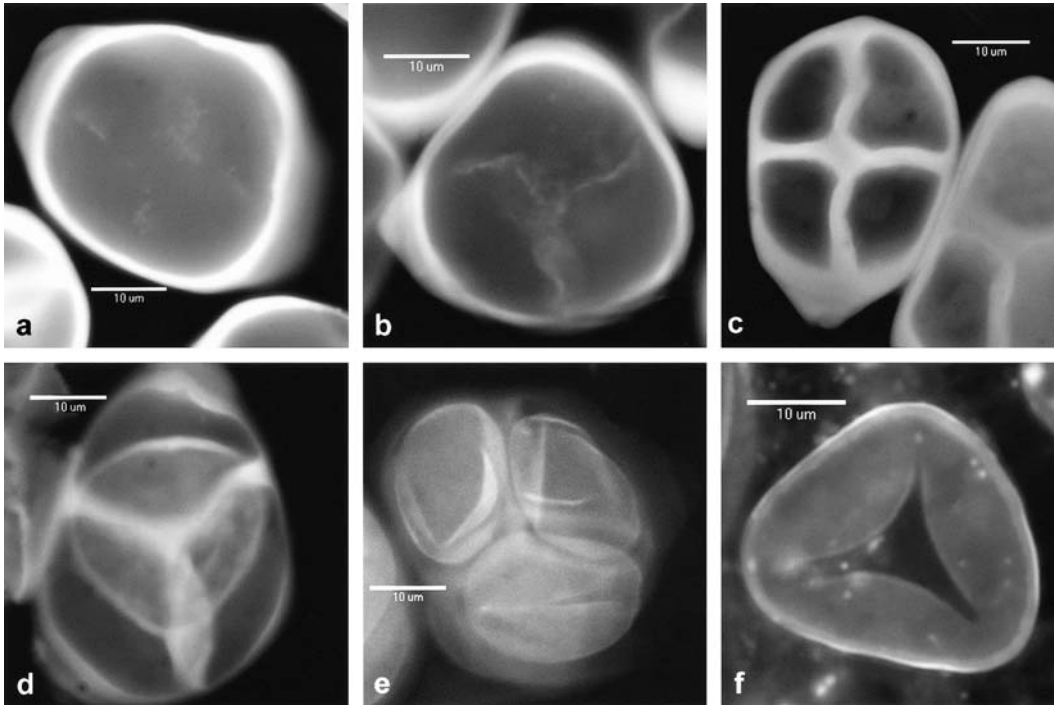
**Fig. 2 a–i.** Microsporogenesis in *Dianella tasmanica*. AB, aniline blue; CR, congo red. Bars: 10 µm. **a** Three unequal cell plates developing centrifugally (AB). **b** Tetrads with a shape intermediate between tetragonal and decussate (AB). **c** Mature tetrad with apertures visible on the microspores, indicated by arrow (CR). **d** Six cell plates developing centrifugally, joining in the centre of the tetrad (AB). **e** Tetrahedral tetrad with three intersporal walls visible (AB). **f** Tetrahedral tetrad (CR). **g** Symmetric trichotomosulcate pollen (CR). **h** Monosulcate pollen (AB). **i** Asymmetric trichotomosulcate pollen (AB)

and Weber 2000). In nearly all species with successive cytokinesis the tetrads were predominantly tetragonal or decussate, occasionally T-shaped, and additional callose was laid on the cell plates in most cases (Fig. 1f) except in *Wachendorfia paniculata*, in which the cell plates were left bare in mature tetrads. In both *Strelitzia* species, a large variety of tetrad shapes was observed. Many tetrads were of irregular shapes, called here Z-shaped suggesting that there are no constraints on the shape of the tetrad in these species which produce pollen grains with no apertures. Further investigation of species producing inaperturate pollen will be needed to test whether there is indeed a correlation between the production of inaperturate pollen grains and the release of constraint on tetrad shape. As for *Anthurium hookeri* (of

which the type of cytokinesis is described here for the first time) and *Alisma canaliculatum*, which produce polyporate pollen, our observations support the hypothesis of Wodehouse (1935), who suggested that for high aperture numbers the aperture pattern is disconnected from meiosis.

#### *Microsporogenesis with intermediate cytokinesis*

Cytokinesis in *Dianella tasmanica* involved the formation of centrifugal cell plates. This situation is found in all species of the Hemerocallidaceae and the Asphodelaceae for which intersporal wall formation has been described (Ressayre et al. 2005, Penet et al. 2005). These two closely related families are sister group to the higher Asparagales clade



**Fig. 3 a–f.** Microsporogenesis in *Gaussia attenuata*. AB, aniline blue; CR, congo red. Bars: 10 µm. **a** Microsporocyte with four cell plates developing centrifugally (AB). **b** Microsporocyte with six cell plates developing centrifugally and joining in the centre of the microsporocyte (AB). **c** Tetragonal tetrad with extra callose laid on the cell plates (AB). **d** Tetrahedral tetrad with the six cell plates visible (AB). **e** Asymmetric tetrahedral tetrad of four monosulcate microspores (AB, fluorescein isothiocyanate filter). **f** Trichotomosulcate pollen grain (AB, fluorescein isothiocyanate filter)

(Chase et al. 2000) characterised by successive cytokinesis involving centrifugal cell plates. The literature is conflicting about the type of cytokinesis in *Dianella tasmanica*, described as simultaneous (Schnarf and Wunderlich 1939) or successive (Huynh 1971). Our observations reveal that cytokinesis in this species can be achieved by the formation of either 3 or 6 cell plates. When six cleavage planes were involved, all cell plates were formed simultaneously. When three cleavage planes were involved (like in successive cytokinesis), the cell plates formed were unequal, with one larger than the two others. Our interpretation is that the large cell plate separates the nuclei resulting from meiosis I and the two smaller cell plates separate the nuclei resulting from meiosis II. Unlike in successive cytokinesis, a true dyad stage was never observed, the cell plates formed after meiosis II being initiated before the first cell plate was completed. Cytokinesis in *Dianella tasmanica* should then be considered as intermediate between simultaneous and successive, which could explain the conflicts found in the literature.

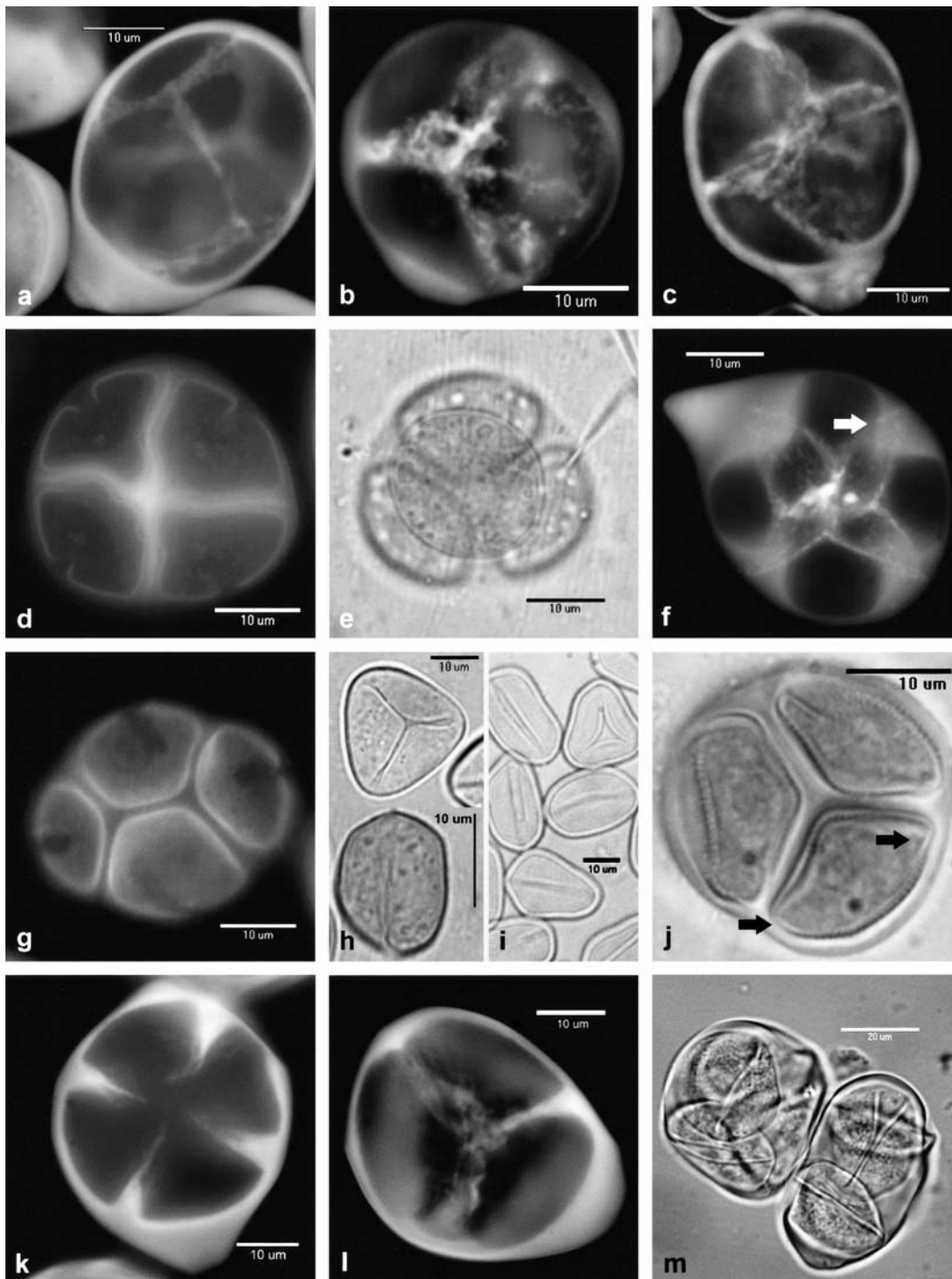
#### *Microsporogenesis with simultaneous cytokinesis*

Different ways of separating the four microspores were found in species with simultaneous cytokinesis (Table 2).

Intersporal wall formation was a two-step process, with first callosic cell plates developing either centrifugally or centripetally, and then additional callose laid onto the cell plates once the four nuclei were completely separated. One exception was found, however, in the case of *Dietes grandiflora*. In this species there was no stage with bare cell plates; the cell plates were covered with extra callose while they were developing. This situation, which is a rule in the eudicots (Brown and Lemmon 1991), had never been described in the monocots so far. The diversity displayed by monocots concerning intersporal wall formation when cytokinesis is simultaneous contrasts greatly with the conserved pattern observed in the eudicots. It also contrasts with the situation observed in the case of successive cytokinesis, in which intersporal wall formation always involved cell plates forming centrifugally.

#### *Tetrad shape and aperture pattern*

In the literature, simultaneous cytokinesis is generally considered to be associated with the formation of tetrahedral tetrads, especially in the eudicots (Blackmore and Crane 1998, Harley 1999b). However, almost all simultaneous-cytokinesis species examined here displayed a large variety in



**Fig. 4 a–m.** Simultaneous cytokinesis with centripetal intersporal wall formation. AB, aniline blue; CR, congo red. Bars: a–l, 10  $\mu\text{m}$ ; m, 20  $\mu\text{m}$ . **a–c** Developing cell plates. **a** *Chamaedorea microspadix*. **b** *Sisyrrinchium striatum*. **c** *Dioscorea communis*. **d** and **e** *Chamaedorea microspadix*. **d** Tetragonal tetrad of four monosulcate microspores, with apertures indicated by arrow (AB, fluorescein isothiocyanate filter). **e** Symmetric tetrahedral tetrad, apertures not visible (CR). **f** Asymmetric tetrahedral tetrad in *Sisyrrinchium striatum* with callose ingrowths indicated by arrow (AB). **g** and **h** *Chamaedorea microspadix*. **g** Rhomboidal tetrad of four monosulcate microspores in (AB, fluorescein isothiocyanate filter). **h** Monosulcate and trichotomosulcate pollen grains (CR). **i** Monosulcate and trichotomosulcate pollen grains in *Sisyrrinchium striatum* (CR). **j** Mature tetrad in *Dioscorea communis* with equatorial apertures indicated by arrows. **k–m** *Diates grandiflora*. **k** Four cell walls developing centripetally toward the centre of the tetrad (AB). **l** Six cell walls developing centripetally toward the centre of the tetrad (AB). **m** Two tetrads with zonosulci visible on microspores (CR)

**Table 2.** Main features of microsporogenesis in the examined species

Cytokinesis and intersporal wall formation and order and species <sup>a</sup>	Aperture type	Apertures within tetrad
Successive and centrifugal		
Alismatales		
<i>Alisma canaliculatum</i>	polyporate	no data
<i>Anthurium hookeri</i>	diporate or triporate	no data
Asparagales		
<i>Beschorneria yuccoides</i>	monosulcate	
Commelinales		
<i>Tradescantia</i> × <i>andersoniana</i>	monosulcate	polar
<i>Commelina erecta</i>	monosulcate	polar
<i>Wachendorfia paniculata</i>	monosulcate	
Zingiberales		
<i>Strelitzia reginae</i>	inaperturate	
Simultaneous and centrifugal		
Asparagales		
<i>Dianella tasmanica</i>	monosulcate and trichotomosulcate	polar
Arecales		
<i>Gaussia attenuata</i>	monosulcate and trichotomosulcate	polar
Simultaneous and centripetal		
Dioscoreales		
<i>Dioscorea communis</i>	disulcate	equatorial
Asparagales		
<i>Dietes grandiflora</i>	zonasulcate	polar
<i>Sisyrinchium striatum</i>	monosulcate and trichotomosulcate	polar
Arecales		
<i>Chamaedorea microspadix</i>	monosulcate and trichotomosulcate	polar

<sup>a</sup> Orders are according to Angiosperm Phylogeny Group (2003)

the shape of postmeiotic tetrads. The tetrad shape ranged from tetragonal to tetrahedral (symmetric or asymmetric), including rhomboidal. Theoretically, the shape of a tetrad is defined by the number of cleavage planes which divide the internal space of a spherical or elliptical volume: three or four planes result in tetragonal or decussate tetrads (depending on the relative orientation of the cleavage planes), five planes result in rhomboidal tetrads, six equal planes result in symmetric tetrahedral tetrads, six unequal planes result in asymmetric tetrahedral tetrads. The variation in tetrad shape in our sampling of species should therefore result from the occurrence of variation in the number and size of the cleavage planes that divide the four haploid nuclei after meiosis. Such a variation was recorded in several species of our sampling, for example, in *Gaussia attenuata* or *Dietes grandiflora*. Interestingly, the variation in tetrad shape was associated with variation in pollen aperture pattern in four species examined here, namely, *Chamaedorea microspadix* and *Gaussia attenuata* (Arecaceae), *Dianella tasmanica*

(Hemerocallidaceae), and *Sisyrinchium striatum* (Iridaceae). These species produced a mixture of monosulcate and trichotomosulcate pollen grains, a situation that is not uncommon in the palm family (Harley 1999a) or in the “lower” asparagoids with simultaneous cytokinesis (Rudall et al. 1997). *Dianella tasmanica* was known to produce trichotomosulcate pollen (Huynh 1971, Roth et al. 1987) arranged in tetrahedral tetrads at the postmeiotic stage, a general feature of Hemerocallidaceae with the exception of *Hemerocallis* sp. (Roth et al. 1987). The specimen of *Dianella tasmanica* studied here produced a small proportion of monosulcate pollen mixed with symmetric trichotomosulcate pollen grains and occasional asymmetric trichotomosulcate pollen grains. The position of the apertures within the tetrad shows that monosulcate pollen results from tetragonal or decussate tetrads, as would be expected if cytokinesis were successive, whereas trichotomosulcate pollen is associated to tetrahedral tetrads. The record of both symmetric and asymmetric tetrahedral tetrads (not shown) in *Dianella tasmanica* could account for the record of symmetric and asymmetric trichotomosulcate pollen grains. In *Gaussia attenuata* and *Chamaedorea microspadix*, all mature tetrads observed consisted of monosulcate pollen grains assembled in tetragonal, rhomboidal, decussate, or asymmetric tetrahedral tetrads. If tetrad shape has an impact on aperture shape, as suggested by our observations, then we would expect to find the trichotomosulcate pollen grains of *Gaussia attenuata* and *Chamaedorea microspadix* associated in symmetric tetrahedral tetrads. Unfortunately, we have not been able to observe this pollen type in tetrads, probably due to the rare occurrence of trichotomosulcate pollen in these two species. In *Sisyrinchium striatum*, the observation of a continuum of pollen shapes ranging from symmetric monosulcate to symmetric trichotomosulcate (Fig. 4i), together with tetrad shapes ranging from tetragonal to tetrahedral, including rhomboidal, also supports the hypothesis of a link between aperture pattern and tetrad shape. In this species, like in *Dianella tasmanica*, trichotomosulcate pollen grains were assembled in tetrahedral tetrads, whereas monosulcate pollen grains was found in tetragonal or decussate tetrads.

#### Convergences in microsporogenesis

As already mentioned, cytokinesis involved centrifugal cell plates in *Dianella tasmanica* and *Gaussia attenuata*, whereas in *Chamaedorea microspadix* and *Sisyrinchium striatum*, cell plate formation was centripetal, a feature generally associated with the eudicots (Brown and Lemmon 1988, 1991). It is noteworthy that four species belonging to different orders converged in producing both

monosulcate and trichotomosulcate pollen grains in spite of differences in the formation of intersporal walls. In contrast, cell plate formation was achieved similarly in three species that belonged to different clades of the monocots and produced pollen grains differing in aperture pattern: *Chamaedorea microspadix*, *Sisyrinchium striatum* (monosulcate and trichotomosulcate pollen), and *Dioscorea communis* (disulcate pollen). In these three species, as well as in *Gaussia attenuata* and *Dianella tasmanica*, the observation of mature tetrads showed that extra callose was deposited onto the cell plates after their completion. This aspect was not studied here, but it would be interesting to conduct further observations in order to identify precisely the exact points where callose is last deposited among the microspores, since this parameter has been shown to be involved in aperture pattern determination (Ressayre et al. 2002). For example, in *Pontederia cordata*, which has a successive cytokinesis, all cell plates are covered by callose deposited centrifugally from the centre of the tetrad toward the junctions between the intersporal walls and the outer wall of the tetrad, precisely where the apertures are located in the mature tetrad (Ressayre 2001). Another example is *Phormium tenax*, a species producing trichotomosulcate pollen through simultaneous cytokinesis: in the tetrad, the three branches of the trichomosulcus are directed toward the last points where callose is deposited among the microspores (Ressayre et al. 2005). This phenomenon was actually observed in the case of the *Sisyrinchium* species studied here: ingrowths of callose developed toward the junction of the cell plates and the outer wall of the tetrad once cytokinesis was achieved (Fig. 4f). If such a phenomenon is a rule in aperture pattern determination in angiosperms, then we expect to find no differences in the way callose is laid onto the cell plates among *Dianella tasmanica*, *Gaussia attenuata*, *Chamaedorea microspadix*, and *Sisyrinchium striatum* (all produce similar pollen morphologies in spite of differences in cell plate formation). On the other hand, differences are expected between *Chamaedorea* or *Sisyrinchium* and *Dioscorea* species (similar cell plate formation but different pollen morphologies).

### Conclusion

Despite the small size of the sampling of species examined in this study, our results point out the existence of convergences in the different features of microsporogenesis in monocots. This was already known concerning cytokinesis: there are numerous transitions from successive to simultaneous cytokinesis in the different clades of the

monocots. We show here that this phenomenon also concerns the formation of cell plates during simultaneous cytokinesis: centripetal cell plates occur in the Arecaceae, Iridaceae and Dioscoreaceae (three unrelated families), and centrifugal cell plates are found in the Arecaceae as well as in “higher” Asparagales and the related Hemerocallidaceae. Detailed observations are now needed to know whether convergences occur concerning extra callose deposition within the monocots. It will also be necessary to examine the link between callose deposition on the plasma membrane of the microspores and intracellular features such as the orientation of the meiotic spindles and the position of endoplasmic-reticulum shields. Further investigations of microsporogenesis will then improve our understanding of the evolution of aperture pattern ontogeny in monocots.

### Acknowledgments

We are grateful to the various botanic gardens which provided us with most of the plant material studied here (MNHN, JBVP, CBNB, and Parc Botanique de Launay, France; Royal Botanic Gardens Kew, U.K.; Kirstenbosch National Botanical Garden, Republic of South Africa). We thank two anonymous reviewers for their helpful comments on the manuscript. This work was financially supported by the Institut Français de la Biodiversité (grant number 041 18 19 00).

### References

- Angiosperm Phylogeny Group (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Bot J Linn Soc* 141: 399–436
- Arens K (1949) Prova de Calose por meio da microscopia a luz fluorescente e aplicações do metodo. *Lilloa* 18: 71–75
- Blackmore S, Barnes SH (1990) Pollen wall development in angiosperms. In: Blackmore S, Knox RB (eds) *Microspores: evolution and ontogeny*. Academic Press, London, pp 173–192
- Blackmore S, Crane PR (1998) The evolution of apertures in the spores and pollen grains of embryophytes. In: Owens SJ, Rudall PJ (eds) *Reproductive biology*. Royal Botanic Gardens, Kew, pp 159–182
- Brown RC, Lemmon BE (1988) Microtubules associated with simultaneous cytokinesis of coenocytic microspores. *Am J Bot* 75: 1848–1856
- Brown RC, Lemmon BE (1991) The cytokinetic apparatus in meiosis: control of division plane in the absence of a preprophase band of microtubules. In: Lloyd CW (ed) *The cytoskeletal basis of plant growth and form*. Academic Press, London, pp 259–273
- Buchner R, Weber M (2000) PalDat – a palynological database: descriptions, illustrations, identification, and information retrieval. <http://paldat.botanik.univie.ac.at/>
- Chase MW, Soltis DE, Soltis PS, Rudall PJ, Fay MF, Hahn WH, Sullivan S, Joseph J, Molvray M, Kores PJ, Givnish TJ, Sytsma KJ, Pires JC (2000) Higher level systematics of the monocotyledons: an assessment of current knowledge and a new classification. In: Wilson KL, Morrison DA (eds) *Monocots: systematics and evolution*. Commonwealth Scientific and Industrial Research Organisation, Sydney, pp 3–16
- Erdtman G (1952) *Pollen morphology and plant taxonomy: angiosperms*. Almqvist and Wiksell, Stockholm

- Furness CA, Rudall PJ (1999a) Inaperturate pollen in monocotyledons. *Int J Plant Sci* 160: 395–414
- Furness CA, Rudall PJ (1999b) Microsporogenesis in monocotyledons. *Ann Bot* 84: 475–499
- Furness CA, Rudall PJ (2000) Aperture absence in pollen of monocotyledons. In: Harley MM, Morton CM, Blackmore S (eds) *Pollen and spores: morphology and biology*. Royal Botanic Gardens, Kew, pp 249–257
- Goldblatt P, Le Thomas A (1993) Pollen morphology of Madagascan *Aristea* and *Geosiris* (Iridaceae-Nivenoideae) in relation to systematics and phylogeny. *Adansonia* 14: 223–233
- Goldblatt P, Manning JC, Rudall PJ (1998) Iridaceae. In: Kubitzki K (ed) *Flowering plants: monocotyledons. The families and genera of vascular plants*, vol 3. Springer, Berlin Heidelberg New York, pp 295–335
- Grayum MH (1992) Comparative external pollen ultrastructure of the Araceae and putatively related taxa. *Monogr Syst Bot Missouri Bot Gard* 43: 1–167
- Harley MM (1999a) Palm pollen: overview and examples of taxonomic value at species level. In: Henderson A, Borchsenius F (eds) *Evolution, variation, and classification of palms*. New York Botanical Garden Press, Bronx, NY, pp 95–120
- Harley MM (1999b) Tetrad variation: its influence on pollen form and systematics in the Palmae. In: Kurmann MH, Hemsley AR (eds) *The evolution of plant architecture*. Royal Botanic Gardens, Kew, pp 289–304
- Harley MM (2004) Triaperturate pollen in the monocotyledons: configurations and conjectures. *Plant Syst Evol* 247: 75–122
- Harley MM, Baker WJ (2001) Pollen aperture morphology in Areaceae: application within phylogenetic analyses, and a summary of the fossil record of palm-like pollen. *Grana* 40: 45–77
- Heslop-Harrison J (1963) An ultrastructural study of pollen wall ontogeny in *Silenependula*. *Grana Palynol* 4: 7–24
- Huynh K-L (1971) Etude de l'arrangement du pollen dans la tétrade chez les Angiospermes sur la base de données cytologiques. III. Le pollen trilète du genre *Dianella* Lam. (Liliaceae). *Beitr Biol Pflanz* 47: 277–286
- Longly B, Waterkeyn L (1979a) Etude de la cytotinèse. II. Structure et isolement des plaques cellulaires microsporocytaires. *Cellule* 72: 227–242
- Longly B, Waterkeyn L (1979b) Etude de la cytotinèse. III. Les cloisonnements simultanés et successifs des microsporocytes. *Cellule* 73: 65–80
- Penet L, Nadot S, Ressayre A, Forchioni A, Dreyer LD, Gouyon PH (2005) Multiple developmental pathways leading to a single morph: monosulcate pollen (examples from the Asparagales). *Ann Bot* 95: 331–343
- Ressayre A (2001) Equatorial aperture pattern in monocots: same definition rules as in eudicots? The example of two species of Pontederiaceae. *Int J Plant Sci* 162: 1219–1224
- Ressayre A, Godelle B, Raquin C, Gouyon P-H (2002) Aperture pattern ontogeny in angiosperms. *J Exp Zool (Mol Dev Evol)* 294: 122–135
- Ressayre A, Mignot A, Siljak-Yakovlev S, Raquin C (2003) Postmeiotic cytokinesis and pollen aperture number determination in eudicots: effect of the cleavage wall number. *Protoplasma* 221: 257–268
- Ressayre A, Dreyer LD, Triki-Teurtroy S, Forchioni A, Nadot S (2005) Post-meiotic cytokinesis and pollen aperture pattern ontogeny: comparison of development in four species differing in aperture pattern. *Am J Bot* 92: 576–583
- Roth JL, Walker JW, Walker AG (1987) The distribution and systematics of trichotomosulcate pollen within the Lilialean complex. *Am J Bot* 74: 751
- Rudall PJ, Furness CA, Chase MW, Fay MF (1997) Microsporogenesis and pollen sulcus type in Asparagales (Liliana). *Can J Bot* 75: 408–430
- Schnarf K, Wunderlich R (1939) Zur vergleichenden Embryologie der Liliaceae: Asphodeloideae. *Flora* 133: 297–327
- Sheldon JM, Dickinson HG (1983) Determination of patterning in the pollen wall of *Lilium henryi*. *J Cell Sci* 63: 191–208
- Sheldon JM, Dickinson HG (1986) Pollen wall formation in *Lilium*: the effect of chaotropic agents and the organization of the microtubular cytoskeleton during pattern development. *Planta* 168: 11–23
- Stainier F, Huard D, Bronkers F (1967) Technique de coloration spécifique de l'exine des microspores jeunes encore groupées en tétrade. *Pollen Spores* 9: 367–370
- Till-Bottraud I, Mignot A, De Paepe R, Dajoz I (1995) Pollen heteromorphism in *Nicotiana tabacum* (Solanaceae). *Am J Bot* 82: 1040–1048
- Wodehouse RP (1935) *Pollen grains*. McGraw Hill, New York