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APERTURE PATTERN AND MICROSPOROGENESIS IN ASPARAGALES

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ABSTRACT

The aperture pattern of pollen grains is a character defined as the number, shape, and position of apertures. Although this character is highly variable in angiosperms, two states are particularly widespread. Pollen grains with one polar aperture occur frequently in basal angiosperms and monocots while tricolpate pollen is a synapomorphy of the eudicots. Many morphological characters are the result of a compromise between selective forces (acting on morphology) and developmental constraints (limiting the range of possible morphologies). To investigate what are the respective roles of development and selection in the determination of aperture pattern in angiosperms, we have chosen to study the characteristics of cell division during male meiosis, since it has been shown that aperture pattern is determined during microsporogenesis. The present study focuses on Asparagales. From a selection of species belonging to the major families of Asparagales, we described the type of cytokinesis, the way callose is deposited, the shape of the tetrad, as well as the shape and position of apertures within the tetrad. We show that although pollen morphology is quite uniform in Asparagales (most species produce monosulcate pollen), the characteristics of cell division during male meiosis vary among families. A highly conserved developmental sequence is observed in higher Asparagales whereas lower Asparagales, and particularly Iridaceae, display different ways of achieving cell division.

Key words: aperture pattern, Asparagales, microsporogenesis, pollen.

INTRODUCTION

Pollen morphology is extremely diversified in angiosperms. Many studies have focused on the structure of pollen grains and particularly on the ornamentation of the pollen wall. However, a high diversity is also found in the apertures, and particularly in the aperture pattern (Erdtman 1952; Huynh 1976; Thanikaimoni 1986; Harley and Zavada 2000). Variation in aperture pattern is expressed in the shape and number of apertures, as well as the position of apertures within the tetrad, visible when microsporogenesis is completed. The pollen grains in monocots are characterized by a relatively conserved aperture pattern since many species produce monosulcate pollen. However, the monosulcate type is not a strict rule in monocots (Harley and Zavada 2000) and variation occurs in several families such as Alismataceae (Furness and Rudall 1999), Araceae (Grayum 1992), Liliaceae and Tofieldiaceae (Huynh 1976; Rudall et al. 2000), Arecaceae (Harley and Baker 2001), Dioscoreaceae (Caddick et al. 1998), and Hemerocallidaceae (Huynh 1971). The Hemerocallidaceae family belongs to the order Asparagales, which has been subject to detailed phylogenetic analyses (Chase et al. 1995; Rudall et al. 1997; Fay et al. 2000). Rudall et al. (1997) surveyed the evolution of cytokinesis and pollen aperture type in Asparagales and concluded that simultaneous cytokinesis is apomorphic for the order, with a reversal to the successive type in higher Asparagales. As with the rest of monocots, most asparagalean species produce monosulcate pollen grains. Other aperture types have

been recorded in families such as Hemerocallidaceae, characterized by the predominance of trichotomosulcate pollen (Roth et al. 1987), or Iridaceae, in which among others, zonasulcate, disulcate, trichotomosulcate, or inaperturate types are encountered (Goldblatt and Le Thomas 1992, 1993). It is noteworthy that variation is mostly found in families belonging to the lower asparagalean grade, whereas taxa from the higher Asparagales clade consistently produce monosulcate pollen. Moreover, monosulcate pollen grains can be produced by either successive or simultaneous cell division, whereas trichotomosulcate pollen occurs only when cytokinesis is of the simultaneous type (Rudall et al. 1997).

The type of cytokinesis is one of the different parameters that have been shown to play a role in the determination of aperture pattern (Ressayre 2001; Ressayre et al. 2002). Other parameters involved are the characteristics of intersporal wall formation during male cytokinesis (centrifugal or centripetal callose deposition), the shape of the tetrad (tetragonal, tetrahedral, or rhomboidal), and the position of apertures within the tetrad (polar or grouped at the last point of contact between the microspores).

Little information is available concerning intersporal wall formation in Asparagales. The process has been described in detail for a handful of species, such as *Convallaria majalis* L. (Waterkeyn 1962), *Hemerocallis fulva* L., and *Sansevieria trifasciata* Prain. (Longly and Waterkeyn 1979). Cytokinesis is described as being successive for all three species, and cell division involves the formation of three centrifugal cell plates (one formed after the first meiotic division, two formed after the second meiotic division) meeting with callose ingrowths at the junction with the callose wall surrounding the tetrad. Cell plates are covered by additional callose

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Table 1. List of species surveyed in this study. Kirstenbosch = Kirstenbosch National Botanical Garden (Cape Town, South Africa); Paris = Muséum National d'Histoire Naturelle (Paris, France); Orsay = campus of Université Paris-Sud (Orsay, France); Stellenbosch = Stellenbosch Botanical Garden (South Africa); Brest = Conservatoire Botanique National de Brest (France); Kew = Royal Botanic Gardens, Kew (UK).

Family	Species	Living collection number	Origin
<i>Higher Asparagales</i>			
Agapanthaceae	<i>Agapanthus caulescens</i> Spreng.	134/53	Kirstenbosch
	<i>A. umbellatus</i> L'Hér.	n/a	Paris
Agavaceae	<i>Beschorneria yuccoides</i> C. Koch	22/764	Paris
Alliaceae	<i>Allium ursinum</i> L.	n/a	Orsay
Amaryllidaceae	<i>Narcissus poeticus</i> L.	n/a	Orsay
Anthericaceae	<i>Arthropodium cirrhatum</i> R. Br.	1985–706	Kew
Convallariaceae	<i>Convallaria majalis</i> L.	n/a	Orsay
Hyacinthaceae	<i>Eucomis autumnalis</i> (Mill.) Chitt.	671/83	Kirstenbosch
	<i>Veltheimia bracteata</i> Harv. ex Baker	n/a	Stellenbosch
<i>Lower Asparagales</i>			
Asphodelaceae	<i>Aloe globulifera</i> Graebn.	7280	Paris
	<i>Bulbine alooides</i> Willd.	431/35	Kirstenbosch
Hypoxidaceae	<i>Hypoxidia maheensis</i> F. Friedmann	810/110	Brest
Iridaceae	<i>Moraea bipartita</i> L. Bolus	1102/71	Kirstenbosch
	<i>M. aristata</i> (Houtt.) Asch. & Graebn.	1252/82	Kirstenbosch
	<i>Libertia formosa</i> Graham	1980–773	Kew

deposition. So far, only centrifugally extending cell plates have been described in monocots (Longly and Waterkeyn 1979; Ressayre 2001).

This paper presents the first large-scale investigation of intersporal wall formation in a group of plants—Asparagales—conducted using simple methods of staining and observation. Our main objective was to explore the developmental pathway of microsporogenesis from a cellular point of view, in a range of species representing the taxonomic diversity of Asparagales, with a focus on higher Asparagales, in order to understand the conservativeness of aperture pattern.

MATERIALS AND METHODS

For this study, 14 species were selected, representing most major families of the higher Asparagales, as well as a few families of the lower Asparagales. Fresh floral buds of different sizes, and therefore at different stages of microsporogenesis, were collected from plants grown in botanical gardens or found on the campus of the Université Paris-Sud (Table 1). Floral buds were immediately dissected to extract the anthers. One anther per bud was squashed and mounted in aceto-carmin in order to identify the stage of the bud (Fig. 1, 2). When meiosis was in progress, the remaining anthers were squashed in aniline blue (modified from Arens 1949), which revealed callose wall formation by epifluorescence (Fig. 3, 4). When the tetrad stage was observed, half of the remaining anthers were squashed in congo red (Stainier et al. 1967), in order to visualize the position of apertures within the tetrad (Fig. 5, 6), and the remaining half were mounted in aniline blue, to visualize callose walls just before the release of pollen grains. Intersporal wall formation of the first and second meiotic divisions is represented in Fig. 7–18 and 19–30, respectively. Mature pollen was also mounted in congo red. 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 345, emission 425 nm long pass) for aniline blue staining.

RESULTS

Higher Asparagales

This clade was represented in this study by nine species from seven families: Agapanthaceae (Fig. 17, 18, 28), Agavaceae (Fig. 11–14), Alliaceae (Fig. 3), Amaryllidaceae (Fig. 7–10, 27), Anthericaceae (Fig. 19–22, 29), Convallariaceae (Fig. 15, 16, 30), and Hyacinthaceae (Fig. 25–26). All species examined produced monosulcate pollen and presented the same characteristics concerning the progress of microsporogenesis. Cytokinesis was of the successive type, as shown by the occurrence of dyads (Fig. 10, 14, 18). Cell walls separating the four microspores after both first and second meiotic divisions were formed by callosic cell plates expanding centrifugally (Fig. 7–9, 11–12, 15–16, 17–18, 19–21, 23–26). The tetrads were mostly tetragonal, with all four microspores in the same optical plane (Fig. 22, 24, 28) or decussate, with one pair of microspores forming a right angle with the other pair (Fig. 29). T-shaped tetrads were seldom recorded. The sulcus, generally visible on the wall of the microspores in post-meiotic tetrads, was in a polar position and parallel to the cleavage planes (Fig. 27–30).

Lower Asparagales

Six species belonging to three different families (Fig. 1, 2, 4–6, 31–42) and presenting either successive or simultaneous types of cytokinesis were examined for microsporogenesis. All species produced monosulcate pollen grains, arranged in tetrads of irregular shape. In three of the species examined, namely *Hypoxidia maheensis* (Hypoxidaceae) (Fig. 34–36), *Moraea aristata*, and *M. bipartita* (Iridaceae)

Fig. 1–6.—Examples of differential staining during microsporogenesis. Fig. 1–2. Aceto-carmin staining.—1. *Moraea bipartita*.—2. *Libertia formosa*.—3–4. Aniline blue staining.—3. *Allium ursinum*.—4. *L. formosa*.—5–6. Congo red staining; stars indicate the position of sulci.—5. *Hypoxidia maheensis*.—6. *L. formosa*.

(Fig. 31–33), the pattern observed for microsporogenesis was strictly similar to the pattern described for higher Asparagales: successive cytokinesis (Fig. 1), centrifugal cell plates (Fig. 31, 32, 34, 35), tetragonal or decussate tetrads (Fig. 33, 36) and polar apertures parallel to cleavage planes (Fig. 5, 33). The three other species examined, *Aloe globulifera* (Fig. 37–30), *Bulbine alooides* (Asphodelaceae) (Fig. 40–42), and *Libertia formosa* (Iridaceae) (Fig. 2, 4, 6) had a cytokinesis of the simultaneous type, as shown by the observation of four nuclei in the same cytoplasm right after both meiotic divisions (Fig. 40). In the two Asphodelaceae species, intersporal cell walls were formed by centrifugal cell plates (Fig. 37, 41), similar to higher Asparagales. The only difference was that all cell plates grew simultaneously, forming six walls separating the four microspores assembled in a tetrahedral tetrad (Fig. 38, 42). In *Bulbine alooides*, additional callose deposits occur at the intersection of cell plates (Fig. 42). Cell wall formation seems to occur differently in *Libertia formosa*: callose ingrowths starting from the wall surrounding the future tetrad were observed (Fig. 4), and progress centripetally towards the center. In spite of the differences concerning cytokinesis and cell wall formation, all three species displayed a distal sulcus (Fig. 6, 39) as in higher Asparagales.

DISCUSSION

The microsporogenesis pathway leading to monosulcate pollen, a feature of monocots and basal angiosperms, was known to involve either successive or simultaneous cytokinesis. The results obtained in this study show that in higher

Asparagales, a clade characterized by successive cytokinesis (Rudall et al. 1997), the whole process of microsporogenesis is highly conserved. All species examined displayed identical features concerning the formation of the callosic cell walls and the distribution of sulci within the tetrad. Intersporal walls are formed by centrifugally expanding cell plates, with slight ingrowths of callose at the junction between the cell plates and the callose wall surrounding the tetrad (indicated by an arrow on Fig. 22). What had been described for two species (Longly and Waterkeyn 1979) can therefore be considered as a general feature of the higher Asparagales.

The clade consisting of Asphodelaceae, Hemerocallidaceae, and Xanthorrhoeaceae is sister to higher Asparagales (Fay et al. 2000). Asphodelaceae, which produce monosulcate pollen through simultaneous cytokinesis (Rudall et al. 1997), achieve the cell wall formation by means of centrifugal cell plates as in higher Asparagales. This is also the case for Hemerocallidaceae (data not shown) in which, interestingly, *Hemerocallis* displays a type of cytokinesis intermediate between successive and simultaneous cytokinesis (Cave 1955). *Xanthorrhoea* is reported as being successive (Rudall et al. 1997). Our results show that in lower Asparagales, the microsporogenesis pathway associated with a successive type of cytokinesis is achieved by way of centrifugally growing cell plates, exactly like in higher Asparagales. A simultaneous cytokinesis is, however, the most common condition in lower Asparagales. In this case, cell wall formation can be achieved by centrifugal cell plates, a situation encountered in Asphodelaceae and related families,

Fig. 7-18.—Intersporal wall formation after the first meiotic division in higher Asparagales.—7-10. *Narcissus poeticus*.—7-9. Microsporocytes stained with aniline blue.—10. Dyad stained with aceto-carmin.—11-14. *Beschorneria yuccoides*.—11-13. Microsporocytes stained with aniline blue.—14. Dyad stained with aceto-carmin.—15-18. Dyads stained with aniline blue.—15-16. *Polygonatum multiflorum*.—17-18. *Agapanthus caulescens*.

Fig. 19–30.—Intersporal wall formation after the second meiotic division in higher Asparagales.—19–26. Tetrads stained with aniline blue.—19–22. *Arthropodium cirrhatum*.—22. Ingrowth of callose indicated by the arrow.—23–24. *Eucomis autumnalis*.—25–26. *Veltheimia bracteata*.—27–30. Tetrads stained with congo red; stars indicate the position of sulci.—27. *Narcissus poeticus*.—28. *Agapanthus umbellatus*.—29. *Arthropodium cirrhatum*.—30. *Convallaria majalis*.

Fig. 31–42.—Intersporal wall formation in lower Asparagales (staining: aniline blue unless stated otherwise).—31–32. *Moraea bipartita*.—33. Tetrad of *M. aristata* stained with congo red; the stars indicate the position of the sulci.—34–36. *Hypoxidia maheensis*.—37–39. *Aloe globulifera*.—39. Tetrad stained with congo red; stars indicate the position of sulci.—40–42. *Bulbine alooides*.—40. Microsporocyte stained with aceto-carmin. —42. Additional callose deposit indicated by the arrow.

or by callose growing centripetally (data not shown), such as in Iridaceae or Tecophilaeaceae. In spite of this variation observed in microsporogenesis, the pollen produced is monosulcate in most Asparagales. This raises the question of the role played by cytokinesis and cell wall formation during microsporogenesis in the determination of aperture pattern (Ressayre et al. 2002). A more thorough investigation of microsporogenesis in lower Asparagales is currently being conducted and will allow us to further explore this point.

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