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Microsporogenesis variation in *Codiaeum* producing inaperturate pollen grain

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Abstract

A study of microsporogenesis (the earliest stage of pollen ontogeny) was undertaken in seven cultivars of *Codiaeum variegatum* var. *pictum*, a eudicot species that produces inaperturate pollen grains. Microsporogenesis appears highly variable for the developmental events suspected to be implicated in the determination of aperture pattern. Most eudicots have tri-aperturate pollen grains and microsporogenesis is described as highly conserved in this clade. The observed burst of variation in *C. variegatum* therefore appears especially remarkable. A plausible hypothesis to explain the variation is that the pollen being inaperturate, the selective forces applying on the ontogeny of the aperture pattern are relaxed. ***To cite this article: B. Albert et al., C. R. Biologies 332 (2009).***

Résumé

Variations au cours de la microsporogénèse chez *Codiaeum*. Une étude de la microsporogénèse (les premières étapes de développement du pollen) a été entreprise chez sept cultivars de *Codiaeum variegatum* var. *pictum*, une Eudicotylédone produisant du pollen inaperturé. La microsporogénèse s'est révélée très variable pour les caractères qu'on pense être impliqués dans la détermination du nombre et de la position des apertures à la surface des grains de pollen. La plupart des Eudicotylédones possèdent du pollen tri-aperturé et une microsporogénèse très conservée. Les variations observées sont donc remarquables. Une explication pourrait être que le pollen étant inaperturé, la sélection sur l'ontogénèse de la distribution des apertures est relâchée. ***Pour citer cet article : B. Albert et al., C. R. Biologies 332 (2009).***

Keywords: *Codiaeum variegatum* var. *pictum*; Callose; Cytokinesis; Microsporogenesis; Pollen

Mots-clés : *Codiaeum variegatum* var. *pictum* ; Callose ; Cytocinèse ; Microsporogenèse ; Pollen

1. Introduction

The male gametophytes of the flowering plants, the pollen grains, are composed of two or three cells surrounded by a complex multilayered protective wall

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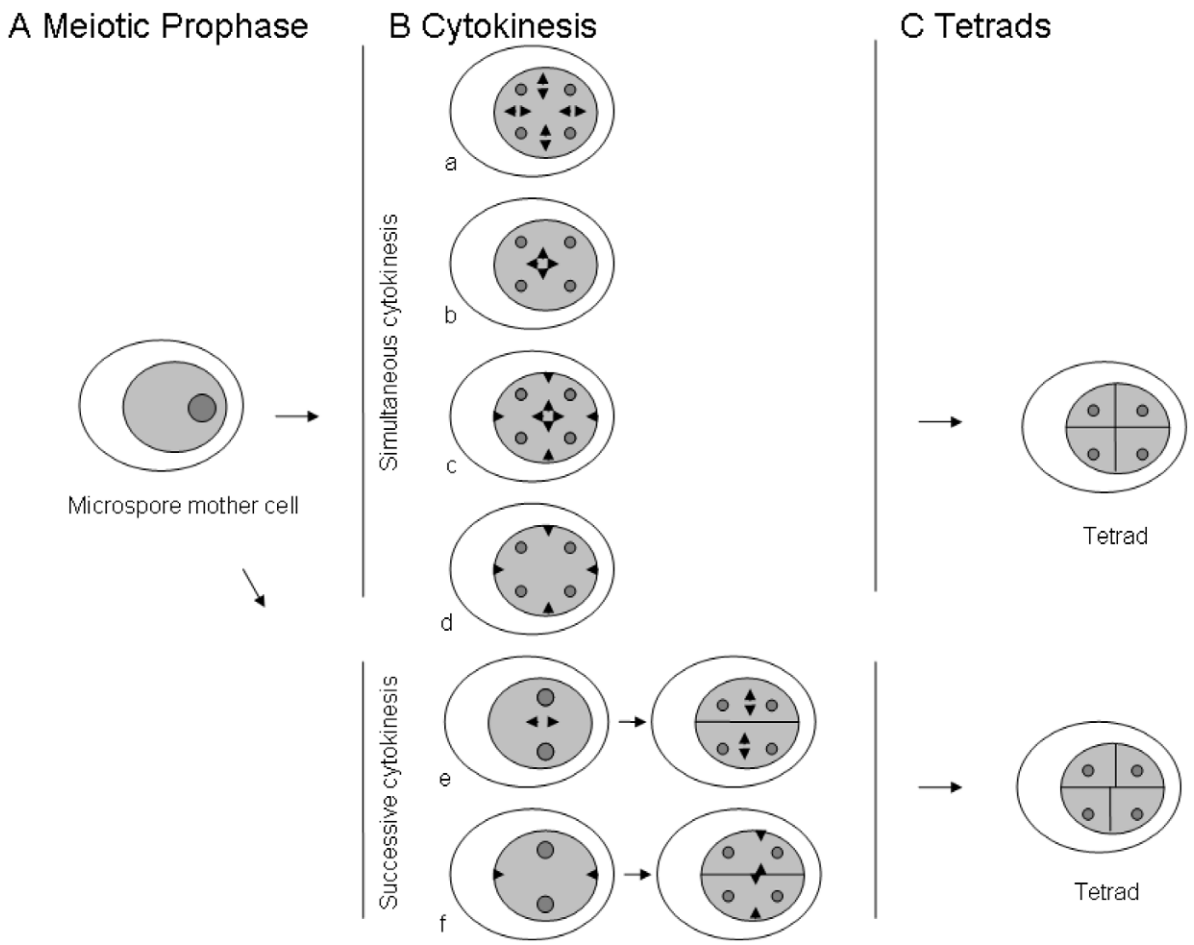

Fig. 1. Microsporogenesis in angiosperms. Meiosis begins with the production of a callosic wall surrounding each of the microspore mother cells (A). Both nuclear divisions take place within the microspore mother cell wall. Cytokinesis (B) can take place after the completion of the two nuclear divisions (simultaneous cytokinesis) or after each of the nuclear divisions (successive cytokinesis) by the formation of the intersporal callosic walls. The intersporal callosic walls are made up of a cell plate that remains almost naked (no or few additional callose deposits) or that can be embedded either simultaneously or subsequently in thick additional callose deposits (not shown). There are four different ways of forming the cell plates and the additional callose deposits in simultaneous cytokinesis. Each cell plate can be formed: a) centrifugally at the cleavage plan level, i.e. starting from the middle of each wall, b) centrifugally at the microspore mother cell level, i.e. starting from the middle of the microspore, c) centripetally at the cleavage plan level, i.e. starting from the middle and the border of the microspore mother cell, d) centripetally at the microspore mother cell level, i.e. starting from the periphery of the microspore mother cell. In successive cytokinesis, a first intersporal callose wall is formed after the first nuclear division, and a second after the second nuclear division. These walls can be formed e) centrifugally or f) centripetally. At the end of microsporogenesis, the microspores remained assembled for a while in tetrad (C) within the microspore mother cell wall before the microspores are released in the anther locule after digestion of the callose. Tetrad shape depends on the type of cytokinesis and on the orientation of the second meiotic axes (not shown).

made of sporopollenin. Pollen grains display a wide range of variation in all their morphological characters (size, shape, and wall macro- and ultrastructure [1]). Apertures are special areas of the pollen wall, characterised by a thinning or an absence of the outer layer (exine). The aperture pattern is defined as the structure, number and position of apertures on the surface of the pollen grain. Like any pollen characteristic, the aperture pattern is variable. Apertures vary in structure (pore, furrow or both), in number (from no aperture to more than one hundred) and in location on pollen surface (polar or equatorial for low aperture number). They are flexible and permeable areas preventing pollen wall breakage and permitting water and gas exchange, and are thereby strongly involved in all the processes of fertilization, from pollen survival during pollination to germination of the pollen tube [2]. Selection on pollen morphology has already been pointed out or suggested for different species of flowering plants. For example, in the genus *Viola*, differences in viability and fertility

among pollen grains differing by their aperture number have been observed [3–5]. In addition, there is a general trend in angiosperms to an increase in pollen aperture number, suggesting that pollen aperture pattern is under selection [6]. Besides this global trend, selection on aperture pattern has been suggested to explain why several microsporogenesis pathways all leading to monosulcate pollen evolved in various monocot families [7,8].

The ontogeny of aperture pattern takes place during microsporogenesis. Microsporogenesis begins with the formation of a thick callosic wall enclosing microspore mother cell (Fig. 1). Microspores are produced by meiosis and cytokinesis takes place by the formation of intersporal callosic walls leading to the formation of a tetrad. Aperture often becomes visible soon after cytokinesis while the microspores are still enclosed within the former microspore mother cell callose wall. Aperture pattern ontogeny has been linked to post-meiotic cytokinesis, which is variable in angiosperms [9–14]. Variation concerns: (1) cell plate formation (Fig. 1) which can take place centrifugally at the cleavage plane level [15], centrifugally at the microspore mother cell level [16], centripetally at the cleavage plan level [15] or centripetally at the microspore mother cell level [17]; (2) tetrad shape can be tetrahedral, tetragonal, rhomboidal, linear, T-shaped or decussate [18–20] (partially constrained by the type of cytokinesis, tetrahedral and rhomboidal tetrads cannot be produced in successive cytokinesis); and (3) position of apertures within tetrad which can be polar or equatorial [10,21] (global aperture patterns are formed by a different mechanism [9, 22]). This variation has been shown to be linked with variation in aperture pattern. An ontogenetic model [23] suggests that the combination of the variation in these four developmental events can explain most of the aperture pattern diversity observed at the level of angiosperms. In some species, additional callose deposition on the cell plates has also been shown to be involved in the determination of the aperture pattern [16, 24–26].

Up to now, aperture pattern ontogeny has been studied mostly in species with aperturate pollen grains. In aperturate species, microsporogenesis leads to microspore production and is also implicated in aperture pattern determination. In these species, apertures perform essential functions for both pollen viability and fertility. Therefore, the ontogenetic changes that alter aperture pattern determination are probably constrained by the necessity to preserve pollen performances, explaining why the features of microsporogenesis are generally conserved within species. On the other hand, in inaperturate species microsporogenesis only leads to microspore production. As a result in inaperturate species, the mutations that affect the developmental events implicated in aperture pattern determination in aperturate species, but that do not impair microspore formation are not under selection, allowing the occurrence of developmental variation that would be eliminated in aperturate taxa. One can therefore expect a higher range of variation in microsporogenesis in inaperturate taxa compared with other taxa. The observation of Furness [27] showing that at the scale of angiosperms, inaperturate pollen is not associated with a particular tetrad shape or cytokinesis type, supports our hypothesis of relaxation of the selective forces applying on aperture pattern. In this paper, we examine microsporogenesis in taxa producing inaperturate pollen grains, in order to find out if some developmental variation occurs when the selective pressures linked to the ontogeny of aperture pattern are removed. Core eudicots usually display a conserved microsporogenesis and appear then as a good candidate for studying the effect of a relaxation of constraints on aperture pattern. Seven cultivars of *Codiaeum variegatum* var. *pictum*, a core eudicot species belonging to the Euphorbiaceae family that produces inaperturate pollen [28], were studied. Microsporogenesis appears to be remarkably variable in the various cultivars: each of the different developmental steps that are suspected to be involved in aperture pattern ontogeny (type of cytokinesis, cell plate formation, additional callose deposition and tetrad shape) were found variable in at least one of the cultivars.

2. Materials and methods

Plant material—Flower buds were sampled from the *Codiaeum variegatum* var. *pictum* collection of the Jardin Botanique de la ville de Paris, France. Seven cultivars were studied: ‘Carrierei’, ‘Flambeau’, ‘Excellent’, ‘Comte de Winseck’, ‘Mer de glace’, ‘Petra’, and ‘Souvenir de Laken’.

Microsporogenesis observation—Fresh flower buds were collected at different developmental stages. Several flower buds per individual and several stamens per flower bud were sampled and observed for each developmental stage. The anthers were extracted and immediately squashed and mounted in aniline blue, according to a protocole modified from the method of Arens [29], with 15% of glycerol. With this method, the callose of the intersporal walls (cell plates and additional callose deposits) becomes fluorescent when illuminated by UV light (DAPI filter; excitation at 345, emission at 425 nm long pass). For each cultivar, the progression of the cell

plates, additional callose deposition, and the resulting tetrad shape were recorded. In addition, the inaperturate condition of the pollen was checked.

3. Results

In the seven cultivars studied, only inaperturate pollen grains were produced. Despite this lack of difference in pollen morphology, variation in cell wall formation, additional callose deposits, and the shape of the tetrads were recorded (summarised in Table 1).

Codiaeum variegatum* var. *pictum 'Carrierei' and Codiaeum variegatum* var. *pictum 'Flambeau'—In these two cultivars, cytokinesis is simultaneous and cell plate formation begins in the middle of the microspore mother cell and displays a centrifugal progression (Fig. 2: 1–4, 10–12, 13–14). Most tetrads are tetrahedral (Fig. 2: 5–6, 7–8, 15–16, 17). Tetragonal tetrads were observed in Flambeau (Fig. 2: 18). Conspicuous additional callose deposits were observed in 'Carrierei' only (Fig. 2: 7–8, arrows). The pollen is inaperturate (Fig. 2: 9, 19).

Codiaeum variegatum* var. *pictum 'Comte de Winseck' and Codiaeum variegatum* var. *pictum 'Excellent'—In these two cultivars, cytokinesis is simultaneous and cell plate formation begins at the periphery of the microspore mother cell and progresses centripetally (Fig. 3: 1–3, 9–10). Most tetrads are tetrahedral (Fig. 3: 4–5, 6–7, 11). Tetragonal tetrads were found in 'Excellent' (Fig. 3: 12). Conspicuous additional callose deposit was observed in 'Comte de Winseck' only (Fig. 3: 6–7). The pollen is inaperturate in both cultivars (Fig. 3: 8, 13).

Codiaeum variegatum* var. *pictum 'Mer de glace'—Cytokinesis is simultaneous and cell plate formation begins at the border of the cleavage planes and progress centripetally (Fig. 4: 1–4). Only tetrahedral tetrads were observed (Fig. 4: 8). Conspicuous additional callose deposits were observed (Fig. 4: 5–7). Mature inaperturate pollen grains are dispersed in tetrad (Fig. 4: 8).

Codiaeum variegatum* var. *pictum 'Petra'—Cytokinesis is simultaneous. Variation in cell plate formation was observed in this cultivar. Cell plates progress either centripetally from the periphery of the cleavage planes (Fig. 4: 9), or centripetally starting from the callose wall surrounding the microspore mother cell (Fig. 4: 10–11). Most tetrads are tetrahedral (Fig. 4: 10–15). Additional callose deposits were observed (Fig. 4: 14–15). The pollen is inaperturate (Fig. 4: 16).

Codiaeum variegatum* var. *pictum 'Souvenir de Laken'—Variation in both cytokinesis type (successive or simultaneous), and cell plate formation were observed. In some of the microspore mother cells, cytokinesis was successive, i.e., takes place in two steps: formation of a dyad and after, formation of two callose walls to complete the cytokinesis (Fig. 5: 1, 2–3 and 4). In these cases, the first wall and the second walls are formed centrifugally from the middle of the microspore mother cell (Fig. 5: 1, 2–3). Three-walled tetragonal tetrads were observed (Fig. 5: 4). In the other microspore mother cells, the cytokinesis was simultaneous (Fig. 5: 6, 7, 10). In these cases, the formation of callose walls starts from the edge of the cleavage plane and progress centripetally (Fig. 5: 5). The tetrahedral shape was the most frequently observed (Fig. 5: 7, 8–9), nevertheless rhomboidal with five cleavage planes (Fig. 5: 6) and tetragonal tetrad with four cleavage plans (Fig. 5: 10) were also found. Additional callose deposit was observed (Fig. 5: 4, 10). The pollen is inaperturate (Fig. 5: 11).

4. Discussion

In eudicots, microsporogenesis is usually considered as a fixed developmental sequence associated with the production of tri-aperturate pollen [6]. In monocots, variation during microsporogenesis is observed only be-

Table 1
Summary of the features of microsporogenesis for the cultivars of *Codiaeum variegatum* var. *pictum* studied. CP: cell plate, Th: tetrahedral tetrad, Tg: tetragonal tetrad, Rh: rhomboidal tetrad, ACD: additional callose deposit, MMC: microspore mother cell.

Cultivars	Cytokinesis	CP start	CP progression	Tetrads	ACD
'Carrierei'	Simultaneous	Middle of the MMC	Centrifugal MMC	Th	Yes
'Flambeau'	Simultaneous	Middle of the MMC	Centrifugal MMC	Th + Tg	No
'Comte de Winseck'	Simultaneous	Periphery of the MMC wall	Centripetal MMC	Th	Yes
'Excellent'	Simultaneous	Periphery of the MMC wall	Centripetal MMC	Th + Tg	No
'Mer de glace'	Simultaneous	Periphery and middle of the MMC	Centripetal cleavage plan	Th	Yes
'Petra'	Simultaneous	Periphery of the MMC wall	Centripetal MMC	Th	Yes
		Periphery and middle of the MMC	Centripetal cleavage plan		
'Souvenir de Laken'	Simultaneous	Periphery and middle of the MMC	Centripetal cleavage plan	Th + Tg + Rh	Yes
	Successive	Middle of the MMC	Centrifugal MMC		

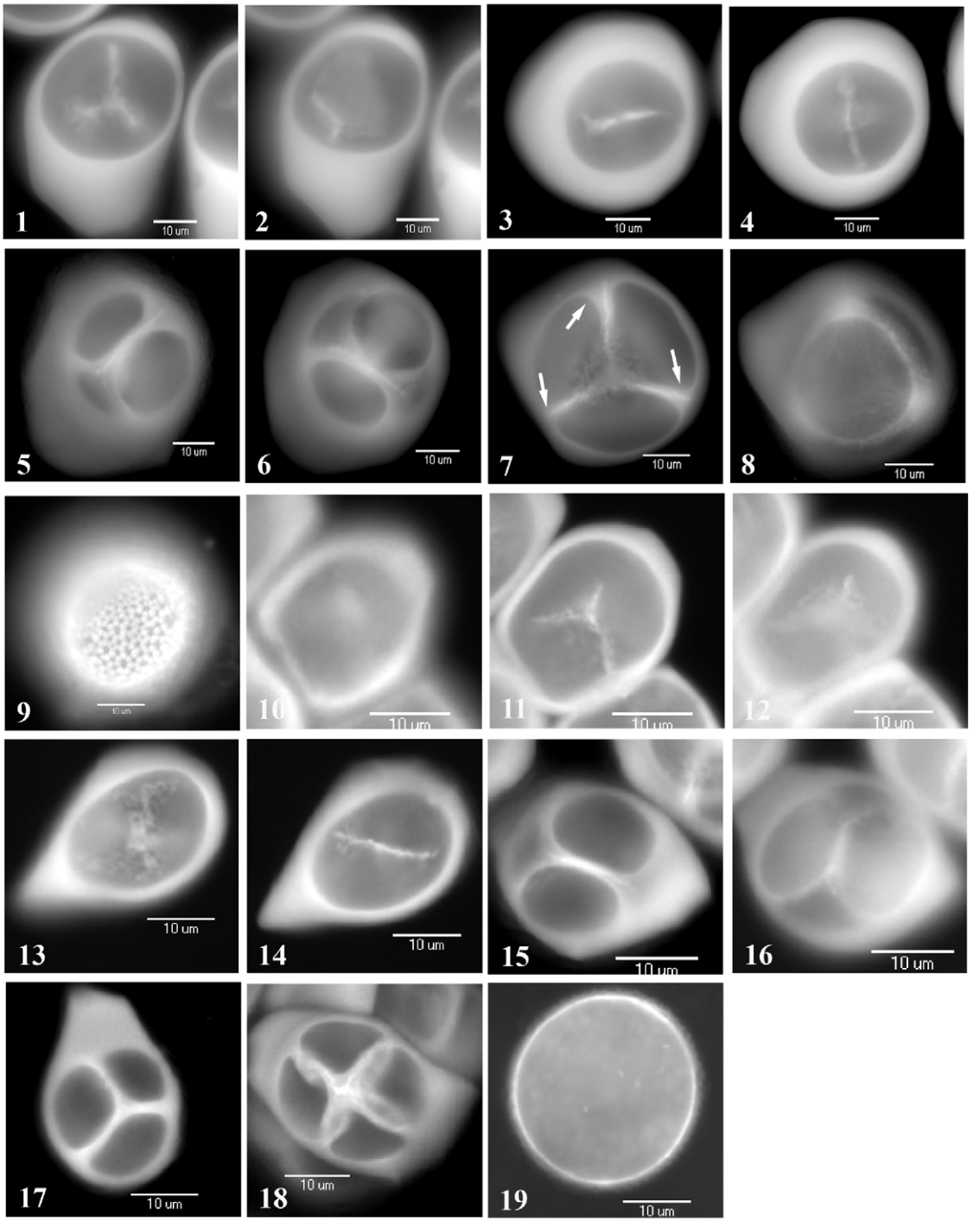

Fig. 2. Tetrad formation and mature pollen grains in *Codiaeum variegatum* var. *pictum* 'Carrierei' and 'Flambeau'. 1–9: 'Carrierei'. 1–4: centrifugal cell plate formation at the microspore mother cell level in two different microspore mother cells (1–2 and 3–4 are two different views of each microspore mother cell). 5–8: additional callose deposits (arrows) can be observed on the cell plates (5–6 and 7–8 are two different views of two different tetrahedral tetrads). 9: inaperturate pollen grain. 10–19: 'Flambeau'. 10–12, 13–14: centrifugal cell plate formation at the level of the microspore mother cell in two different microspores mother cell (10–12 and 13–14). 15–17: tetrahedral tetrad (15–16 two views of the same tetrad). 18: tetragonal tetrad. 19: inaperturate pollen grain.

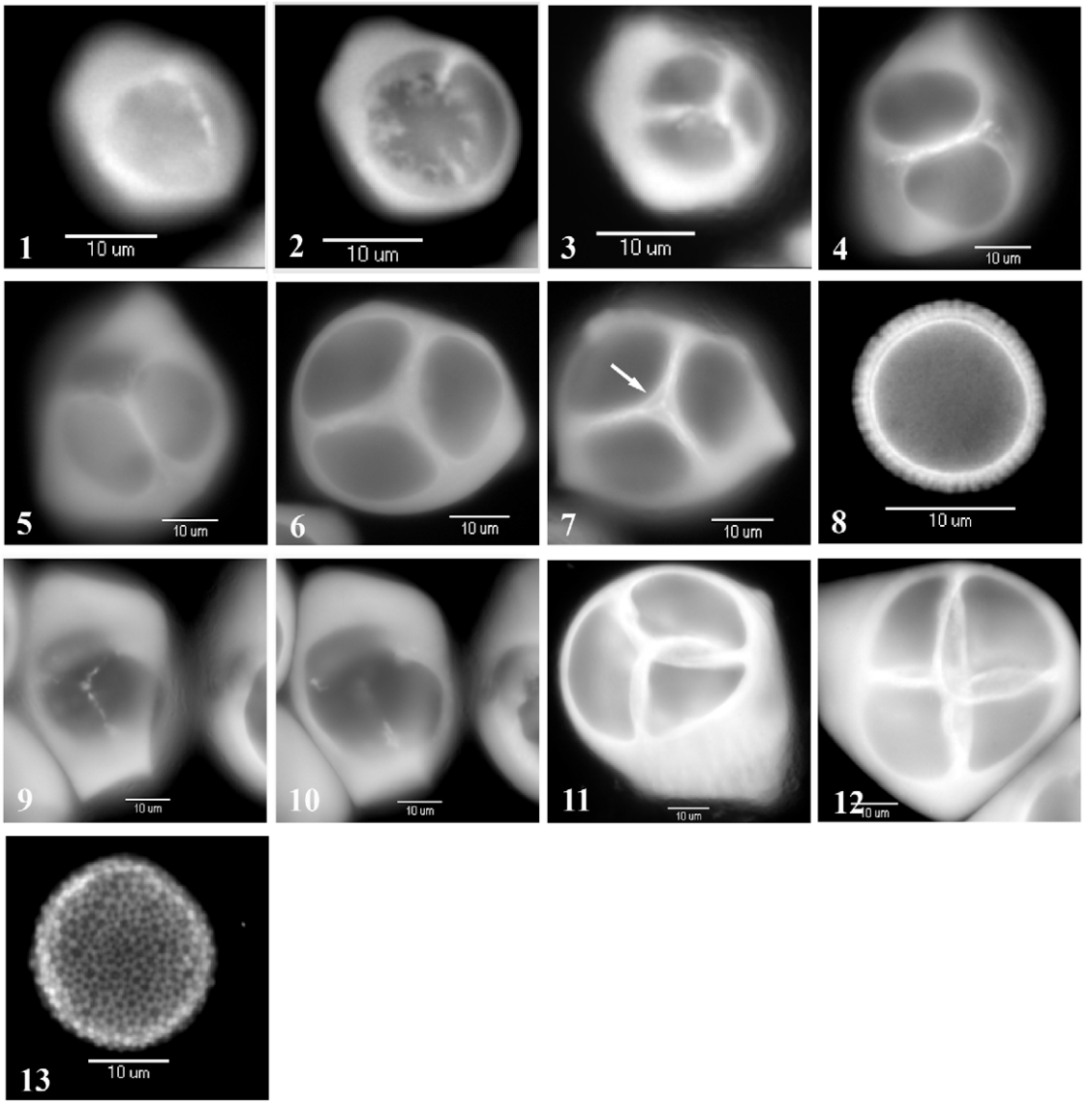

Fig. 3. Tetrad formation and resulting mature pollen grains in *Codiaeum variegatum* var. *pictum* 'Comte de Winseck' and 'Excellent'. 1–8: 'Comte de Winseck'. 1–3: centripetal formation of the cell plates at the microspore mother cell level (3 different views of the same microspore mother cell). 4–7: two tetrahedral tetrads (top and bottom of the same tetrad: one 4–5, the other 6–7). Additional callose deposits can be seen on 7 (arrow). 8: mature inaperturate pollen grain. 9–13: 'Excellent'. 9–10: two different views of centripetal cell plate formation at the microspore mother cell level. 11: tetrahedral tetrad. 12: tetragonal tetrad. 13: inaperturate pollen grain.

tween species and not within species (except for variation in tetrad shape that can be observed between microsporocytes within an anther). The burst of variation observed among and within the seven inaperturate cultivars of *C. variegatum* is therefore highly remarkable (Table 1). Three out of the four developmental steps suspected to be involved in aperture pattern ontogeny (Fig. 1) were found to be variable (the last one, aperture position cannot be considered for inaperturate pollen). The type of cytokinesis, although mostly simultaneous, was also found to be partly successive in one cultivar. Intersporal wall formation was found to vary between and within cultivars. Different patterns of additional callose deposits were recorded. Last, tetrad shape was also found variable. However, unlike the preceding developmental variation, tetrad shape is known to vary in eudicots, most species displaying a vast majority of tetrahedral tetrads and a few tetragonal and rhomboidal tetrads [30]. The range of variation of tetrad shapes observed in the different cultivars is not exceptional.

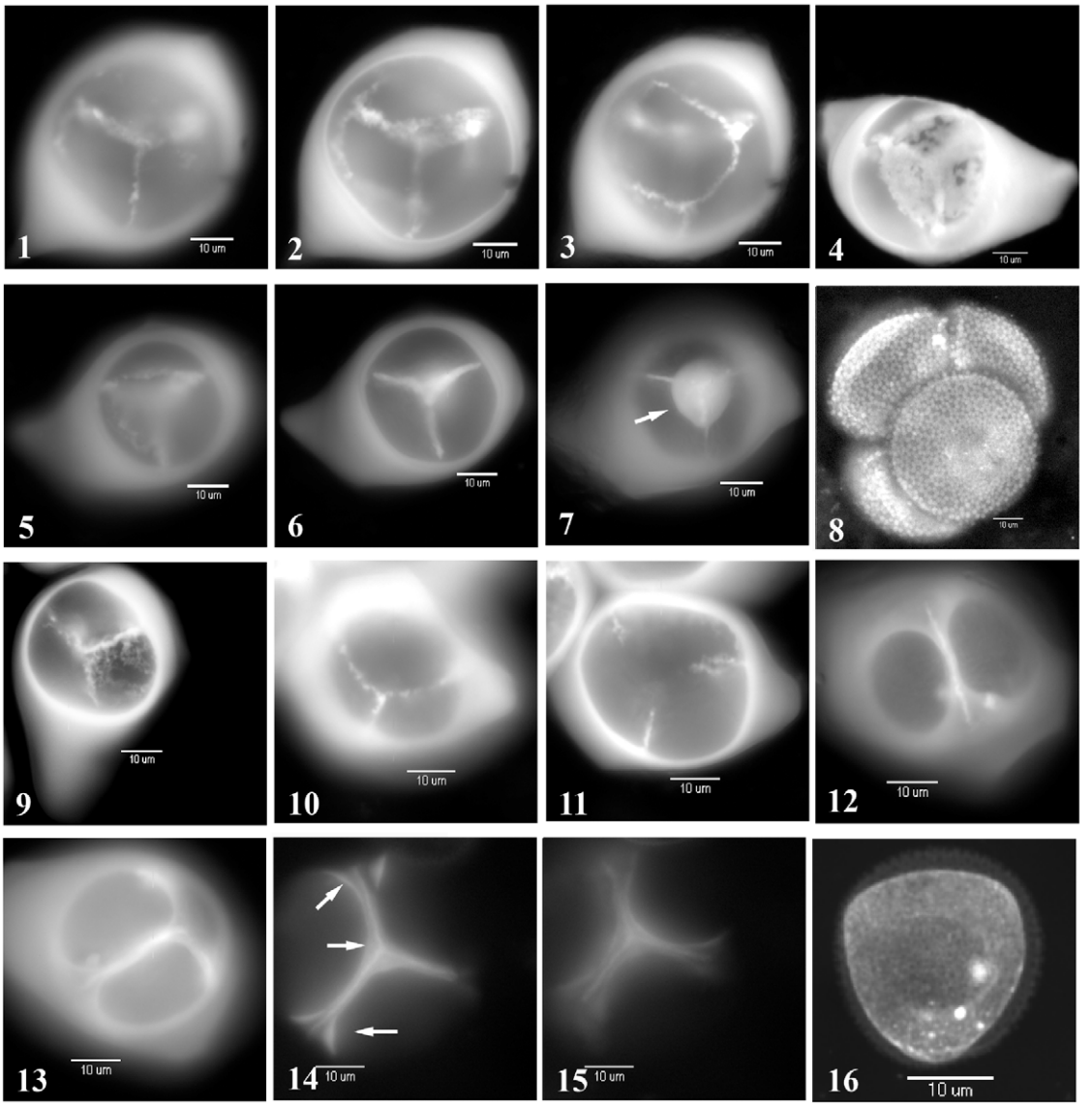

Fig. 4. Tetrad formation and resulting mature pollen grains in *Codiaeum variegatum* var. *pictum* 'Mer de glace' and 'Petra'. 1–8: 'Mer de glace'. 1–4: centripetal cell plate formation started at the border of the cleavage plan (1–3: same microspore mother cell at different level of observation). 5–7: different views of a tetrahedral tetrad with additional callose deposit (arrow). 8: inaperturate pollen grain release in tetrad. 9–16: 'Petra'. 9: centripetal cell plate formation started at the border of the cleavage plan. 10–11: centripetal cell plate formation at the microspore mother cell level (same tetrad). 12–13: two views of a tetrahedral tetrad. 14–15: two views of a tetrahedral tetrad with additional callose deposit (arrow). 16: inaperturate pollen grain.

In 'Souvenir de Laken', both successive and simultaneous cytokinesis were observed within the same anther. This is highly unusual for a eudicot species, since in higher eudicots, cytokinesis is uniformly described as simultaneous [9,18,30–37], with only four exceptions recorded. In the Proteales, an early diverging order of the eudicots, the cytokinesis is reported as successive in one genus [38]. In rosids, three cases of successive cytokinesis are described, one in a species of *Rafflesia* [6], and the two others in *Podostemaceae* [6,39]. In *Nelumbo* the cytokinesis was described as successive by Kreunen and Osborn [40], but Banks et al. [41] demonstrated that the cytokinesis is actually simultaneous. Simultaneous cytokinesis is therefore highly conserved in eudicots, although as observed in the *tam* mutant of *Arabidopsis thaliana* [42] with only one mutation a switch from simultaneous to successive cytokinesis is possible. In light of this mutant, the co-occurrence of both

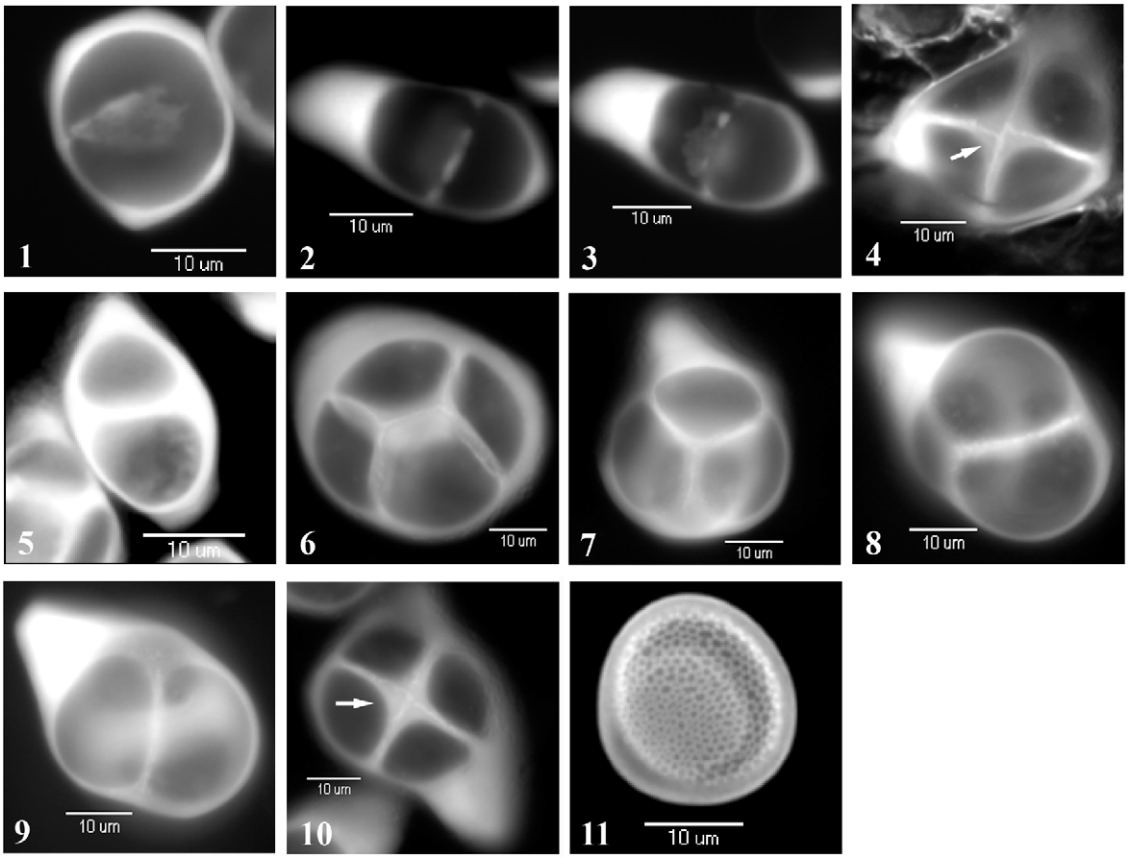

Fig. 5. Tetrad formation and resulting mature pollen grains in *Codiaeum variegatum* var. *pictum 'Souvenir de Laken'*. 1: centrifugal cell plate formation of the first cleavage plan starting from the middle of the microspore mother cell, 2–3: centrifugal cell plate formation of the second cleavage plan starting from the middle of the microspore mother cell (two views of the same microspore mother cell). 4: tetragonal tetrad from successive cytokinesis. Additional callose deposit (arrow). 5: centripetal cell plate formation starting from the border of the cleavage plans. 6: rhomboidal tetrad, 7–9: tetrahedral tetrad (8–9: same tetrad). 10: tetragonal tetrad from simultaneous cytokinesis. Additional callose deposit (arrow). 11: inaperturate pollen grain.

cytokinesis types in 'Souvenir de Laken' appears not so extraordinary, although no other reports of such a phenomenon has ever been described in eudicots. In contrast to eudicots, cytokinesis type is a highly labile character both in monocots (a review in [43]) and in basal angiosperms (a review in [44]). In monocots, the two types of cytokinesis are found in different species, and a few cases of variation in cytokinesis type within a species were described (in three palm species, simultaneous cytokinesis was observed in association with successive cytokinesis [8] and in Dioscoreaceae as well [45]).

In all core eudicot species that have been examined so far, cell plate formation is synchronised with additional callose deposits [9,15,18,33]. Both begin at the periphery of the cleavage plane and progress centripetally. A few exceptions are described in basal eudicot species. In *Protea lepidocarpodendron* and in *Helleborus foetidus*, the synchronisation between cell plate formation and additional callose deposits is broken [25,46]. In *Codiaeum*, additional callose deposits are observed only in some of the cultivars (the others apparently display "naked" cell plates, lacking additional callose deposits). Within the cultivars where additional callose deposits are observed, there is a desynchronisation of cell plate formation and additional callose deposits as in *Protea lepidocarpodendron* and in *Helleborus foetidus*. The additional callose deposits are produced at the intersection of the cleavage walls and differed mostly quantitatively by their thickness. They are similar to those observed in *Helleborus* [25,46] except for 'Conte de Winseck' where the additional callose deposits are especially large and do not stick to the cleavage wall as it is the case in the other cultivars. In addition to this variation in additional callose deposits, the different cultivars of *C. variegatum* display several ways

of forming cell plates (Table 1). In short, cell plate progression can be centripetal (within the cleavage planes or towards the middle of the dividing microspore mother cell) or centrifugal from the middle of the dividing microspore mother cell. The variation is found among cultivars and also within ('petra', 'souvenir de laken'). Intersporal wall formation in eudicots is known to be centripetal [9,15,30,32–34,47] with two exceptions in Proteaceae [38]. Variation in callose wall formation within a species has never been recorded before in angiosperms and, in eudicots, centrifugal progression of cell plates from the middle of the dividing microspore mother cell as observed in 'Carrieri' and 'Flambeau' has not yet been described. So far, such an extent of variation in cell plate formation had only been observed between species in monocots [7,8] or basal angiosperms [17,20, 48–52] where both centripetal and centrifugal formation of intersporal wall are described. Interestingly, in eudicots the usual mode of callose progression is composed of both a centrifugal progression from the periphery of the dividing microspore mother cell and a simultaneous production of callose in the center of the dividing microspore mother cell which progresses centripetally leading to the eudicot-typical so-called wall formation by infurrowing [15]. As a result, the different modes of cleavage wall formation (centripetal from the center of the microspore mother cell, centrifugal from the periphery of the cleavage wall or both) described in *C. variegatum* appear to be variation around the one usual in eudicots. This tends to indicate that the eudicot usual mode of cell wall formation is due in fact to the combination of two distinct independent processes (centripetal from the center of the microspore mother cell and centrifugal from the periphery of the cleavage wall). Our study thus provides some new clues about the links between all the different ways of intersporal wall formation in angiosperms. Monocots as well as basal angiosperms experience either centripetal or centrifugal progression of cell plates while eudicots appear to cumulate both modes in a synchronised way.

To explain the remarkable burst of variation of microsporogenesis observed within our studied species (*C. variegatum*), a tempting hypothesis is that the pollen being inaperturate, selection on the ontogenesis of aperture pattern is relaxed in this species. As a result, the developmental steps involved in aperture patterns are no more constrained. Mutations modifying this part of microsporogenesis could accumulate randomly leading to the observed variation. Mutation accumulation could be exceptionally large in the studied species for two reasons. Firstly, the cultivars are essentially propagated by cuttings, removing almost completely selection on pollen morphology. Secondly, the cultivars were probably exposed to mutagens to obtain several different phenotypes of ornamental interest. Even if, due to the "ornamental treatment", mutation is particularly large in *C. variegatum*, the development is so variable, that a large number of genes (and thus numerous targets for mutation) can be suspected to be involved in the features of microsporogenesis examined here.

Our study is consistent with the observation of Furness [27] that showed that at the scale of angiosperms, inaperturate pollen is not associated with any particular tetrad or cytokinesis type. Altogether, our hypothesis of relaxation of the selective forces applying on aperture pattern received a strong support. Alternatively, it is possible although unlikely that in at least part of the Euphorbiaceae species, the microsporogenesis is variable and varies independently of aperture pattern. To reject such an hypothesis, a large survey of both aperturate and inaperturate taxa in Euphorbiaceae family would be necessary.

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