Postmeiotic cytokinesis and pollen aperture number determination in eudicots: effect of the cleavage wall number

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Summary. In eudicot postmeiotic tetrads, apertures are usually joined in pairs in highly conserved areas. These appear to be located at the last points of contact persisting at the end of cytokinesis between the cytoplasm of the future microspores. In order to investigate the relationship between cytokinesis and aperture formation, aperture distribution within postmeiotic tetrads and the progression of meiosis were studied in Nicotiana tabacum cv. Ambalema. This variety (inbred line) produces about 85% tricolporate pollen and 15% tetracolporate pollen grains. In addition, about 7% of tetrads are composed of four equal-sized microspores and a supernumerary pseudomicrospore of small size and an equal proportion of tetrads exhibit unpaired apertures (these apertures are not joined in pairs within tetrads). Observation of cytokinesis indicates that both unpaired apertures and pseudomicrospores could result from the persistence of late communications between microsporocytes. Observations of tetrads indicate that an increase in the number of elements that are separated during cytokinesis is correlated with an increase in microspore aperture number. All data converge to support the hypothesis that aperture site determination is partly controlled by the number of walls formed to separate the different elements of the tetrad.

Keywords: Pollen; Aperture pattern; Meiosis; Cytokinesis; Tetrad; Nicotiana tabacum.

Introduction

Angiosperm pollen grains are highly patterned organisms with two distinct levels of patterning (Wodehouse 1935, Walker and Doyle 1975, Schmid et al. 1996, Blackmore and Crane 1998). Pollen grains are surrounded by the exine, a complex multilayered wall, which can display an elaborate ornamentation. Further, in one or several usually well-defined regions of this wall, called the apertures, the exine is reduced or absent. The structure, number, and distribution of apertures on the pollen surface constitute the aperture pattern. Aperture distribution on the pollen surface is usually extremely regular (Wodehouse 1935; Van Campo 1976; Pozhidaiev 1998, 2000). Functionally, apertures act as harmomegathic structures, permit water uptake during pollen rehydration and exchanges of intine-bound recognition substances, and are sites of exit for the pollen tube (Heslop-Harrison 1975, Muller 1979, Blackmore and Barnes 1986).

Pollen grains are produced by meiosis. Before meiosis, the microsporocytes are enclosed in a thick callosic wall which persists after the completion of the two divisions. The four microspores stemming from the same meiosis remain assembled in a tetrad until they build a primary template of their exine wall. In eudicot postmeiotic tetrads, apertures are generally observed in pairs, at points of contact between the microspores (first described by Fischer [1890] and further named Fischer’s distribution of apertures by Wodehouse [1935]). Study of meiosis has shown that aperture pairs are formed at the last points of contact persisting between the cytoplasm of the future microspores at the end of cytokinesis (Wodehouse 1935). As a consequence it has been suggested that the spatial cues leading to aperture pattern determination result from cytokinesis. However, no formal demonstration of such a causal link has been provided to date. In this

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paper, the link between cytokinesis and aperture pattern ontogeny is investigated in a heteromorphic species, *Nicotiana tabacum* cv. Ambalema, that produces two main pollen morphs displaying three or four apertures.

Reread with current knowledge, Wodehouse’s hypothesis can be described as follows. In eudicots, cytokinesis is simultaneous, which means that the two nuclear divisions are completed before cytokinesis starts. The second meiotic axes are generally orthogonal, leading to a tetrahedral configuration of the four haploid nuclei. Radial arrays of microtubules extend from the nuclear envelope define four cytoplasmic domains centered on the nuclei (Brown and Lemmon 1988, Traas et al. 1989, Tiezzi et al. 1992, Ressayre et al. 2002a). Six cleavage planes are formed at the borders of these domains. Cytokinesis is completed by the formation of callose walls in the cleavage planes. Callose deposition begins at the periphery of the cleavage planes and in the center of the dividing cell and converges toward the middle of the arrays of microtubules (Longly and Waterkeyn 1979, Blackmore and Barnes 1988, Brown 1991, Ressayre et al. 2002a). At the end of cytokinesis, the cytoplasms of the future microspores remain attached by six narrow junctions, one per cleavage plane, connecting each future microspore to the three others. According to Wodehouse (1935), these narrow junctions, one per cleavage plane, define future aperture sites. Each microspore of a tetrad has thus three apertures, each aperture being joined in pairs with one of the apertures belonging to the three other microspores present in the tetrad (Wodehouse 1935). In most species, the last junction sites are marked by endoplasmic reticulum shields that prevent primexine formation (Heslop-Harrison 1963, Bandhari 1984, Schmid et al. 1996), and they become apertures. One could note that other mechanisms may also be involved (Rowley 1975, Guzzo et al. 1994, Ressayre 2001).

In species producing only tetracolporate pollen, the mechanism proposed by Wodehouse (1935) must be adapted but can still apply. The fourth aperture of each microspore results from the duplication of the pair of apertures that is placed between sister microspores (Huynh 1968). The apertures are thus also joined in pairs within the tetrads. Variation in cytokinesis leading to the maintenance of two cytoplasmic junctions within cleavage planes could explain these duplications (Ressayre et al. 1998, 2002a).

In addition, Wodehouse’s hypothesis can be extended to most angiosperm species and theoretically permits production of most of the aperture patterns with small aperture numbers observed in angiosperms (Ressayre et al. 2002b). The coincidence of aperture sites with the places where cytokinesis is completed has been observed in members of the family Proteaceae, which display an aperture pattern fundamentally different from the other eudicots (Blackmore and Barnes 1995). Proteaceous pollen displays Garside’s distribution of apertures: apertures are joined three by three within the tetrads (Garside 1946). Moreover, in several species of monocots, variation in tetrad geometry has been correlated with aperture pattern variation (Harley 1999, Rudall et al. 1997, Furness and Rudall 1999), and in two monocot species that strongly deviate from eudicot microsporogenesis, apertures are also formed in regions where cytokinesis is completed (Ressayre 2001).

Although an increasing body of data confirm the involvement of the meiotic division in aperture pattern ontogeny (Blackmore and Crane 1998, Ressayre et al. 2002b), no formal evidence has been presented until now to transform the correlation between places where cytokinesis is completed and apertures into a causal relationship. In addition, the hypothesis of Wodehouse appears to be challenged by the existence of numerous exceptions, such as the observation of unpaired apertures, apertures that are not joined in pairs within tetrads (Wodehouse 1935, Huynh 1968, Mignot et al. 1995). Up to now (to our knowledge), no alternative hypothesis has been proposed for eudicots against the pioneer theory of Wodehouse (1935). On the other hand, part of this theory is still not confirmed by experimental observations. The aim of this paper is to look for evidence in favor of a causal relationship between aperture pattern ontogeny and cytokinesis progression.

To achieve this, aperture pattern ontogeny has been investigated within *Nicotiana tabacum* cv. Ambalema. This species displays natural disturbances of meiosis producing a variation in cleavage plane numbers by the formation of additional pseudomicrospores in about 7% of the tetrads. It also exhibits a similar proportion of tetrads displaying unpaired apertures. If the Wodehouse (1935) theory is correct, two predictions should be verified. First, an increase in cleavage plane number should be associated with an increase in aperture number. Second, unpaired apertures should be formed in pairs.

The presence of pseudomicrospores in addition to the four “normal” microspores permits direct testing of the effect on aperture pattern of an increase in the