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PREMEIOTIC MICROSPOROCTE CELL SHAPE INFLUENCES SHAPE OF TETRADS DURING MICROSPOROGENESIS

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Angiosperm microspores are grouped into tetrads before they mature into functional pollen grains. This tetrad stage is an important step in microsporogenesis. Tetrad shapes are diverse across angiosperms, with high levels of variation sometimes occurring within species, reflecting variation in early developmental events of nuclear and cell division (i.e., meiosis and cytokinesis). Among these developmental influences, the shape of the microsporocyte (pollen mother cell; hereafter PMC) is likely to influence tetrad shape. This article presents a test of this hypothesis in 19 species of higher Asparagales with a successive cytokinesis by inferring PMC shape from tetrads observed with epifluorescence microscopy. Sampled species show differences in PMC shape between groups of regular or irregular tetrads at the species level, and there is a strong component of PMC shape variation at the onset of meiosis on resulting tetrad shape, with more elongated PMCs yielding significantly more irregular tetrads.

Keywords: Asparagales, cell shape, microsporogenesis, pollen mother cell, tetrad.

Introduction

Pollen grains are vital components of plants' life cycles (Blackmore 2007) because they govern male function and are a key factor of reproductive success (Furness and Rudall 2004). At the molecular level, many studies have investigated specific pollen genes and proteins and are beginning to unveil the details of cell structure, organization, and patterning during pollen development (Wilson and Zhang 2009; Ma and Sundaresan 2010; Grossniklaus 2011). Also, the description of key features of pollen development (i.e., microsporogenesis) revealed the connection between pollen grain development diversity and the resulting phenotypic diversity (e.g., size, shape, ornamentation, and specialized pollen germination structures such as apertures; Ressayre et al. 2002; Albert et al. 2009, 2011a). Furthermore, it has been shown that pollen diversity may functionally relate to the ecology of pollination (Hesse 2000; Sannier et al. 2009).

Microsporogenesis is a relatively straightforward developmental process that involves only a few key steps before mature pollen is produced (i.e., meiosis typically followed by one or two mitotic divisions). Microsporogenesis is initiated in microsporocytes (also called pollen mother cells; hereafter referred to as PMCs), which are diploid cells undergoing meiosis to form a tetrad of microspores. An important callose deposit constrains the shape of the PMC (Angold 1967; Abramova et al. 2003). Each further microsporogenesis step is then characterized by great developmental diversity (Furness et al. 2002). First, cytokinesis, the physical division into separate

cells, may occur at different stages of nuclear meiotic divisions, leading to successive, simultaneous, or even intermediate or unusual types of cytokinesis (Sampson 1969; Caddick et al. 1998; Ghimire et al. 2011). Second, cell wall formation can be achieved centripetally or centrifugally (Ressayre et al. 2005) or even both ways (Penet et al. 2005), and it plays a key role during pollen development (Albert et al. 2010). Third, postmeiotic sister cells are grouped into tetrads with a dramatic diversity of shapes (Penet et al. 2007) that may stay cohesive until anther dehiscence and pollen dispersal (Copenhaver 2005). Fourth, aperture number and exine ornamentation of pollen grains vary dramatically within and among plant families or even at lower taxonomic levels (Nadot et al. 2008). Finally, mitoses leading to mature pollen grains with differentiated vegetative and sperm nuclei may be postponed to various degrees following the young microspore stage (Brewbaker 1967).

Tetrad shape is a character of long-standing interest in studies of microsporogenesis and systematics, especially because of its strong association with cytokinesis type (Nadot et al. 2006, 2008). Indeed, a successive cytokinesis (by which cell division occurs between each meiotic nuclear division) usually produces tetragonal and decussate tetrad shapes (Rangaswamy et al. 2001), while simultaneous cytokinesis (whereby cell division occurs after meiotic nuclear division is fully completed) results mostly in tetrahedral tetrad shape and more rarely in rhomboidal shape (Rudall et al. 1997). Other irregular tetrad shapes may also be produced in plants, especially in species with a successive cytokinesis. Irregular tetrad shapes usually occur at very low frequencies and at times were considered teratological. However, in some species, irregular tetrad shapes can be produced at somewhat higher rates or even become the majority (Penet et al. 2005; Albert et al. 2011b). I further investigated whether tetrad shape regularity could be explained

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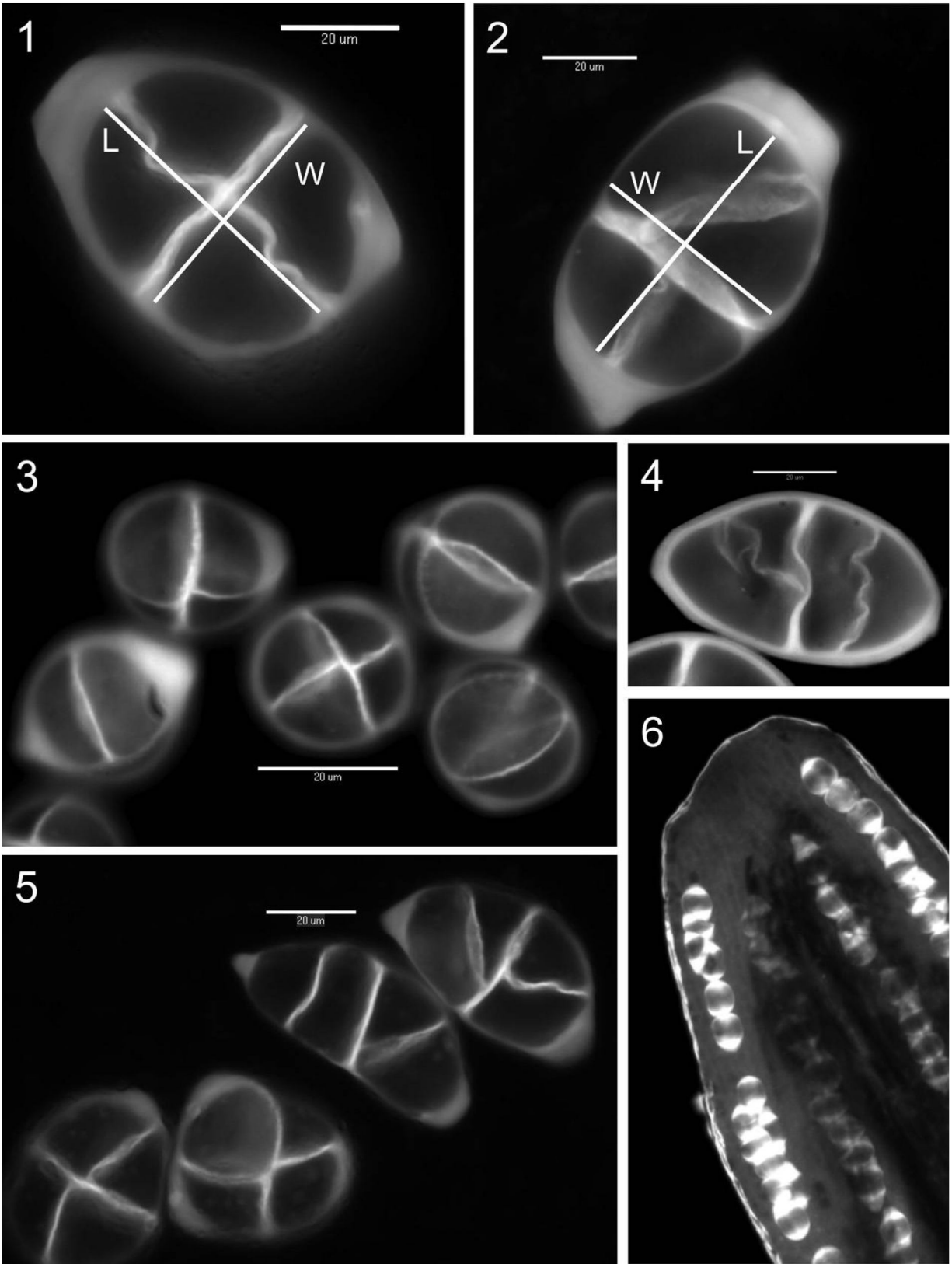


Fig. 1 Tetrads stained with aniline blue. Figs. 1.1, 1.2, Tetrads from *Hosta* sp. (tetragonal tetrad with regular shape and irregular decussate tetrad with irregular shape, respectively). Fig. 1.3, Population of tetragonal (regular) tetrads from *Hypoxidia mabeensis*. Fig. 1.4, T-shaped (irregular) tetrad from *Albucca nelsonii*. Fig. 1.5, Regular and irregular tetrads from *Moraea aristata*. Fig. 1.6, Anther of *Agapanthus praecox* with microsporocytes filling the locular space. Measures recorded on tetrads are illustrated in figs. 1.1 and 1.2 (L = length; W = width). Scale bars represent 20 μ m.

by specific developmental features, specifically, whether they had a link with the PMC shape.

I tested this developmental hypothesis in Asparagales (Angiosperm Phylogeny Group 1998, 2003), a clade of plant species that is characterized by an apomorphic successive cytokinesis and exhibits diversity in tetrad shape (Rudall et al. 1997; Furness and Rudall 1999, 2001). In this study, I specifically address these questions: (1) To what extent do PMC shapes differ among species? and (2) Do tetrad shape groups (regular vs. irregular) originate from different PMC shapes? The results show that few species produce PMCs with a high diversity of shapes and that premeiotic PMC shape does have an impact on resulting tetrad shape.

Material and Methods

The influence of PMC shape on tetrad shape during microsporogenesis was investigated in 19 species belonging to eight families from Asparagales (Angiosperm Phylogeny Group 2003): family Hypoxidaceae: *Hypoxidia maheensis* Friedmann; Iridaceae: *Moraea aristata* (Houtt.) Asch. & Graebn.; Alliaceae: *Allium altaicum* Pall., *Allium ursinum* L.; Amaryllidaceae: *Narcissus poeticus* L., *Agapanthus umbellatus* L'Hér.; Themidaceae: *Triteleia ixioides* ssp. *scabra* Greene; Hyacinthaceae: *Albuca nelsonii* N.E.Br., *Eucomis autumnalis* (Mill.) Chittenden, *Hyacinthus non-scriptus* L., *Ornithogalum longibracteatum* Jacq., *Veltheimia bracteata* Harv. ex Baker; Agavaceae: *Yucca agavoides* Horl. ex Carr., *Arthropo-*

dium cirrhatum G. Benn., *Beschorneria yuccoides* K.Koch, *Chlorophytum pauciflorum* Dammer, *Hosta* sp.; Convallariaceae: *Polygonatum multiflorum* Kunth, *Convallaria majalis* L. Voucher references are available in Penet et al. (2005).

For each species, I squashed anthers from several young buds in aniline blue and performed observations under epifluorescence microscopy (Nadot et al. 2006). Aniline blue specifically stains callose and allows observations of the callose wall surrounding PMCs and young tetrads before the wall disappears before microspore release (Angold 1967). Very early shapes for sporogenous cells, before they reach the stage of premeiotic microsporocytes, are usually isodiametric (Furness 2008) or even already very elongated in some species (e.g., in Asclepiadoideae; dan Dicko-Zafimahova 1980), and shape may vary from early sporogenous cells to premeiotic microsporocytes. Nevertheless, once callose deposition is thick enough, changes in cell shape are much less dramatic. The callose deposit is continuous and mostly even, thus keeping a similar length to width ratio between the premeiotic PMC stage (just before meiosis and cytokinesis are initiated) and young tetrad stage (just after meiosis and cytokinesis are completed). In fact, when I compared the length : width ratio at both stages in a species with mostly regular tetrads (*Agapanthus praecox*) and another with mostly irregular tetrads (*A. nelsonii*), ratios did not differ between premeiotic PMC and young tetrad stages (Welch *t*-test for unequal variances, $t = -0.87$, $P = 0.39$, $n = 60$, and $t = 0.40$, $P = 0.69$, $n = 60$, respectively).

In this study, I therefore used the tetrad length : width ratio (figs. 1.1, 1.2) as a proxy for premeiotic PMC shape. The clas-

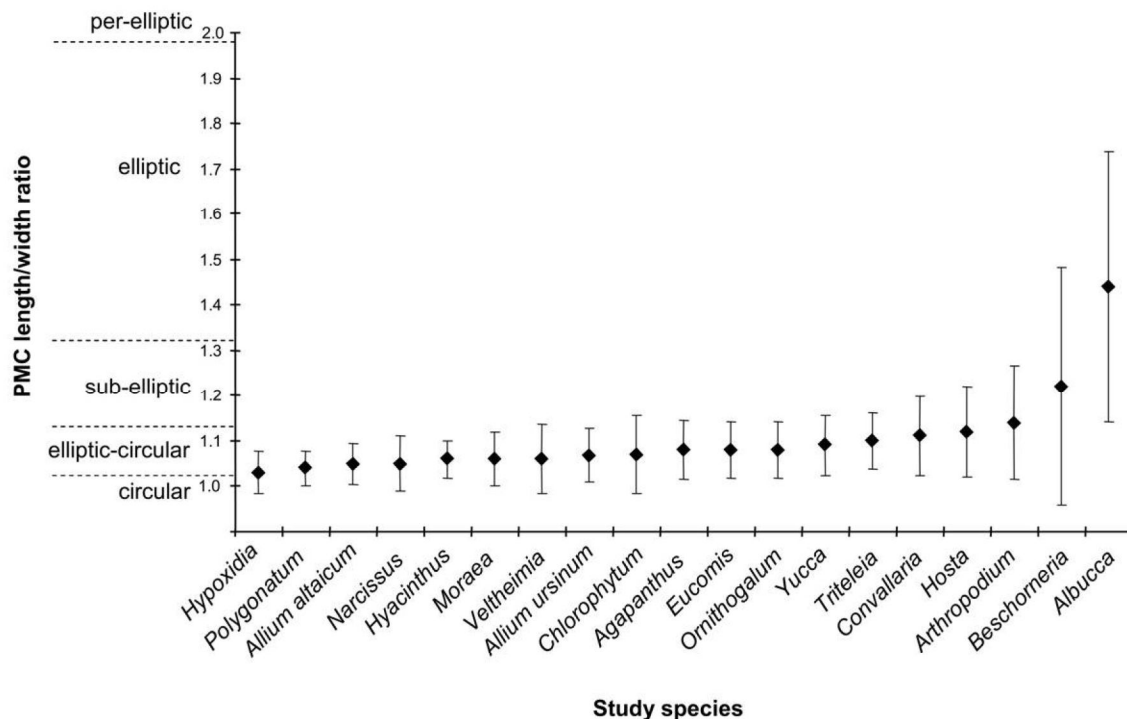


Fig. 2 Mean (\pm SD) pollen mother cell (PMC) length : width ratio and corresponding shapes in the 19 study species of Asparagales. Study species are indicated by their genus name, except for *Allium* congeners. Species are ranked from lowest to highest average PMC length : width ratio.

sification suggested by Erdtman for pollen grains was followed (Erdtman 1969, cited in Harley and Dransfield 2003). According to the ratio value, PMC shape falls into one of five classes: circular (ratio = 1.00), elliptic-circular (1.01–1.13), subelliptic (1.14–1.32), elliptic (1.33–2.00), and per-elliptic (>2.00), the latter being very rare. I measured the length and width (in μm), excluding the callose deposit, of 30–60 tetrads per species ($n = 643$ tetrad observations). The shape of each measured tetrad was also recorded. Tetrad shapes were then classified into two groups: regular (i.e., tetragonal and decussate; see figs. 1.1, 1.3, 1.5) or irregular (linear tetrads, which are rare [fig. 1.5] and intermediate ones, such as T-shaped [figs. 1.2, 1.4, 1.5]).

For the six species where at least five irregular tetrads were found, I tested whether the regular tetrad group differed from the irregular group in their mean PMC length:width ratio, using Welch t -tests for unequal variances in R (R Development Core Team 2011). A G-test of independence across all 19 species was conducted to investigate the relationship between PMC shapes and tetrad shapes (regular vs. irregular groups). Because of sampling limitations with per-elliptic PMC shape, elliptic and per-elliptic shapes were pooled to fit the requirements of the test.

Results

PMC shapes were variable among the 19 study species of Asparagales (fig. 2). In most species, this variation resulted in average circular to elliptic-circular PMCs, but a few species also produced subelliptic PMCs. Average subelliptic PMCs were found in *Convallaria majalis*, *Hosta* sp., *Arthropodium cirrhatum*, and *Beschorneria yuccoides*, and only *Albucca nelsonii* produced mainly elliptic PMCs (fig. 2). In the latter two species, PMCs spanned the full range of shapes and varied from circular to elliptic.

As for tetrad shape (see examples in fig. 1), I found irregular tetrads in 10 of the study species (table 1), with *A. nelsonii* and *B. yuccoides* the only ones presenting irregular tetrads with a linear shape (fig. 1.4). When plotting tetrad shapes according to length and width measures corresponding to PMCs for these two species, linear and regular tetrads segregate distinctly, whereas tetrads of intermediate shape span along the species PMC size range (fig. 3). This distribution was not observed in the other species with lower variation in PMC shape (data not shown).

Overall, I found a larger mean PMC length:width ratio (i.e., PMCs were more elongated) for irregular tetrads than

Table 1

Difference in Pollen Mother Cell (PMC) Length:Width Ratio between Groups of Regular and Irregular Tetrads at the Species Level

| Plant species and tetrad group | Proportion | PMC ratio (SE) | n | t | P |
|---------------------------------|------------|----------------|-----|------|--------|
| <i>Albucca nelsonii</i> | | | 60 | 5.48 | <.0001 |
| Regular | .25 | 1.21 (.14) | | | |
| Irregular | .75 | 1.52 (.30) | | | |
| <i>Beschorneria yuccoides</i> | | | 30 | 2.60 | .017 |
| Regular | .40 | 1.10 (.08) | | | |
| Irregular | .60 | 1.30 (.31) | | | |
| <i>Hosta</i> sp. | | | 60 | 1.11 | .27 |
| Regular | .63 | 1.11 (.10) | | | |
| Irregular | .37 | 1.14 (.10) | | | |
| <i>Convallaria majalis</i> | | | 30 | .42 | .68 |
| Regular | .80 | 1.11 (.09) | | | |
| Irregular | .20 | 1.13 (.09) | | | |
| <i>Hypoxidia maheensis</i> | | | 30 | .50 | .63 |
| Regular | .83 | 1.02 (.05) | | | |
| Irregular | .17 | 1.03 (.03) | | | |
| <i>Moraea aristata</i> | | | 30 | 2.70 | .059 |
| Regular | .87 | 1.05 (.05) | | | |
| Irregular | .13 | 1.13 (.06) | | | |
| <i>Triteleia ixioides</i> | | | 30 | ... | ... |
| Regular | .90 | 1.09 (.06) | | | |
| Irregular | .10 | 1.16 (.09) | | | |
| <i>Allium altaicum</i> | | | 60 | ... | ... |
| Regular | .93 | 1.04 (.05) | | | |
| Irregular | .07 | 1.06 (.03) | | | |
| <i>Chlorophytum pauciflorum</i> | | | 30 | ... | ... |
| Regular | .93 | 1.06 (.06) | | | |
| Irregular | .07 | 1.24 (.21) | | | |
| <i>Agapanthus umbellatus</i> | | | 30 | ... | ... |
| Regular | .97 | 1.08 (.07) | | | |
| Irregular | .03 | 1.03 (na) | | | |

Note. Species are ordered by the observed proportion of irregular tetrads. Proportion of tetrads, group mean PMC length:width ratio, and sample size are indicated. In species where a t -test was possible, significant differences between groups are underlined, and a marginally significant difference is italicized. na = not available.

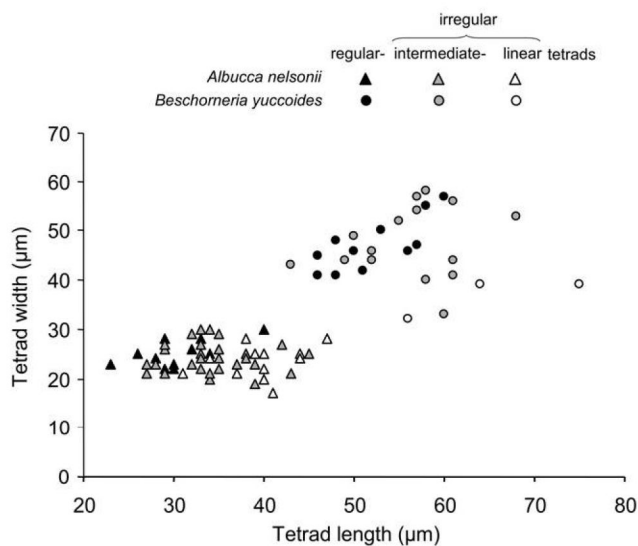


Fig. 3 Segregation of tetrad shape groups in relation to measured length and width of pollen mother cells for two species producing linear tetrads. Triangles = *Albucca nelsonii*; circles = *Beschorneria yuccoides*. For both species, black symbols represent regular tetrads, gray symbols represent intermediate tetrads, and white symbols represent linear tetrads.

for regular tetrads (table 1). This difference in mean PMC length:width ratio between tetrad groups was tested for the six species with more than 10% irregular tetrads and was statistically significant for *A. nelsonii* and *B. yuccoides* and marginally significant for *Moraea aristata* (table 1). With all species combined, the relationship between PMC shape and tetrad shape is highly significant ($G = 146.4$, $P < 0.0001$; fig. 4).

Discussion

Our results show that premeiotic PMC shape has an impact on the following tetrad shapes during microspore development in Asparagales species with successive cytokinesis. Rounder PMCs usually translate into regular tetrad shapes (tetragonal, decussate), while more elongated PMCs tend to produce tetrads with less regular shapes such as linear or T-shaped tetrads (fig. 4; see also fig. 1.5). This pattern is general in our sample and is likely to be found in other monocots, a clade with a high frequency of species with successive cytokinesis (Rudall et al. 1997).

Cytokinesis type is already known to influence tetrad shape: tetrahedral (or more rarely rhomboidal) tetrads are usually produced when cytokinesis is simultaneous, whereas successive cytokinesis is associated with the production of tetragonal or decussate tetrads (Rudall et al. 1997). Here, I describe a novel factor involved in tetrad shape diversity: in species with successive cytokinesis, irregular tetrads seem to originate from elongated PMCs, while rounder PMCs yield mostly regular shapes (fig. 4). Irregular tetrads may thus not be teratological, as has been suggested in the literature but instead may be the result of variation in premeiotic PMC shape.

Whether the same pattern exists in species having simultaneous cytokinesis requires further study. Rhomboidal tetrads, for example, also seem to be produced by more elongated PMCs in species with simultaneous cytokinesis (L. Penet, personal observation).

Two of the study species (*Albucca nelsonii* and *Beschorneria yuccoides*) had PMC shapes that departed significantly from average PMC shapes in our sample (fig. 2). Given that these species belong to different families (Duvall et al. 1993), the increase in PMC shape diversity has arisen independently twice. Variation in PMC shape may have evolved because of selection, although this hypothesis remains unclear with regard to pollen morphology since all of the study species produce monosulcate pollen grains (Penet et al. 2007). PMC shape variation may also arise as a by-product of other processes, such as polyploidy or genome size (Ressayre et al. 2002). However, because DNA content is highly variable among our study species, one would have expected greater PMC shape diversity in our sample than was actually observed. Another nonexclusive hypothesis would take into account intraspecific sources of variation in PMC shape such as locular spatial constraints within anthers (fig. 1.6). Indeed, pollen number is correlated with stamen size (Ritland and Ritland 1989), and tetrad shape is known to vary along anthers (Wilson 1949), as well as with differences in microsporocyte shape (dan Dicko-Zafimahova 1980; Furness 2008). It would be of interest to study PMC and tetrad shape variation within anther locules, contrasting, for instance, closely related species with different stamen sizes or species with anther size polymorphisms.

Tetrad diversity is highly relevant in the study of microsporogenesis because it is associated with diversity of apertures in mature pollen grains, a feature that is under selective influence (Dajoz et al. 1991, 1993). Depending on how cell walls are formed during cytokinesis to separate microspores, different tetrad shapes generate different aperture patterns (Ressayre et al. 2002). In all of our study species, successive cytokinesis is as-

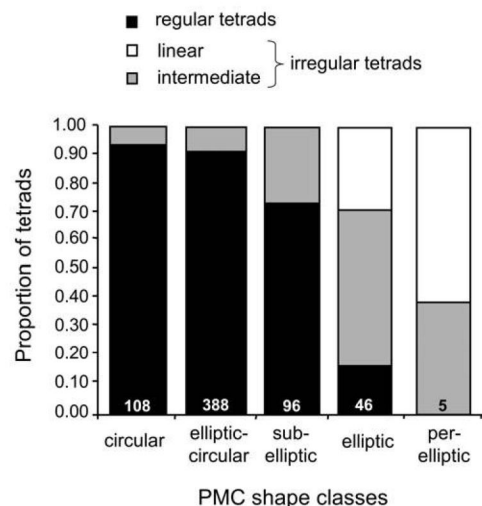


Fig. 4 Proportion of tetrads produced by pollen mother cell (PMC) shape classes: black = regular tetrads; gray = intermediate tetrads; white = linear tetrads. Numbers at the base of bars indicate the number of tetrads characterized (total observations: $n = 643$).

sociated with centrifugal cell division that forces pollen into monosulcate shape (Penet et al. 2007). Tetrad diversity therefore did not translate into pollen morphological differences, even in species producing frequent irregular tetrads (table 1). In those species, selection on tetrad shape may be relaxed, resulting in more developmental variation, as is observed for microsporogenesis in other species (Albert et al. 2009; Nunes et al. 2009). In contrast, such a diversity of tetrads would probably result in greater aperture diversity in species with simultaneous cytokinesis, unless alternative pollen morphologies are counter-selected. This topic needs further exploration in species with simultaneous cytokinesis. In conclusion, PMC shape at the onset

of meiosis impacts tetrad shapes produced after meiosis. Some species with successive cytokinesis have a greater PMC shape diversity and therefore greater tetrad diversity, and we still need to understand whether this diversity is selected for. Packaging constraints within locules in anthers is a promising hypothesis.

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