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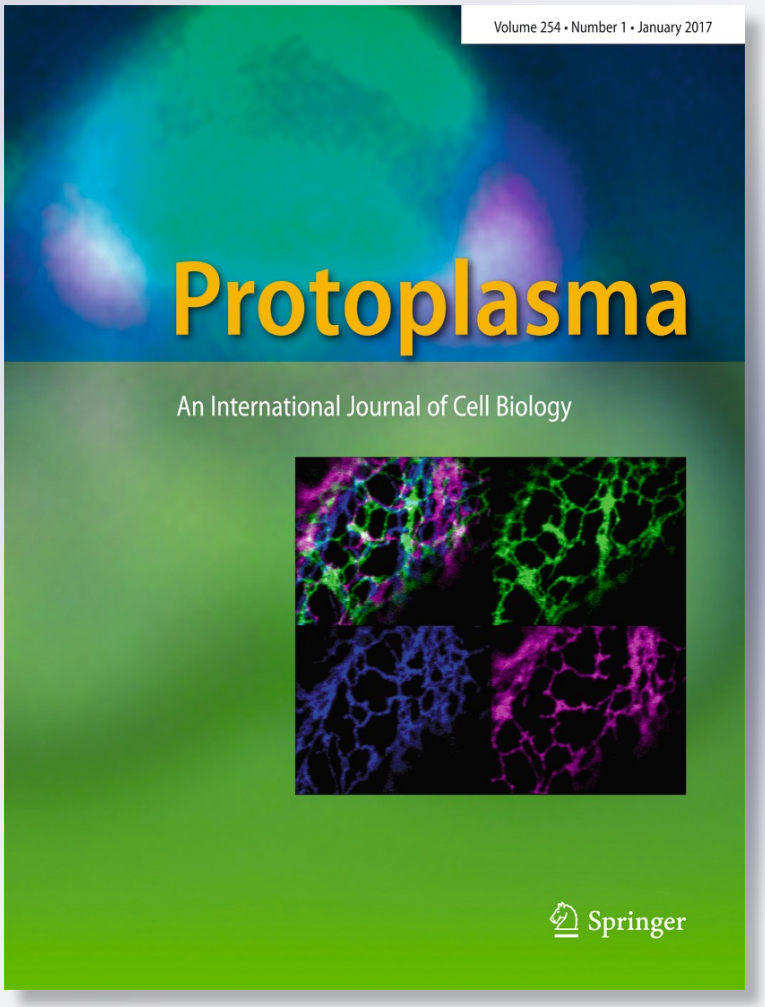

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REVIEW ARTICLE

It is a matter of timing: asynchrony during pollen development and its consequences on pollen performance in angiosperms—a review

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Abstract Functional pollen is needed to successfully complete fertilization. Pollen is formed inside the anthers following a specific sequence of developmental stages, from microsporocyte meiosis to pollen release, that concerns microsporocytes/microspores and anther wall tissues. The processes involved may not be synchronous within a flower, an anther, and even a microsporangium. Asynchrony has been barely analyzed, and its biological consequences have not been yet assessed. In this review, different processes of pollen development and lifetime, stressing on the possible consequences of their differential timing on pollen performance, are summarized. Development is usually synchronized until microsporocyte meiosis I (occasionally until meiosis II). Afterwards, a period of mostly asynchronous events extends up to anther opening as regards: (1) meiosis II (sometimes); (2) microspore vacuolization and later reduction of vacuoles; (3) amylogenesis, amylolysis, and carbohydrate interconversion; (4) the first haploid mitosis; and (5) intine formation. Asynchrony would promote metabolic differences among developing microspores and therefore physiologically heterogeneous pollen grains within a single microsporangium. Asynchrony would increase the effect of competition for resources during development and pollen tube growth and also for water during (re)hydration on the stigma. The differences generated by developmental asynchronies may have an adaptive role since more efficient pollen grains would be selected with regard to homeostasis, desiccation tolerance, resilience, speed of (re)hydration, and germination. The performance of each pollen grain which landed onto the stigma will be the result of a series of selective steps determined by its development, physiological state at maturity, and successive environmental constrains.

Keywords Asynchrony · Competition · Development · Homeostasis · Microspores · Pollen

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Introduction

Angiosperm pollen is the male gametophyte responsible to carry the male sperm cells to the female counterpart, the pistil, to achieve fertilization. Functional pollen is needed to successfully complete fertilization. Pollen grains may look like simple structures, but each pollen grain is an independent unit that underwent a precise developmental program and it is involved in a delicate relationship with the sporophytic and the external environments, both during development and presentation/dispersal (Fig. 1). In fact, developmental failure and/or environmental stresses may cause male sterility (Fig. 1).

In angiosperms, pollen grains develop inside the anthers of stamens, in closed cavities called microsporangia, which are delimited by the anther walls (Fig. 2). Anthers are supported by a filament, usually a slender structure of parenchymatic nature that transports water and nutrients to the anther via a vascular bundle connected to the flower and the sporophytic vascular system. The microsporangia are kept together by the connective tissue, where the vascular bundle arriving from the filament ends (Fig. 2). Pollen development follows a specific sequence, which involves both the anther wall tissues and the

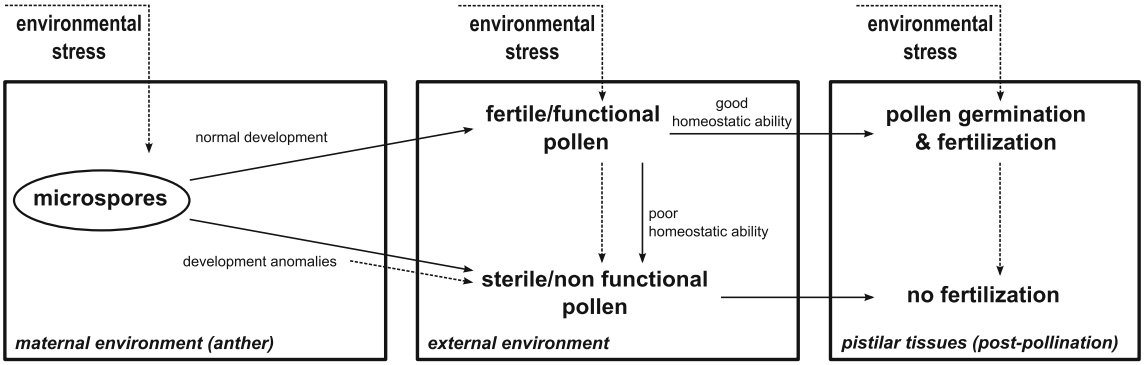

Fig. 1 Scheme summarizing the environmental contexts where microspores/pollen can be found and the final outcome according to different conditions faced. *Full lines* represent the course of normal development; *dashed lines* indicate the impact of environmental stresses, which can be of different types and intensities

microsporocytes/microspores (Sanders et al. 1999; Scott et al. 2004; Fig. 2), and therefore, several developmental stages can be clearly distinguished (e.g., Borg and Twell 2011; Zhang and Yang 2014). Microsporocytes differentiate in the center of the microsporangia; microsporocytes are diploid cells that undergo meiosis to form a tetrad of unicellular haploid microspores enclosed by a callose wall (Fig. 2 (a, b)). Single microspores are separated from the tetrads after callose digestion and differentiate as pollen grains following several definite changes in the cytoplasm, the cell wall, and one or two haploid mitotic divisions (Fig. 2 (b)). The final maturation of microspores involves different degrees of cytoplasm dehydration, before and/or after anther opening (Fig. 2 (b, c)). Therefore, mature pollen can be released/dispersed with different water content (Fig. 2 (c)), in a dormant or in a more or less active state, depending on the species (Franchi et al. 2011). After arrival on the stigma, pollen grains establish a close relationship with the pistilar tissues in order to (re)hydrate, germinate, and form the pollen tubes, and their metabolism is reactivated if dormant (Heslop-Harrison 1987; Van Aelst et al. 1993).

All the processes from microsporocyte meiosis to pollen release may not be synchronous within a flower, an anther, and even a microsporangium (e.g., González et al. 2001; Jacobs and Lersten 1994; Liu et al. 2007; Sunderland and Huang 1987; Taylor et al. 2013; Teng et al. 2005). Likewise, the timing of the events that follow pollen arrival to the stigma is not uniform and depends on the responsiveness of single pollen grains. The occurrence of asynchrony at different stages has been registered in many cases, but it has been infrequently quantified and/or pondered (e.g., Sunderland and

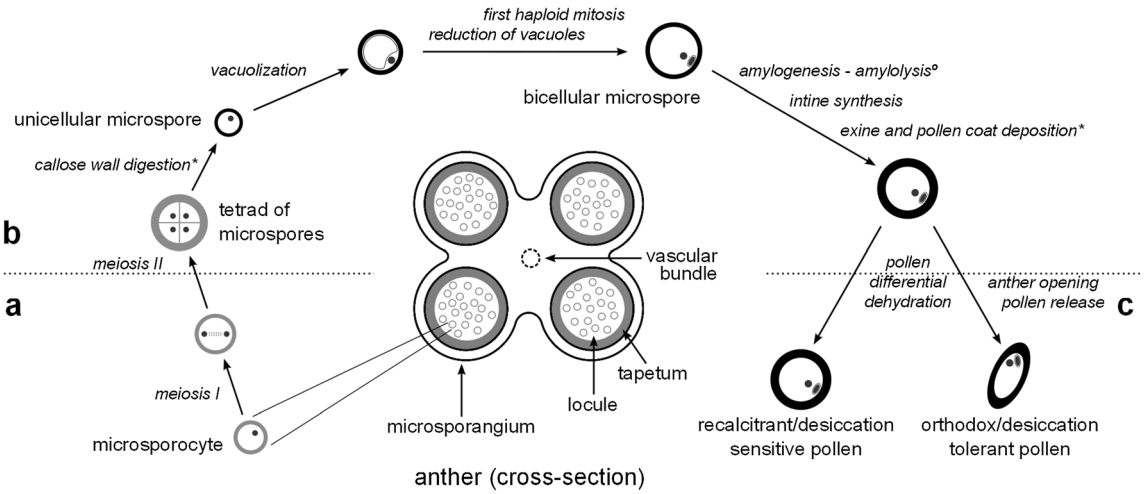

Fig. 2 Schematic anther structure and pollen development (from microsporocyte, clockwise direction). A cross section of a tetrasporangiate anther is represented in the center. *a* Initial synchronous processes until meiosis I. *b* Succession of mainly asynchronous processes until pollen final maturation and anther opening. *c* Anther opening, pollen dehydration (different degrees according to the species), and pollen release (two-celled pollen is represented); pollen dehydration is largely synchronous, but mature pollen can be found in slightly different states of maturation as a consequence of the asynchrony registered along *b*. Processes are referred in *italics*; *dashed horizontal lines* separate periods with differences in the synchrony of the processes. *degree symbol* variable among species; *asterisk* the event depends on the tapetum development and therefore is a synchronous event

Huang 1987) and its biological consequences have not been yet assessed. In this review, we will try to summarize different processes during pollen development and lifetime (until germination), according to the environmental contexts where pollen grains can be found (Fig. 1), stressing on the possible consequences of their differential timing on pollen performance.

Pollen in the sporophytic environment

Timing of events during pollen development

The series of processes from the stage of microsporocyte to that of mature pollen may be synchronous or asynchronous among all the microsporocytes or microspores within a microsporangium. In general, the development is synchronized until microsporocyte meiosis I and sometimes until meiosis II, particularly when meiosis is of simultaneous type (e.g., *Cymbopetalum bailloni* R.E.Fr. (Tsou and Fu 2007)). Synchronous cell divisions would be under genetic control (Magnard et al. 2001; Wang et al. 2004; Li et al. 2015), and it has been suggested that the presence of cytoplasmic connections between microsporocytes would facilitate synchrony of meiotic events within a microsporangium (Heslop-Harrison 1966; Mamun et al. 2005b; Whelan et al. 1974). Nevertheless, meiosis II can be asynchronous, when meiosis is either of successive type (e.g., Ekici 2014; Teng et al. 2005) or of simultaneous type (e.g., González et al. 2001; Liu et al. 2007; Pacini and Juniper 1984; Vesselina and Mateu-Andrés 2010). In some cases, asynchrony has been considered a meiotic instability related to the formation of sterile pollen (e.g., *Vicia rigidula* Royle (Kaur and Singhal 2010)) or diploid gametes (Bieling et al. 2003; Ghorbani et al. 2015). Besides, asynchrony is common in intraspecific hybrids and related to meiotic anomalies (e.g., *Eucalyptus* spp. (Yang and Kang 2015)). After the meiotic division of microsporocytes, there is a period of mostly asynchronous events that extends up to anther opening (Fig. 2 (b)), although there are exceptions (e.g., some Orchidaceae spp., due to the persistence of cytomictic channels (Pacini 2009a), or some Winteraceae and Monimiaceae spp. having tetrads as pollen dispersal units (Sampson 1977, 1981)). The asynchronous events involve, apart from meiosis II (Fig. 2 (b)), the following: (1) microspore vacuolization (e.g., *Hordeum vulgare* L. (Shim et al. 2009)), followed by new cytoplasm formation and later reduction of vacuoles (Fig. 3a; although it may also be very synchronous, as in *Arabidopsis thaliana* (L.) Heynh. (Zhang et al. 2002)); (2) amylogenesis (Clément and Pacini 2001; Fig. 3b), eventually amylolysis (e.g., "more or less synchronous" in *Tradescantia paludosa* E.S. Anderson & Woodson (Maruyama 1968); Fig. 3c, e), carbohydrate inter-conversion, and storage; (3) the first haploid mitosis to form the generative cell that remains enclosed by the vegetative cell (Fig. 3d) and the change of shape and migration of the generative cell (Fig. 3e; e.g., Kant et al. 2013; Sampson 1981); and (4) intine formation, which is under microspore control (Borg and Twell 2011) and depends on microspore development because it may be synthesized before (e.g., Jung et al. 2006; Owen and Makaroff 1995; Regan and Moffatt 1990) or after the first haploid mitosis (e.g., Mirgorodskaya et al. 2015; Sharma et al. 2015; Ubera Jiménez et al. 2006; Vinckier et al. 2012). By contrast, during the same period, digestion of the callose wall that encloses the tetrad of microspores and exine and pollen coat deposition are synchronous processes among all microspores (Fig. 2 (b)). This is because callase, which digests the callose walls (Scott et al. 2004, and references therein), and (part of the) materials for exine and pollen coat are provided by tapetal cells (Blackmore et al. 2007; Goldberg et al. 1993), whose development and degeneration are synchronous across a microsporangium.

The male gametes will be formed after a mitotic division of the generative cell, but the timing of this division is species specific, so pollen grains can be dispersed as two celled (vegetative cell enclosing the generative cell, with mitosis occurring after pollination inside the pollen tube) or three celled (vegetative cell enclosing the two gametes, formed by dispersal). A very particular case of asynchronous development has been registered in *Annona cherimola* Mill., which has bicellular and tricellular pollen at the time of anther opening. Thus, even the second haploid mitotic division is asynchronous in this species, if that division occurs before pollen dispersal (Lora et al. 2009).

Altogether, there may be some differences in the metabolic state of single pollen grains by the time of anther opening as a consequence of the eventual asynchronies during their development. Actually, particular cases of pollen dimorphism have been registered as the outcome of drastic desynchronization at different stages of microspore development (e.g., Sunderland and Huang 1987). On this regard, it has been reported time after time that environmental stress (such as extreme temperatures or drought) may cause developmental failures and partial or total pollen impairment (Fig. 1; e.g., Koonjul et al. 2005; Parish et al. 2012; Saini 1997; Song et al. 2015). Asynchronies may be favored during development under environmental stress (e.g., meiotic abnormalities in *Ulex* spp. (Misset 1992) or delayed amylogenesis in rice (Han et al. 2006)), which may result in the formation of variable percentages of fertile and sterile pollen grains, as an extreme case of differences among mature pollen grains.

Flow of fluids and nutrients during pollen development

Pollen behaves as a sink during its development in the anther and depends on an external supply of nutrients (Schwacke et al. 1999) that is under control of the tapetum, a special

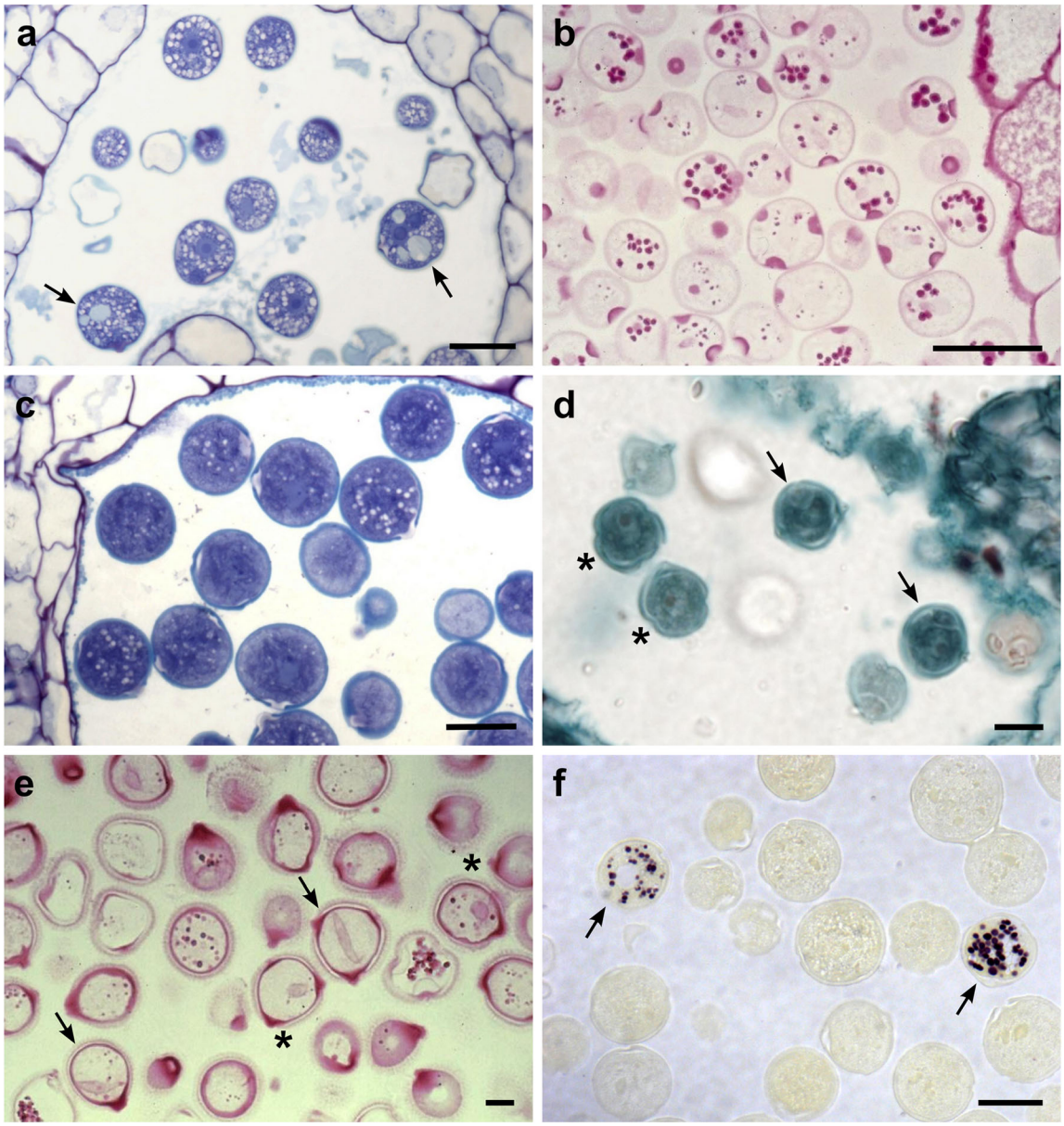

Fig. 3 Asynchronies during pollen development. **a** Reduction of vacuoles; observe a few microspores still containing vacuoles (*arrows*) while amylogenesis has occurred (starch not colored). **b** Amylogenesis; note the different contents of amyloplasts (*strongly colored*) among microspores. **c** Amylolysis; observe the different numbers of amyloplasts among microspores, already absent in some of them (starch not colored). **d** First haploid mitosis; observe some microspores at the unicellular stage (*asterisks*) and others at the bicellular stage (*arrows*). **e** Differentiation of the generative cell and differential amylolysis; note the asynchronic migration and change to fusiform shape of the generative cell (*arrows*), still rounded in some microspores (*asterisks*), and the different amyloplast (*strongly colored*) contents among microspores. **f** Different starch (*strongly colored*) contents among mature pollen grains. **a**, **c**, **f** *Solanum lycopersicum* L.; **b** *Parietaria judaica*; **d** *Solanum neorickii* D.M. Spooner, G.J. Anderson & R.K. Jansen; **e** *Olea europaea* L. **a**, **c** Toluidine blue staining; **b**, **e** Periodic acid-Schiff reaction; **d** Fast green and hematoxylin staining; **f** Lugol's iodine reaction. *Scale bars* = 25 μm (**a**–**c**, **e**, **f**) and 10 μm (**d**)

antheral tissue (Fig. 2). Pollen nutrition during development is supported by the saps that arrive to the anther provided by the sporophyte through the vascular bundle (Fig. 4). The xylem sap moves towards the anther and its microsporangia following the symplast and apoplast through the connective tissue and the anther walls (Fig. 4). The phloem unloading pathway is also active, in which nutrients move from phloem cells to sink cells via plasmodesmata (Imlau et al. 1999; Zhang et al. 2010) or through specific symporters (Stadler and Sauer 1996). Nutritive substances derived from the photosynthetic activity of the anther wall cells, if they contain chloroplasts, may also be provided to the developing microspores (Clément and Pacini 2001). The presence of plasmodesmata facilitates the movement of substances and organelles between cells (e.g., Wang et al. 2002), promoting the coordinated functioning of the tissues, which is particularly observed in the

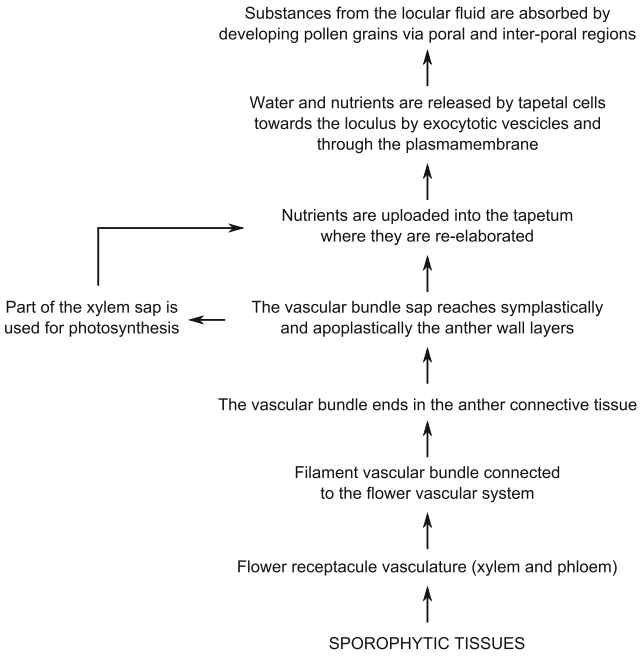

Fig. 4 Schematic pathway followed by fluids and nutrients from the sporophyte towards developing pollen grains

tapetum (Rowley 1993). However, plasmodesmata are absent between microspores and tapetum (e.g., rice (Mamun et al. 2005a), tomato (Schwacke et al. 1999)), although they may be present before meiosis (e.g., *Capsicum annuum* L. (Horner and Rogers 1974)).

The tapetum is a transitory, apoptotic tissue of secretory nature. At least two types of tapeta are recognized according to the cellular structure and the relative relationship with microspores, namely secretory and amoeboid, but their functions are essentially the same (Pacini 1997). There is a third type of tapetum sometimes recognized, called invasive nonsyncytial (Tiwari and Gunning 1986), which may be intermediate between the other two types (Furness 2008). The better known and most common is the secretory tapetum, which encircles the locule, the space formed in the center of the microsporangium and filled with a locular fluid secreted by the tapetum itself (Furness 2008; Pacini 2009b). The microspores are immersed in the locular fluid (Fig. 2) that would work as the connecting medium between the tapetum and the microspores. A functional tapetum is critical for microspores' development; tapetum ablation results in male sterility (Goldberg et al. 1993; Mariani et al. 1990), while a number of male sterile mutants have defects in the tapetum (e.g., Kawanabe et al. 2006; Parish and Li 2010; Sanders et al. 1999).

The locular fluid is released by the tapetal cells via plasma membrane or by vesicles (Clément et al. 1998, and references therein; Owen and Makaroff 1995). The locular fluid may be already detected at the microsporocyte stage (Clément et al. 1998), and it is present at least until the early bicellular microspore stage (Quilichini et al. 2014), i.e., the onset of anther and pollen dehydration in many species. Substances of different natures are secreted by the tapetum into the locule (e.g., pectin (Aouali et al. 2001; Clément et al. 1998), proteins (Huang et al. 2013; Papini et al. 1999), lipidic bodies (Dickinson and Lewis 1973; Hsieh and Huang 2007; Parish and Li 2010; Owen and Makaroff 1995; Paul et al. 1992; Staiger et al. 1994; Wang et al. 2003; Wu et al. 1997)). Soluble carbohydrates are the main substances supplied by the sporophyte through the tapetum to nourish developing microspores (Engelke et al. 2010), which can be detected in the locular fluid even at late stages (Carrizo García et al. 2015). Owing to the demand of different types of substances during microspore development, the composition of the locular fluid varies according to the developmental stage of the microspores (e.g., Castro and Clément 2007; Clément et al. 1998, and references therein; Dunwell and Thurling 1985; Pressman et al. 2012; Quilichini et al. 2014). The tapetum, at least of the secretory type, degenerates by programmed cell death (PCD), beginning by the time when microspores become vacuolated; the process is generally completed around the first microspore haploid mitosis (Sanders et al. 1999). PCD can also involve other anther tissues, such as part of the anther wall and the connective tissue near the locules, whose digested contents would provide additional materials to be used by the microspores (Varnier et al. 2005; Wetzel and Jensen 1992).

Microspores would gradually uptake the substances provided by the tapetum, according to their needs (e.g., soluble carbohydrates to store starch, sporopollenin precursors when exine is formed). Therefore, microspores would compete for the resources within a microsporangium. The eventual metabolic differences created among microspores as a consequence of developmental asynchronies may influence their competitiveness, i.e., some microspores would be at a more advanced stage than others, possibly taking the resources first. As a result, asynchrony of physiological states may be ultimately emphasized. The extent of competition for nutrients during microspore development would also depend on other variables, such as the position of microspores in relation to the tapetal cells, the type of pollen dispersal unit, and the abundance of the locular fluid (Pacini 2010). Indeed, there can be different strategies to reduce competition, which in turn reduce or prevent asynchrony. For instance, the competition for nutrients would be low in the case of Poaceae and allied monocot species where, in a cross section of the anther, microspores are arranged in a single row around the locule, usually with the single pore of each one facing the tapetum (e.g., *H. vulgare* (Charzyńska and Lenart 1989), *Sorghum bicolor* (L.) Moench (Christensen and Horner 1974), and several other species (Kirpes et al. 1996)). By contrast, competition would be higher when the locule contains many dispersed microspores immersed in an abundant locular fluid, although they

can move because of the temporary pulsation of tapetal cells. Pollen movements, in order to facilitate uniform nutrition, are probably faster during early microspore stage and decrease with tapetum degeneration (Pacini 1990). Microspore competition for nutrients may also be low in some cases of compound pollen because of anatomical reasons. For example, in *Acacia* species, each microspore is in contact with the tapetum during development and the locular space is absent (e.g., Kenrick and Knox 1979; Pacini 2010), or some Orchidaceae species in which cytomictic channels may persist until the late microspore stage and then even the first haploid mitosis may be synchronous throughout a microsporangium (Pacini 2009a, Figs. 5–11). However, in massulate species, microspore development may be synchronized within a massula but not necessarily between the massulae formed within each microsporangium (e.g., *Peristylus spiranthes* (Schauer) S.Y. Hu (Zee and Siu 1990)), and therefore, competition may be established between them. Competition would be also low in species with periplasmodial and invasive tapeta, because the tapetal cells occupy the spaces between microspores (Furness 2008; Pacini and Keijzer 1989; Tsou and Fu 2007), ensuring a tight relationship with each one.

The degeneration of the tapetum, independent of the type, marks the starting of the maturation phase of pollen grains. When pollen is mature, there is a general decrease of the water content in different parts of the anther (Nelson et al. 2012). In order to allow the presentation and dispersal of pollen grains, the locular fluid disappears by evaporation through the anther epidermis and/or by resorption towards the stamen filament or other floral parts (Ge et al. 2001; Keijzer 1987; Pacini and Hesse 2004; see below). The subject has been scarcely analyzed, but recently, it has been shown in two Poaceae species (maize and long stamen rice) that the locule dehydrates at an early stage (Tsou et al. 2015), earlier than usually suggested. By then, vacuolization in the microspores is approaching its maximum, and therefore, the authors proposed that the free fluid could be taken up by the microspores themselves (Tsou et al. 2015). On this regard, rapid swelling of pollen grains right before anther opening was registered in rice and barley (Matsui et al. 1999, 2000), which may be in line with the phenomenon suggested by Tsou et al. (2015), although it is a later stage. In the case of Poaceae species, pollen grains are dispersed with high water content (e.g., maize (Kerhoas et al. 1987), rice (Das et al. 2014), and others (Franchi et al. 2011)); thus, the way of water relocation may be different for species with pollen grains dispersed with low water content.

The advanced processes regulated by sporophytic tissues, i.e., tapetum PCD and changes of water content in the anther, are homogeneous across a microsporangium, whereas the microspores may be at different physiological states by then (some more advanced than others). As a result, physiological differences between microspores may be reinforced under the influence of sporophytic events during the final steps of maturation. This is because each microspore would react in a different way according to its particular physiological state (e.g., degree or speed of dehydration depending on the concentration of cytoplasmic osmoregulatory molecules).

Carbohydrate metabolism during microsporogenesis

Among the main features of microspore development are the gradual processes of amylogenesis and amylolysis, which are asynchronous within a microsporangium (Figs. 2 (b) and 3b, c, e, f). The most common trend is a single event of amylogenesis, although one (e.g., Carrizo García 2007; Clément et al. 1994; Pacini et al. 1992; Polowick and Sawhney 1993; Yeung et al. 2011) or two (e.g., Maruyama 1968; Pacini and Franchi 1988; Pacini and Viegi 1995; Santos de Oliveira et al. 2015) cycles of amylogenesis and amylolysis may occur, according to the species. When two cycles of amylogenesis occur, the first one usually takes place in an early microspore stage, before intine formation, and the second one after the first haploid mitosis, being both asynchronous processes (Pacini and Franchi 1988; Pacini and Viegi 1995). It is worth mentioning that there may be another cycle of amylogenesis/amylolysis before microspore formation, either in the pollen mother cells (Lora et al. 2009) or in the microsporocytes during meiosis (Maruyama 1968). The asynchronic nature of these processes could be related to the metabolic differences observed among microspores after meiosis and, as regards amylogenesis, probably to their different capacities to compete for the nutrients provided by the tapetum. The synthesis of starch would depend on the availability of cytoplasmic sucrose and hexoses that relies on the ability of each microspore to uptake and/or metabolize these substances. These processes are under genetic control, involving the expression of specific genes (e.g., *AtSTP6* monosaccharide symporters (Scholz-Starke et al. 2003) and *PmSUC1* sucrose transporter (Lauterbach et al. 2007)).

As regards amylolysis, it may be total or partial towards maturity, which determines the pollen final starch content. Therefore, mature pollen may be starchy or starchless (Baker and Baker 1979). For instance, in several tomato varieties, it has been recorded that the starch stored during pollen development is almost completely hydrolyzed before anthesis; thus, mature pollen has a negligible amount (Fig. 3f; Carrizo García et al. 2010; Polowick and Sawhney 1993; Pressman et al. 2002). By contrast, mature pollen grains of rice, sorghum, and maize are filled with starch, while the failure of starch biosynthesis has been observed in male sterile lines of these species (e.g., Datta et al. 2001, 2002; Jain et al. 2007; Kong et al. 2007). Starch presence was first related to the pollination mechanism (Baker and Baker 1979), but it was suggested later that the variation in starch content could be better explained by the relation between sugars and desiccation and other functional features (Franchi et al. 1996; Roulston and Buchmann

2000). In general, pollen starch content was usually considered uniform within a species, but variations in this trait have also been recorded in fertile plants (e.g., *Capsicum pubescens* Ruiz & Pav. (Bo and Carrizo García 2015), *Parietaria judaica* L. (Franchi et al. 1984), and several Commelinoid monocots (Zona 2001)). Since amylogenesis and amylolysis are not strictly synchronized among microspores within a microsporangium, there may be some differences in the starch and oligosaccharide content of each one at maturity (Fig. 3f; e.g., Bo and Carrizo García 2015; Franchi et al. 1984). Indeed, the presence of starchy and starchless grains in the same anther was observed in 76 out of 901 species studied (Franchi et al. 1996). Because of the eventual differences in the content of carbohydrate of mature pollen, dissimilar physiological reactions of single pollen grains may be presumed with reference to their ability to control desiccation in the atmosphere, to (re)hydrate and to germinate (see below), processes in which carbohydrates are involved. Indeed, the storage of sucrose during the final stage of pollen maturation is important because pollen becomes desiccation tolerant, probably due to the formation of a glassy state, with the stabilizing participation of sucrose (Firon et al. 2012; Hoekstra et al. 2001).

The progress of amylogenesis/amylolysis and their timing also affects other processes not related to the natural life of microspores or pollen such as induced androgenesis. The presence or absence of amyloplasts during specific microspore stages would indicate if a species is androgenetic or not as well as the favorable period to induce androgenesis (Sangwan and Sangwan-Norreel 1987). For instance, in *Nicotiana*, as soon as starch accumulated in the microspores, in vitro androgenesis was not followed (Nitsch and Nitsch 1970). Different embryogenic capacities as well as different sporophytic pathways among microspores could be expected due to the asynchronous microspore development (Croser et al. 2011; Hu and Kasha 1999; Liu et al. 2002). Indeed, different pre-treatments have been applied to favor synchronization of microspore embryogenesis in order to have a higher yield of haploid embryos (e.g., Hu and Kasha 1999; Pechan et al. 1991).

Pollen in the external environment (presentation/dispersal)

Anther opening and pollen release

Anther opening, the process of anther wall rupture at the stomium to allow pollen release (Sanders et al. 1999) is the result of several steps (septum rupture and shrinkage, stomium breakage, stomium opening, and separation and outward bending of anther walls) that involve a precise combination of cell lysis processes and mechanical pressure (Carrizo García et al. 2006; Wilson et al. 2011). A key structure for anther opening and wall outward bending is the endothecium of the anther wall, which is formed by one or more layers of apoptotic cells having lignified wall thickening of different shapes (Manning 1996). A biomechanical model for anther opening has been recently proposed, in which the anther wall dehydration is pointed out as the driving force while the endothecium has a key role in the process (Nelson et al. 2012), as previously suggested (Wilson et al. 2011). On that regard, endothecium impairment can result in failure of anther opening, even though the stomium can open normally (Dawson et al. 1999; Mitsuda et al. 2005; Thangasamy et al. 2011; Yang et al. 2007). Anther opening has always been considered a process that involves tissue desiccation (either resorption or evaporation (Bonner and Dickinson 1989; Keijzer 1987)), although water internal regulation has been poorly studied (but see Bots et al. (2005) and Stadler et al. (1999)). As a general rule, it is agreed that high relative humidity (RH) delays or inhibits anther opening while low RH accelerates the process (Bianchini and Pacini 1996; Carrizo García et al. 2006; Franchi et al. 2007; Keijzer 1987; Linskens and Cresti 1988; Lisci et al. 1994; Yates and Sparks 1993). However, in *Allium triquetrum* L., the anthers would not open until a particular stage is reached despite the environmental conditions (Carrizo García et al. 2006). Actually, signaling of different hormones would be important to regulate the timing of anther opening and pollen final maturation (Cecchetti et al. 2004; Peng et al. 2013; Rieu et al. 2003; Sanders et al. 2000; Scott et al. 2004; Shih et al. 2014). Anther opening is under sporophytic control, and therefore, it is closely timed.

Desiccation is uniform in an anther, and therefore, all the pollen grains developed within each microsporangium will undergo the final dehydration at the same time, even though they could be at different developmental stages. While the anther opens, pollen grains get in contact with the external environment (which is very different from the environment where they have developed) and they are eventually released to achieve pollination. Pollen is particularly vulnerable at this stage, and its relationship with the environment is critical. Pollen is usually dispersed in a dormant state (Footitt and Cohn 2001; Nelson et al. 2012), that is with low water content and reduced metabolic activity. The water content of non-reproductive cells with an active metabolism is generally higher than 50 %, while that of mature pollen grains at presentation is generally lower than 30 % (e.g., *C. annuum* (Carrizo García et al. 2013) and *Juglans* spp. (Luza and Polito 1987)), with well-defined exceptions with higher water content (e.g., several Poaceae and Cucurbitaceae species (Carrizo García et al. 2015; Das et al. 2014; Franchi et al.

2011; Kerhoas et al. 1987)). Although the final maturation of pollen involves different degrees of cytoplasm dehydration, before and/or during presentation after anther opening (Fig. 2; Lisci et al. 1994), at that specific moment, there is usually a hydric shock on pollen. That hydric shock triggers water content fluctuations as well as metabolic reactions to protect pollen grains from sudden changes of water content that generally continue during pollen presentation/dispersal. Pollen must be programmed to mitigate against the more dangerous effects occurring during presentation and dispersal, i.e., it needs a sort of homeostatic ability to maintain viability notwithstanding the physical variations of the environment (Fig. 1; Firon et al. 2012). Several molecules can help to protect cellular integrity during the changes of water content experienced by pollen grains (e.g., sucrose (Hoekstra et al. 2001), proline, and LEA proteins (Firon et al. 2012), and references therein). The inter-conversion of carbohydrates in the last moments of pollen maturation (i.e., starch and/or sucrose storage) may be critical for pollen survival in the environment until pollination is completed. Because single mature pollen grains can show differences in their carbohydrate content and metabolic state by anther opening, as mentioned before, the environmental conditions faced when they are released and eventually dispersed may increase the biological diversity of the pollen population shed by a flower. The features that may be affected are viability-longevity, vigor, and water content that, in the end, may influence pollen germination on the stigma. For instance, pollen viability is gradually reduced over time (e.g., *C. pubescens* (Bo and Carrizo García 2015), *Helleborus* spp. (Vesprini and Pacini 2005), and *Papaver* spp. (Azimi-Motem et al. 2008)) which means that some pollen grains die faster than others. Likewise, when pollen is subjected to an environmental stress (e.g., low relative humidity or high temperature), including controlled storage conditions, pollen viability, and/or germinability may be lost faster but still gradual, meaning that not all pollen grains die at once because some of them would be more resistant (e.g., *Lagerstroemia* spp. (Masum Akond et al. 2012), *Panicum virgatum* L. (Ge et al. 2011), *Trachycarpus fortunei* (Hook.) H. Wendl. (Guarnieri et al. 2006), *Typha latifolia* L. (Hong et al. 1999), and several other species (Bassani et al. 1994; Nepi et al. 2010)).

Mature pollen during presentation/dispersal

Pollen becomes available for pollination after anther opening, but there is a variable period, as regards its length and conditions, until pollination is fulfilled. Pollen is usually presented to dispersing agents (either biotic or abiotic), although presentation is sometimes omitted in some groups of plants (e.g., pollen launched by different mechanisms (Franchi et al. 2007) or in cleistogamous flowers (Márquez-Guzmán et al. 1993)). In general, the longer the period pollen grains are exposed to the environment, the greater the chances it will be damaged by its negative effects (e.g., Hong et al. 1999). Indeed, the environmental conditions during dispersal are a major constraint for pollen survival since pollen is not longer protected by the anther tissues. Therefore, pollen survival and performance largely depend on its homeostatic ability to buffer the environmental effects on the cellular functioning (Fig. 1), which is closely related to the physiological state of mature pollen grains. However, pollen can be protected by the floral structure in some cases, at least for some time. For instance, although pollen presentation is usually continuous, in a few cases, it can be interrupted temporarily by anther closure determined by hostile environmental conditions, such as rain or high RH (e.g., *Laurus nobilis* L. (Pacini et al. 2014) and *Lilium philadelphicum* L. (Edwards and Jordan 1992)). Besides, it was registered that even though pollen longevity was greatly reduced by rain wetting, pollen response to rainy conditions was related to the existence of protective floral structures (Mao and Huang 2009), while the corolla closure or the flower position can protect pollen from rainwash, solar radiation, and/or changing temperatures (e.g., He et al. 2006; Huang et al. 2002; Wang et al. 2010; Franchi et al. 2014 and references therein). The methods of pollen dispersal are variable among species and may influence the performance of the pollen grains that land on the stigmatic surface (see Appendix 1 for further details).

If there is a period of presentation and dispersal, it can be different according to the plant life form (i.e., herbaceous [perennials or annuals] or woody) and the spatial arrangement (e.g., high or low density, distance among individuals). Therefore, it may be hypothesized that pollen grains will have different features as regards survival/homeostatic abilities, such as desiccation tolerance and rehydration capacity, related to (1) the plant life form, (2) the spatial arrangement of plants, (3) the pollination syndrome, (4) the sexual expression and mating system, and (5) the environmental conditions in which the plants bloom. Because pollen needs to survive in the atmosphere for a variable period of time until it reaches the pistilar tissues, another critical feature is pollen tolerance to desiccation through time, after the initial distress occurred at anther opening. Pollen longevity, as the maintenance of viability through time, has been related to the contents of water and carbohydrates. For instance, low water content and high levels of sucrose and total insoluble cytoplasmic polysaccharides would preserve pollen viability in *T. fortunei* (Guarnieri et al. 2006). On this regard, at least two types of pollen grains can be recognized according to their mean lifespan and desiccation tolerance, namely orthodox/desiccation tolerant and recalcitrant/desiccation sensitive, in analogy with seeds

(Franchi et al. 2011). Orthodox/desiccationtolerant pollen grains are found more frequently. Orthodox pollen grains usually equilibrate with the environment at presentation and during dispersal owing to the low water content and the predominance of soluble carbohydrates over starch. By contrast, recalcitrant pollen grains have high water content and low quantities of soluble carbohydrates. Recalcitrant pollen grains lose water passively and quickly until they die when water content is reduced to a certain threshold (Carrizo García et al. 2015; Nepi et al. 2010). Species with recalcitrant pollen (i.e., sensitive to desiccation) would need fast pollination to avoid the quick loss of pollen viability and/or germination capacity, while pollination may take longer in species with orthodox pollen (Nepi et al. 2010). In fact, some herbaceous plants living close one to the other have recalcitrant pollen grains. The flight of pollen between flowers or individuals is short and safe (e.g., *P. judaica*, *Spinacia oleracea* L., Poaceae species (Franchi et al. 2011)) and then pollen would survive until pollination is completed despite being short living and desiccation sensitive. Because of the relevant role of some carbohydrates and water content in relation to pollen survival in the atmosphere, the developmental asynchronies brought to light acquire another dimension as regards pollen viability-longevity, by promoting possible differential resistance. An interesting ecological concept that may be considered and explored for pollen grains is resilience, as the capacity to absorb disturbance and reorganize while undergoing change so as to still retain essentially the same function and structure (after Walker et al. 2004). The homeostatic ability of pollen grains may be closely related to its possible resilience, considering that pollen has to survive in the environment until pollination is completed. Resilience and homeostatic ability would be particularly important as regards pollination in extreme environmental conditions (e.g., Steinacher and Wagner 2013), for pollen storage, and in front of environmental disturbances such as habitat fragmentation and climatic change (e.g., Coast et al. 2016).

Pollen on the pistilar tissues

Pollen (re)hydration and germination

Under natural circumstances, pollen grains land on the stigmatic surface of the pistil after they have survived exposed to the external environment and then they have to be able to (re)hydrate and germinate. Pollen (re)hydration on the stigma seems to be a regulated process of several steps (Hiroi et al. 2013). It is worth mentioning that germination may be prevented in some cases of incompatibility between the pollen and the female tissues, even though pollen hydration may have occurred (e.g., *Brassica* spp. (Elleman and Dickinson 1990; Hiroi et al. 2013; Zuberi and Dickinson 1985)).

The degree of pollen hydration upon landing on the stigma depends on pollen water content. At least at first, water uptake is mainly a passive process, thus ion fluxes would be involved in water entrance to the pollen cytoplasm (Hepler et al. 2006; Pertl et al. 2010). In addition, the concentration of osmoticant molecules inside the pollen, such as sucrose and single hexoses, is also important. Recent studies have shown that the water-absorbing capacity of pollenkitt may be involved in pollen adhesion to the stigma (Lin et al. 2015). Besides, it was inferred that oils facilitate the diffusion of water from the pistil to the pollen grain (Wolters-Arts et al. 2002). Therefore, it can be hypothesized that pollenkitt may also participate in pollen (re)hydration, acting as a means of facilitating water mobilization.

The integrity of pollen membranes is critical to achieve proper (re)hydration and avoid imbibitional damage, i.e., pollen bursting. Sucrose is a protective molecule that may help to reduce the effects of rehydration, in interaction with the membrane (Hoekstra et al. 1989). Once pollen has been (re)hydrated and the metabolism has been reactivated, in the case of dormant pollen grains, it may germinate and form the pollen tube. Pollen carbohydrate and water contents may play a selective role in male competition upon arrival to the stigma in relation to pollen (re)hydration and germination. That is to say, notwithstanding the uniform conditions of the stigma, the possible differences in mature pollen physiological state and carbohydrate reserves may trigger different responses during (re)hydration and germination of individual pollen grains. Pollen grains on the stigma would compete for water, which may rely on their hydric state. However, competition is also influenced by chance because it may depend on the number of pollen grains and the relative position of each one (particularly of the aperture region) on the stigmatic surface. Pollen grains adhering to the stigma with an aperture close to it have a quicker rehydration (Heslop-Harrison 1987) since pollen tubes emerge from the aperture closest to the aqueous phase of the stigma (Lush et al. 2000). Pollen germination involves a series of specific processes, from the polarization of the vegetative cell to the mobilization of reserves and the synthesis of new cell walls, which are regulated by pollen grains and may progress at different rates among them.

Nourishment during pollen germination

Pollen tubes are nourished by the female tissues on their way to the ovules, and the main nutrient for the developing pollen tubes is sucrose (Nakamura et al. 1980). Actually, moderate heat stress altered

carbohydrate balance in the pistil and would be the reason for a slower rate of pollen tube growth in vivo in cotton under such conditions (Snider et al. 2011). However, two phases of pollen tube growth can be distinguished according to the source of nutrients used, namely an initial autotrophic period, when pollen tubes use reserves stored in pollen grains (Carrizo García et al. 2012; Shivanna 2003; Stephenson et al. 2003), and a later heterotrophic phase, when they take nutrients from the surrounding environment (either the pistilar tissues in vivo or a culture medium in vitro (e.g., Labarca and Loewus 1972, 1973; Nakamura et al. 1980; Schlüpmann et al. 1994; Ylstra et al. 1998)). In line with this proposed boundary, pollen germination may be considered essentially an autotrophic process, mostly sustained by pollen reserves, and therefore, the type of reserves, either starch or soluble sucrose, glucose, and fructose, may regulate its speed. Nevertheless, simultaneous heterotrophism cannot be discarded. The distinction between recalcitrant and orthodox pollen, which differ in water and carbohydrate contents and metabolic activity, should be made. Recalcitrant pollen would germinate faster due to its already active metabolism when it lands on the stigma, although it usually has a low quantity of soluble carbohydrates. By contrast, orthodox pollen needs to rehydrate first in order to reactivate its metabolism (Franchi et al. 2011). Therefore, the type of carbohydrate reserves could be more influential on germination in the case of orthodox pollen. For instance, the dominance of glucose and fructose (the molecules needed for energy and cell growth) and the lack of sucrose were related to the fast pollen germination in a tomato cultivar (Carrizo García et al. 2012).

In several cases, it has been observed that the transcripts of specific genes are accumulated during pollen development and only translated at advanced stages or upon germination (e.g., those related to carbohydrate metabolism (Hirose et al. 2010; Schneidereit et al. 2003; Sivitz et al. 2008; Stadler et al. 1999; Truernit et al. 1999)). This fact could evidence an opportunistic disposition of pollen, meaning that it could be ready to germinate and develop the pollen tube as soon as the conditions are favorable. On this regard, in some cases, pollen grains temporarily store starch at the beginning of pollen tube formation (Bellani et al. 1985; Carrizo García et al. 2015; Dickinson 1968; Singh et al. 1978). That phenomenon may be regarded as a strategy to accumulate nutrients right away as they become available, as a stockpile to be eventually used later for the growing pollen tubes. Could starch also work as a reserve in case of exogenous nutrient depletion? These features may also represent a selective advantage to favor fast germination and initial pollen tube growth and to guarantee the success of fertilization.

When pollen grains arrive to the stigma, they have gone through different challenging conditions that could deepen the physiological diversity among them, originated by the developmental asynchronies. Therefore, germination of each pollen grain may be triggered at different speeds, beginning an uneven race among the pollen tubes formed. The asynchronism of pollen germination is clearly evidenced when pollen is cultured in vitro (e.g., tomato (Carrizo García et al. 2012; Karapanos et al. 2010), *Pyrus communis* L. (Tiwari and Polito 1988)). Afterwards, pollen tubes will compete in the stylar transmitting tissue to reach the ovary and fertilize the ovules, which is also correlated with physical constraints involving the stylar transmitting tissue and with the availability of nutrients along the style (Hormaza and Herrero 1996).

Conclusions

Pollen grains formed within a single microsporangium may be physiologically heterogeneous as a consequence of developmental asynchronies, and then a high proportion of all the pollen released by an anther may be metabolically diverse. Asynchronies during development may be favored by the absence of cell-cell connections between microspores, which is a frequent trait. Asynchrony would increase the effects of competition for resources during development (either nutrients or cell wall materials) and pollen tube growth, as well as for water during (re)hydration on the stigma. Competition would be particularly high under adverse circumstances, probably due to the shortage of resources. The degree of competition between microspores, pollen, and/or pollen tubes may be different according to the species and also between stages in a single species (e.g., Poaceae species in which competition for nutrients is reduced between developing microspores but strong between pollen tubes trying to reach the single ovule found in an ovary. This competition is particularly strong in maize because of the long stigma silk (Heslop-Harrison et al. 1985)). Developmental asynchrony would create differences in the metabolic state among the pollen grains released by an anther. Those differences may promote selection of more efficient pollen grains as regards homeostasis, desiccation tolerance, resilience, speed of (re)hydration, and germination, and therefore, the differences may have an adaptive role. In the end, the performance of each pollen grain landed onto the stigma will be the result of a series of selective steps went through by each one, determined by the progression of its development and its physiological state at maturity and by successive environmental constrains.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Appendix 1. Methods of pollen dispersal and relation with pollen performance

The methods of pollen dispersal, which are related to the array of pollen during its presentation, can be roughly summarized as follows:

A. In cleistogamous flowers, pollen is not exposed (presentation and dispersal are absent); pollen may be directly transferred to the stigma, where it germinates, or it germinates inside the anther and pollen tubes crossing the anther wall to reach the adjacent stigma (Culley and Klooster 2007). Environmental pressure on mature pollen would be low.

B. The flower and the anthers open, and pollen is mature and ready for dispersal by that time.

B.1. Pollen grains are isolated, not surrounded/covered by a pollenkitt or other sticky substances.

B.1.1. Pollen grains are arranged in a single layer per loculus, the filament is often slender: all pollen grains leave the anther as it opens (e.g., some Poaceae (Charzyńska and Lenart 1989; Kirpes et al. 1996) and Cyperaceae). Pollination is usually fast.

B.1.2. Many pollen grains fill completely the loculus: pollen grains form an incoherent mass on the anther wall surface when it opens, and they gradually leave the anther removed mostly by air currents (woody and herbaceous anemophilous species, e.g., *Halophytum ameghinoi* (Speg.) Speg. (Pozner and Cocucci 2006) and *Plantago lanceolata* L. (Timerman et al. 2014)). Pollen is orthodox/desiccation tolerant.

B.1.3. Pollen grains in poricidal anthers remain protected within the anthers and are gradually removed by pollinators (e.g., buzz-pollinated species such as *Solanum* spp. and Ericaceae spp.). Pollen dehydration may be gradual, and pollen is available in doses.

B.2. Pollen grains adhere to the inner surface of the anther walls by means of pollenkitt or other sticky substances.

B.2.1. Pollen grains of big size (100 μm or more, usually recalcitrant/desiccation sensitive) are arranged in a one or two layers: many pollen grains of an anther can be easily removed by flower visitors (e.g., *Cucurbita* spp. and *Malva* spp.); pollination has to be fast.

B.2.2. Pollen grains (usually orthodox/desiccation tolerant) form a spongy mass on the anther surface: pollen grains leave the anther gradually because the repeated pollinators' visits or air currents (e.g., Liliaceae, Rosaceae spp.; wind-pollinated *Ambrosia artemisiifolia* L. (Martin et al. 2009)).

C. Pollen is mature by anther opening, but presentation is absent because pollen is launched by the anthers; pollenkitt is present or absent.

C.1. Insects visiting the flowers determine its aperture and gradual or explosive pollen release because anthers are already open (e.g., some Fabaceae (Galloni et al. 2007; López et al. 1999) and Lamiaceae (Brantjes and De Vos 1981); orthodox/desiccationtolerant pollen).

C.2. Pollen is launched because of the sudden movement of the filament caused by the dry environment (e.g., *Cornus canadensis* L. (Edwards et al. 2015), *Urtica* spp. and *Parietaria* spp. (Franchi et al. 2007), and some Moraceae (Williams and Adam 1993; Taylor et al. 2006); recalcitrant/desiccationsensitive pollen).

C.3. Pollen is launched because of the fast movement of the anther wall (*Ricinus communis* L. (Bianchini and Pacini 1996); orthodox/desiccation-tolerant pollen).

D. In cases of compound pollen, the mass of pollen grains stays within the anther until its removal by animals (Orchidaceae and some Asclepiadaceae); all the pollen grains of a flower can be removed contemporaneously by a single pollinator. Pollen development would be synchronized; pollen competition is established upon pollination. Superficially exposed pollen grains may be more susceptible to the environment pressure.

E. In cypsela inflorescences, with a cluster of flowers and anthers with few pollen grains, locules open inwardly and pollen adheres to the growing style owing to the presence of pollenkitt, and then it is placed for dispersal underneath the not yet receptive stigma (secondary pollen presentation, e.g., species of Asteraceae and Campanulaceae (Vranken et al. 2014)). Mature pollen performance mostly related to its development and physiological state by anther opening.

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