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Embryology of *Macroptilium arenarium* (Leguminosae)

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Abstract. *Macroptilium arenarium* (Bacigalupo) S.I.Drewes & R.A.Palacios produces two floral morphs, aerial chasmogamous flowers and cleistogamous flowers in geophyte racemes. A comparative study of the sporogenesis, gametogenesis and the development of the related sporophytic structures in both floral morphs is reported. The anther is tetrasporangiate, its wall consists of epidermis, endothecium, one or two middle layers and an uninucleate secretory tapetum. The mature endothecium presents fibrilar thickenings that are more developed in cleistogamous flowers. Pollen grains are tricolporate, angulaperturate, and are shed at bicellular stage. The ovule is crassinucelate, bitegmic and anacampylotropous. Megaspore tetrads with linear arrangement have been observed in chasmogamous flowers, whereas only megaspore dyads have been found in cleistogamous flowers. In both floral morphs the chalazal megaspore develops into an embryo sac of Polygonum type. Apomixis is considered as a possible replacement for sexual reproduction in cleistogamous flowers.

Introduction

Macroptilium is represented in the north-west and north-east of Argentina by nine species that have medicinal and forage value (Barbosa Fevereiro 1987; Fernández *et al*. 1988). Bacigalupo (1987) and Drewes (1995, 1997) observed the presence of cleistogamous flowers in some species of this genus. *M. arenarium* produces two types of flowers (aerial chasmogamous and cleistogamous in geophyte racemes; Drewes 2001), whereas *M. fraternum* presents pre-anthesis cleistogamous and pseudocleistogamous flowers, both of them aerial (Drewes and Hoc 2000).

Species with chasmogamous and cleistogamous flowers have been described in at least 54 families of Angiospermae (Maheshwari 1962; Lord 1981). However, few investigations on the embryological development of each floral morph have been performed. Papers published on this subject deal only with the differences between anthers and pollen grains of cleistogamous and chasmogamous flowers in species of Gesneriaceae (Pargney and Dexheimer 1976), Labiatae (Lord 1979), Violaceae (Mayers and Lord 1984), Nyctaginaceae (Veselova 1989) and Lacandoniaceae (Márquez-Guzmán *et al*. 1993). Nothing has been published about the ovule and embryo-sac development, or about its fertilisation.

Embryological studies on the subtribe are rare (Johri *et al*. 1992) and restricted to a few species of *Phaseolus* (Weinstein 1926; Mok *et al*. 1978; Rabakoarihanta *et al*. 1979; Briarty 1980; Johns *et al*. 1992; Abad *et al*. 1995; Faigón Soverna *et al*. 2003), *Vigna* (Ojeaga and Samyaolu 1970; Desphande and Bhasin 1974; Lord and Kohorn 1986; Faigón Soverna *et al*. 2003) and *Macroptilium* (Pritchard and Hutton 1972; Lakshmi *et al*. 1987; Faigón Soverna *et al*. 2003).

The embryology of chasmogamous and cleistogamous flowers of *M. arenarium* was studied in order to contribute to the morphological and functional characterisation of both floral morphs.

Materials and methods

The material was collected in a natural population in Argentina, as described below:

Macroptilium arenarium (Bacigalupo) S.I.Drewes & R.A.Palacios. Prov. Entre Ríos. Dpto. Concepción del Uruguay: Médanos, junction of the road and the railway. 10.xi.2001, Hoc *et al.* 373, 374, 375 (BAFC).

From the three individuals that constitute the population, approximately 200 flowers in different stages of development were fixed in FAA and embedded in paraffin. Sections (5–10 μm) were cut and stained with safranin combined with fast green (D'Ambrogio 1986) and observed with a Wild M20 microscope. Photographs were taken with a Zeiss microscope (Zeiss, Göttingen, Germany). The callosic walls were studied with 0.01% aniline blue, which imparts a yellow fluorescence to callose (O'Brien and McCully 1981). Sudan IV was used to detect lipids and ruthenium red to determine the presence of hemicellulose and peptic substances (D'Ambrogio 1986). For scanning electron microscope (SEM) studies acetolysed (Erdtman 1969) and unacetolysed pollen grains were dehydrated in an ascending series of acetone (70, 80, 90 and 100%), sputter-coated with gold-palladium for 3 min (O'Brien and McCully 1981) and observed with a SEM Philips Series XL,

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Model 30, Holland. Descriptions of pollen grains are based on both acetolysed and unacetolysed material since it has been noted that acetolysis partially destroys structures such as pore membranes. Pollen viability was determined following the technique of Greissl (1989). Observations and recounts were made with a Nikon Optiphot-2 epifluorescence microscope (Nikon, Tokyo, Japan).

Results

Descriptions are common to both floral morphs. The distinctive features are specifically mentioned.

Microsporangium

The anther is tetrasporangiate (Fig. 1*g*) and presents longitudinal dehiscence.

The young anther wall consists of epidermis, endothecium, one or two middle layers and and uninucleate secretory tapetum. The endothecium development coincides with the dicotyledonous type (Davis 1966) since the middle layers and the endothecium share the same origin (Figs 1*a*, 2*a*).

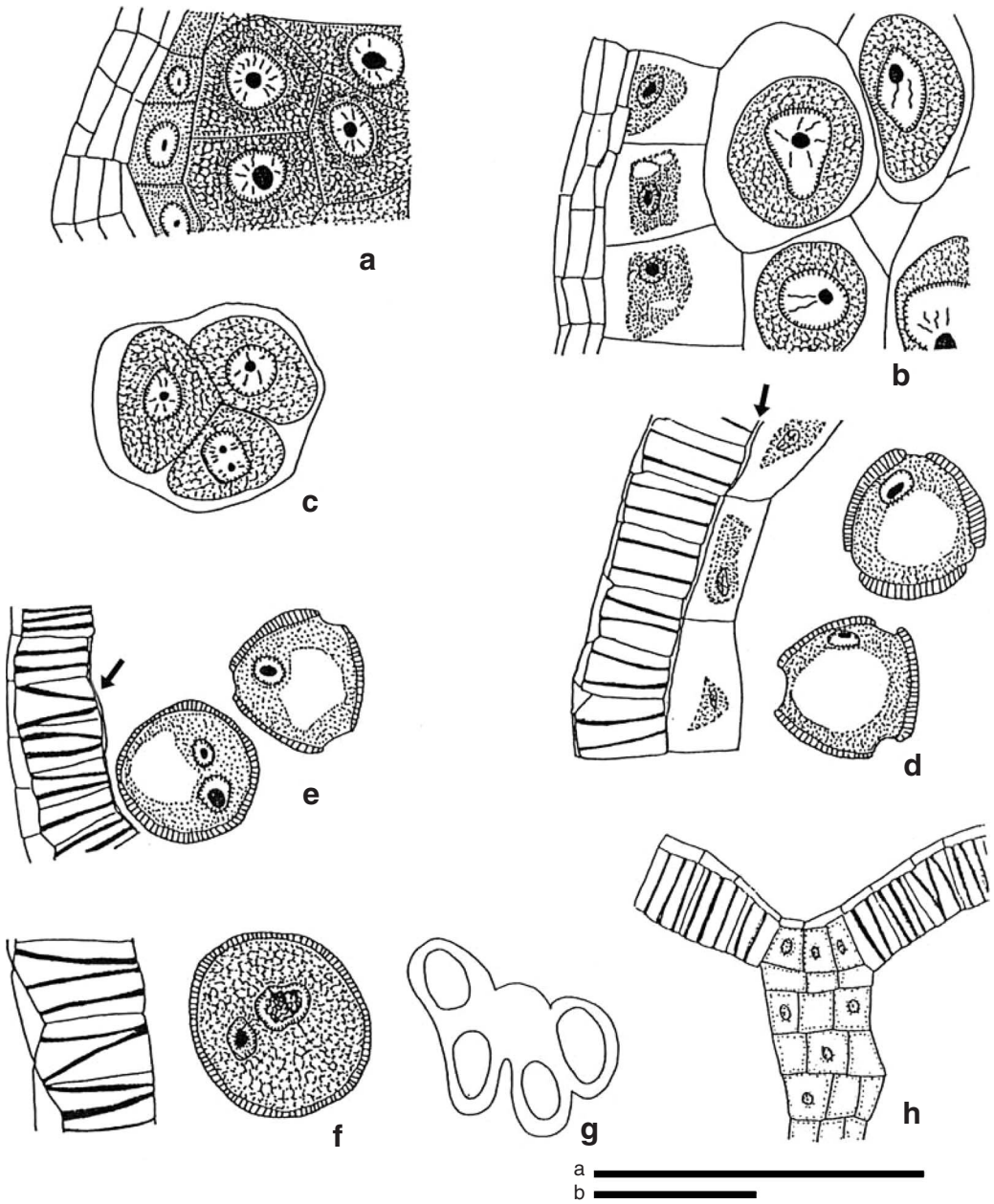

Fig. 1. Chasmogamous flowers. (*a*) Archesporic tissue and young microsporangium wall (scale bar a). (*b*) Microspore mother cells with callose walls (scale bar a). (*c*) Tetrahedral tetrad (scale bar a). (*d*) Young bicellular stage, tapetal cells with obliterating nuclei, middle layers mostly degraded (arrows) (scale bar a). (*e*) Free microspore stage, endothecium cells with fibrous thickenings, tapetum cells absent, middle layers mostly degraded (arrows) (scale bar a). (*f*) Mature bicellular pollen grain, middle layer no longer present (scale bar a). (*g*) Cross-section of a mature anther (sketch) (scale bar b). (*h*) Detail of a mature anther stomiun (scale bar a). Scale bar a = 50 µm, scale bar b = 100 µm.

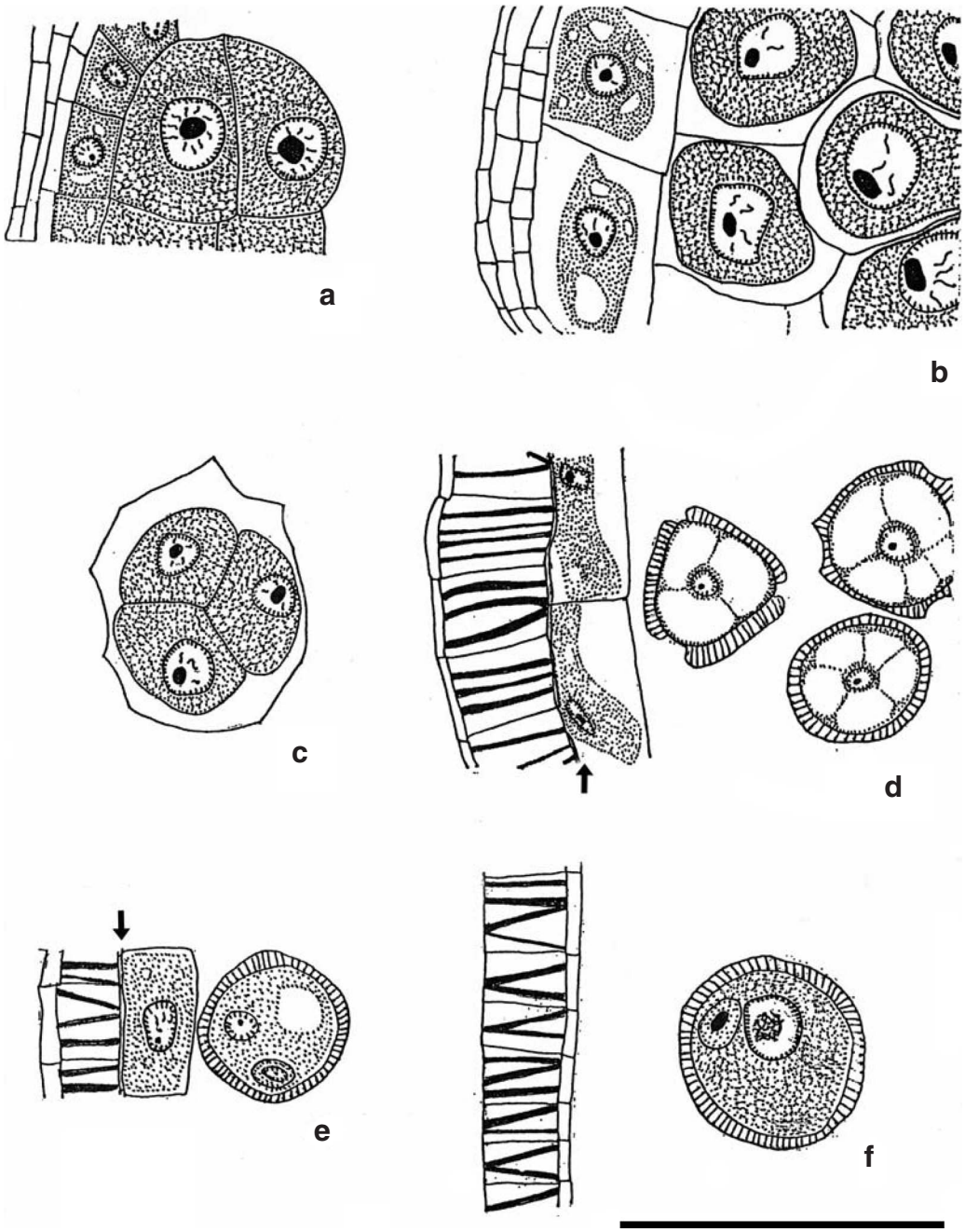

Fig. 2. Cleistogamous flowers. (*a*) Archesporic tissue and young microsporangium wall (scale bar = 50 µm). (*b*) Microspore mother cells with callose walls (scale bar = 50 µm). (*c*) Tetrahedral tetrad (scale bar = 50 µm). (*d*) Free microspore stage, endothecium cells with fibrous thickenings, middle layers mostly consumed (arrow) (scale bar = 50 µm). (*e*) Young bicellular pollen grain stage, persistent tapetal cells, middle layers mostly consumed (arrow) (scale bar = 50 µm). (*f*) Mature bicellular pollen grain, middle layer no longer present (scale bar = 50 µm).

In floral primordia, cells that form the four wall layers have similar size and shape.

Tapetum cells are the first to enlarge and remain uninucleate throughout the ontogeny of the microspores. These cells have dense cytoplasm and are more vacuolated in cleistogamous flowers (Figs 1*b*, 2*b*). They reach their maximum size at an early microspore development stage. In chasmogamous flowers at this stage, tapetum cells start to gradually degenerate and their nuclei to obliterate (Fig. 1*d*). When pollen grains reach the bicellular stage, tapetum cells are no longer observed in the chasmogamous flowers (Fig. 1*e*, *f*) whereas they persist in the cleistogamous ones (Fig. 2*e*), breaking down once the anther has attained its maximum maturity (Fig. 2*f*).

Epidermal and endothecial cells as well as middle layer cells grow radially and tangentially as the anther matures.

Fibrous thickenings develop from the inner tangential and radial walls of the endothecium cells during microgametogenesis. These ribs are interrupted in the outer tangential face (Figs 1*d–f*, 2*d–f*). Therefore, the endothecium acts mechanically in the dehiscence of the anther. In cleistogamous flowers such thickenings are more developed than in the chasmogamous ones.

After the first meiotic division, middle layer cells start a slow degeneration process. At late tetrad stage, most of these cells have been degraded while a few persist restricted to the zone of the locule that contacts the connective tissue until pollen grains reach the bicellular stage (Figs 1*d*, *e*, 2*d, e*, arrows).

In chasmogamous flowers, the septum that separates both locules of an anther lobe breaks down before the opening of the anther. The dehiscence region is formed by a group of parenchymatic cells containing vacuoles. When pollen grains reach maturity these cells disintegrate, allowing anther dehiscence (Fig. 1*h*).

Microsporogenesis and microgametogenesis

The sporogenous tissue differentiates once the four layers of the anther wall have been formed. This tissue is distinguishable by the presence of few intercellular spaces and by isodiametric cells with prominent nuclei, thick walls and a cytoplasm the density of which diminishes from the perinuclei zone towards the walls (Figs 1*a*, 2*a*). Microspore mother cell walls become thicker because of the deposition of callose between the plasmalemma and the primary wall (Figs 1*b*, 2*b*). Subsequently, they come apart by the dissolution of the middle lamella and primary walls that keep the sporogenous tissue together. Each microspore mother cell undergoes simultaneous reductive divisions and gives rise to microspore tetrads with tetrahedral arrangement (Figs 1*c*, 2*c*).

Each individual microspore separates from the tetrad by the sudden dissolution of the callose wall. The deposition of sporopollenin begins immediately after the release of microspores into the anther locule. Consequently, a thick exine wall is formed. The free microspores do not change their size. Young microspores of cleistogamous flowers show a more vacuolated cytoplasm than those formed in chasmogamous flowers (Figs 1*d*, 2*d*).

The first division of the microspore gives rise to a small generative cell and a large vegetative cell. Soon after pollen grains are formed they increase their volume, which generates a stretching and slimming of the exine. The maturation of the pollen grain in cleistogamous flowers is slower than in the chasmogamous ones. That is to say that, at the same stage of the megagametogenesis, bicellular pollen grains of cleistogamous flowers are smaller than those of the chasmogamous ones (Fig. 2*e* cf. Fig. 1*e*). After microspore mitosis the vegetative cell continues to grow, the vacuole gradually disappears and the cytoplasm fills with starch grains. At this stage pollen grains are shed, having the same size in both floral morphs (Figs 1*f*, 2*f*).

Pollen-grain morphology

Mature pollen grains of chasmogamous flowers are suboblate, tricolporate, brevicolpate and angulaperturate with subtriangular amb (= outline of a pollen grain seen in polar view) (Fig. 3*b*). Colpus and pore membranes are spinulate, and fusions between two or three spinules have also been observed (Fig. 3*c*). Mesocolpia, as well as apocolpia, present an irregular tectum surface with spread microperforations (Fig. 3*a*).

Mature pollen grains of cleistogamous flowers are suboblate with subtriangular amb, apparently non-aperturate; the apertures have been observed only under light microscope. The tectum surface is highly irregular and microperforations are so densely arranged that the structure resembles the verrucate type. Spinules are irregularly dispersed (Fig. 3*d*).

Both floral morphs present low pollen viability. However, chasmogamous flowers have a slightly higher viability than cleistogamous ones.

Gynoecium

The gynoecium is unicarpelar and unilocular. The style is laterally inserted and the stigma is apical.

The ovary is surrounded by a membraneous annular disc at its base and is pubescent in both floral morphs, this feature being more conspicuous in cleistogamous flowers. The number of ovules per ovary varies between three and four in cleistogamous flowers and between four and seven in chasmogamous ones.

The stigma surface is composed of papillae (Fig. 4*a*) with a thick cuticle and is surrounded by an incomplete crown of long trichomes. The cuticle usually detaches from the stigmatic surface before the beginning of the anthesis. In chasmogamous flowers it breaks, allowing the release of the stigmatic secretion beneath it, whereas in cleistogamous flowers it is persistent.

The style is hollow with a channel that runs through from the stylar brush towards the base (Fig. 4*b*). In flower buds such channel is coated by an undifferentiated epidermis, whereas in mature flowers it is coated by transmitting tissue.

At the tip of the style, a solid region with a core of transmitting tissue connects the stigmatic surface to the channel of the hollow style.

In chasmogamous flowers pollen germination on the surface of the stigma occurs when a pollen tube emerges from one of the pores, and grows between the stigmatic papillae and reaches the style (Fig. 4*c*, *d*). It grows through the transmitting tissue in the solid region towards this same

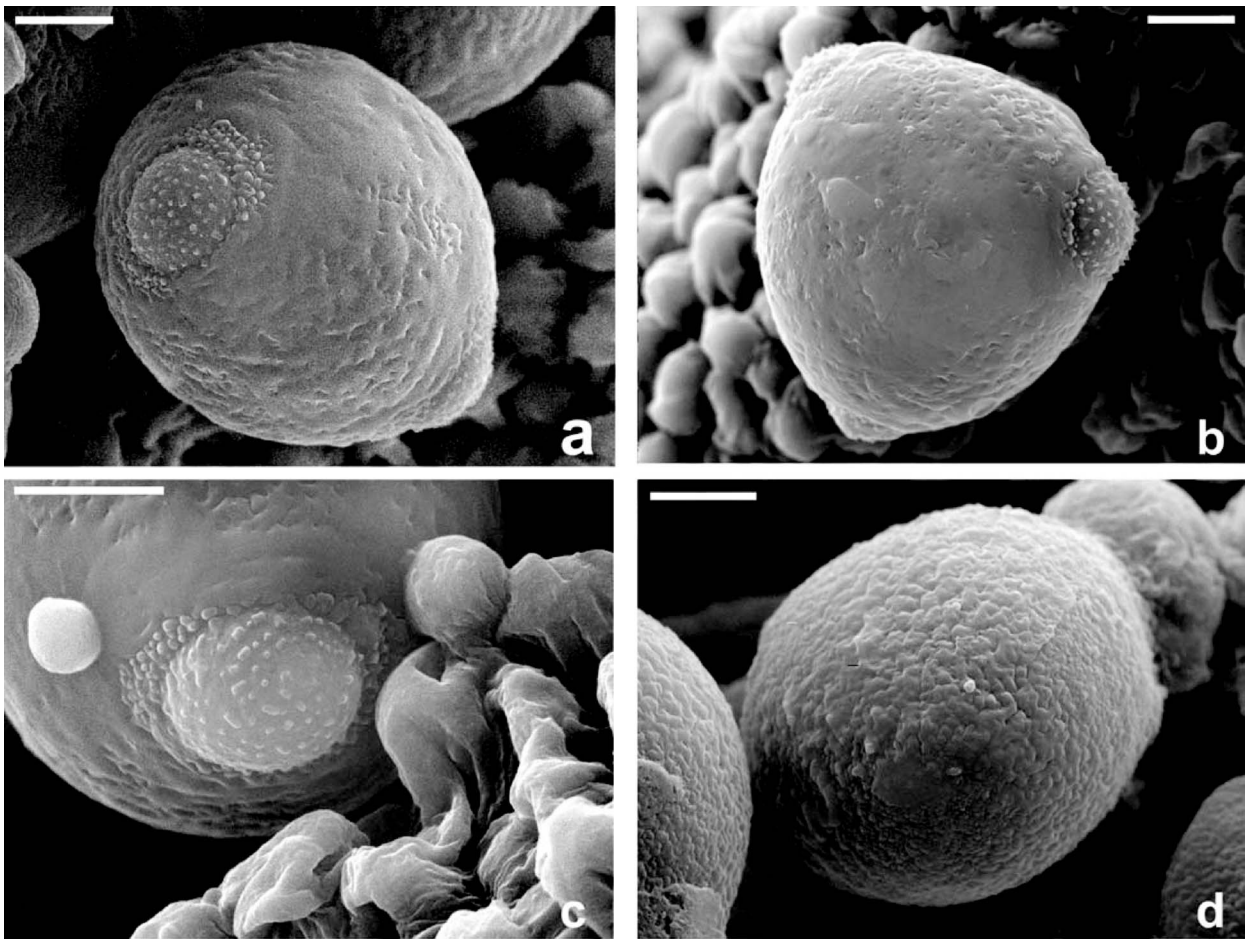

Fig. 3. Scanning electron micrographs of non-acetolysed pollen grains. (*a*–*c*). Chasmogamous flowers. (*d*) Cleistogamous flowers. (*a*) Pollen grain in equatorial view (scale bar = 5000 nm). (*b*) Polar view, general aspect (scale bar = 5000 nm). (*c*) Detail of the colporus (scale bar = 5000 nm). (*d*) General aspect of a pollen grain in equatorial view (scale bar = 5000 nm).

tissue that coats the channel. The cuticle of cleistogamous flowers hardly detaches and pollen grains have not been found on the stigmatic surface or near it.

Ovule

The mature ovule is crassinucelate, anacampylotropous and bitegmic (Fig. 5*a*, *b*). It originates as a small protuberance from the marginal placenta. The ovule primordium is bizonate in longitudinal section. Initially, it seems to be orthotropous; however, it begins to bend at the megaspore mother cell (MMC) stage.

At sporogenous cell stage both integuments differentiate simultaneously. The inner integument is of dermal origin and consists mostly of two layers of cells (Fig. 6*a*). It presents a greater thickness only at the micropylar end on the funicular side. The outer integument, which is of hypodermal origin, is multilayered (Fig. 5*a*, *b*).

The outer integument develops more rapidly than the inner integument, so that at dyad stage the cells of the former enclose the latter and reach the nucellar end. In the mature ovule, a massive structure that covers the inner integument and the micropylar end of the nucellus is formed by repeated divisions on the tip of the outer integument opposite of the raphe. The inner integument never reaches the micropylar end of the nucellus. Therefore, the micropyle consists of an exostome formed by the outer integument and an endostomatic channel delimited by the inner integument at the raphe side and by the internal face of the outer integument at the opposite side (Figs 5*a*, *b*, 6*f*, 7*f*).

In the micropylar end of mature ovules, the epidermal cells of the nucellus become thickened and form an epistase above the embryo-sac. Callose was not detected in these cell walls, which are mostly composed by peptic substances and hemicellulose. In cleistogamous flowers the epistase extends and surrounds two-thirds of the megagametophyte (Figs 5*a*, *b*, 6*f*, 7*f*).

Megasporogenesis and megagametogenesis

A single hypodermal cell of the nucellus, which enlarges considerably, acquires dense cytoplasmic contents

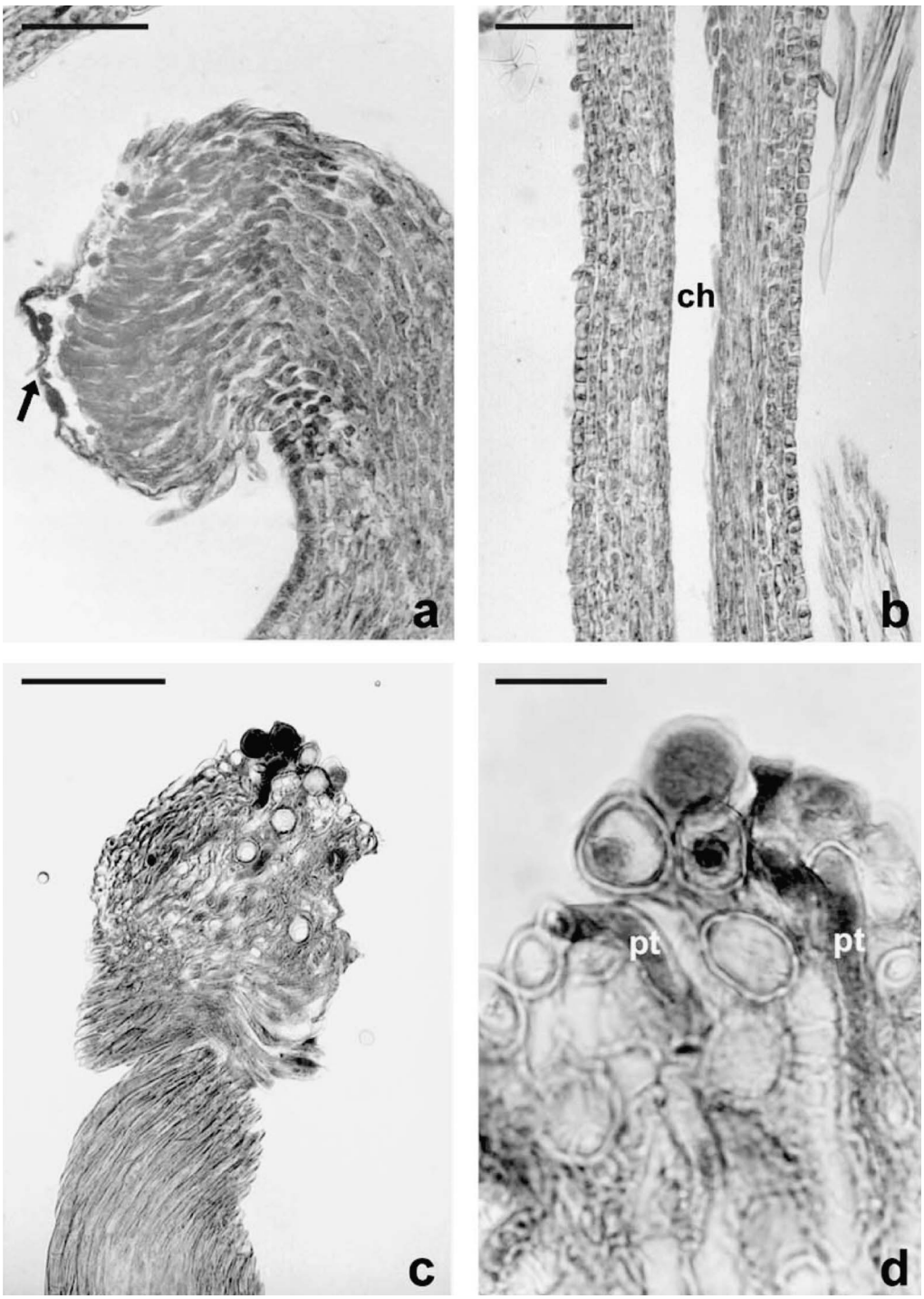

Fig. 4. Chasmogamous flowers. (*a*) Stigma, cuticule (arrow) detaching from the stigmatic surface (scale bar = 100 µm). (*b*) Longitudinal section of the style, channel (ch) and transmitting tissue (scale bar = 100 µm). (*c*) Pollen grains germinating on the stigma before the beginning of the anthesis (scale bar = 100 µm). (*d*) Detail of pollen grains tubes (pt) (scale bar = 25 µm).

and shows a prominent nucleus differentiating into the archesporial cell. The latter divides periclinally, forming an outer primary parietal cell and an inner primary sporogenous cell which functions as MMC. The primary parietal cell undergoes further periclinal divisions. Therefore, the parietal tissue is well developed and the MMC is separated from the nucellar epidermis (crassinucelate ovule) (Figs 6*a*, 7*a*).

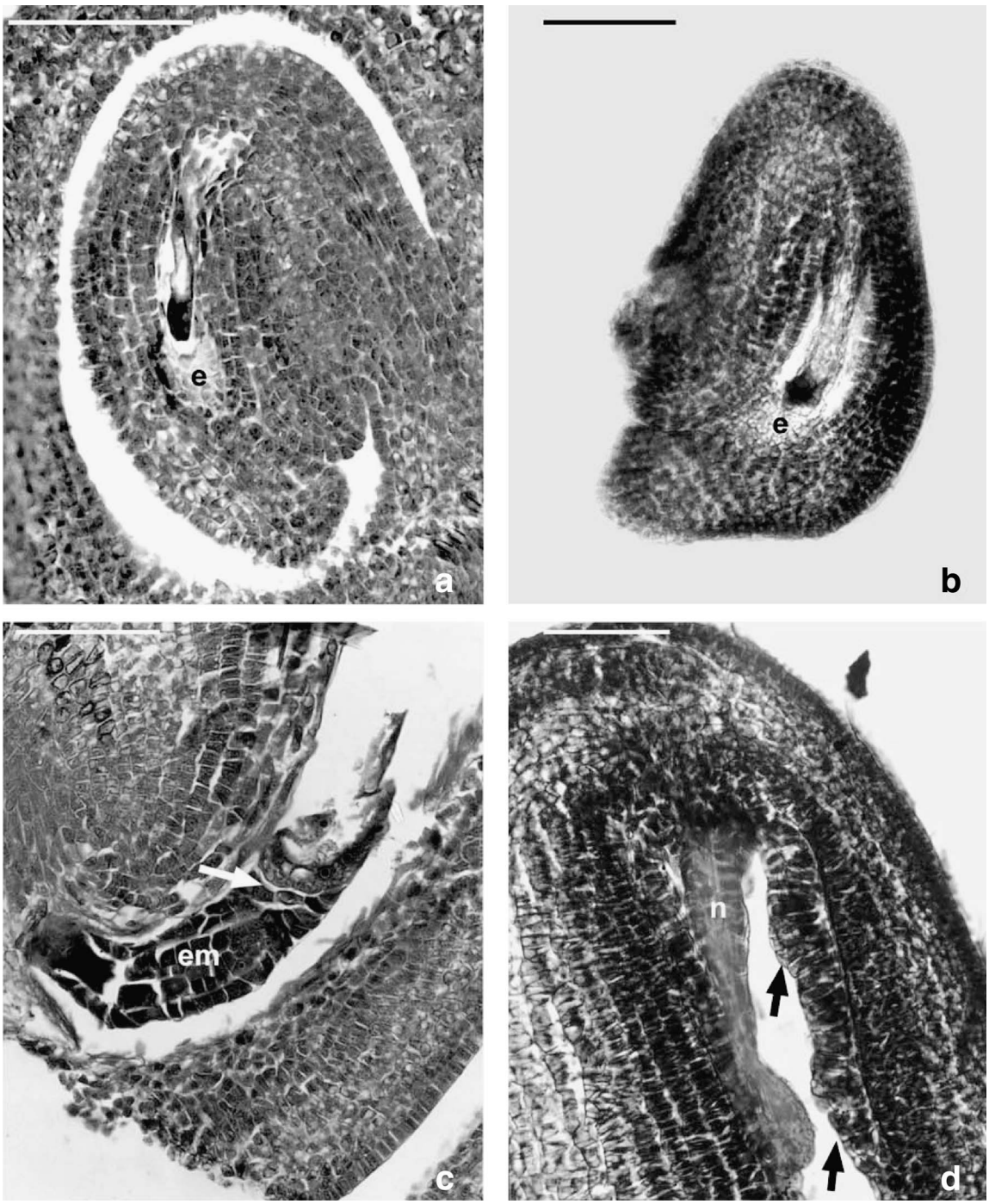

Fig. 5. (*a*, *c*) Chasmogamous flowers. (*b*, *d*) Cleistogamous flowers. (*a*) Longitudinal section of an ovule at four nuclei embryo-sac stage, epistase (e) in the micropylar end (scale bar = 100 µm). (*b*) Longitudinal section of a mature ovule, epistase (e) in the micropylar end and surrounding most of the embryo-sac (scale bar = 100 µm). (*c*) Embryo at torpid stage (em), helobial endosperm and wall (arrow) that separates both chambers (scale bar = 100 µm). (*d*) Nucellus (n), aborted embryo-sac and proliferation of the cells from the inner integument of the ovule (arrows) (scale bar = 100 µm).

More than one MMC have been observed in several ovules of cleistogamous flowers (Fig. 6*b*). In this floral morph, the MMC presents a conspicuous nucleus with one or two nucleoli and remains in this stage for a longer period than in the chasmogamous flowers. Consequently, the MMC increases its volume considerably (Fig. 6*c*).

Ovules with a single MMC have been found in chasmogamous flowers.

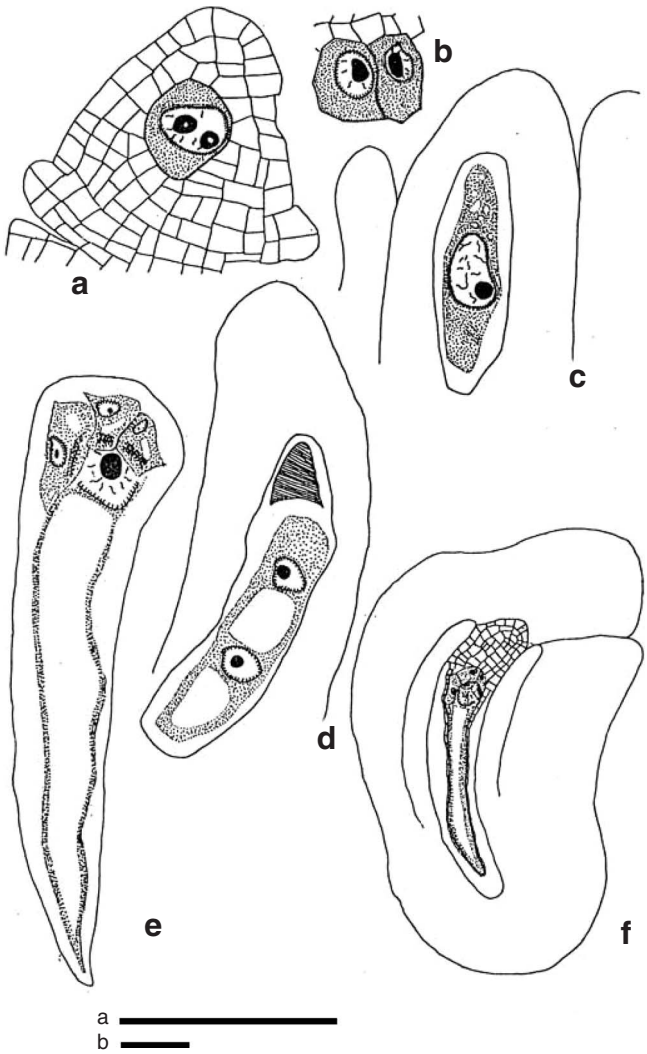

Fig. 6. Cleistogamous flowers. (*a*–*c*) Megasporogenesis (*a*) Megaspore mother cell with two nucleoli in its nucleus (scale bar a). (*b*) Two megaspore mother cells (scale bar a). (*c*) Mature megaspore mother cell (scale bar a). (*d*, *e*) Megagametogenesis. (*d*) Megaspore dyad with the micropylar one degenerated and the functional one developing into the embryo-sac (scale bar a). (*e*) Mature embryo-sac, large secondary nucleus, egg cell with lateral disposition and absent antipodals (scale bar a). (*f*) Longitudinal section of a mature ovule, epistase in the micropylar end and surrounding most of the embryo-sac (scale bar b). Scale bar a = 50 µm, scale bar b = 200 µm.

The MMC divides meiotically giving rise to a linear megaspore tetrad. The three micropylar megaspores degenerate and the chalazal one develops into the megagametophyte (Fig. 7*b*, *c*).

In cleistogamous flowers, only megaspore dyads have been observed (Fig. 6*d*).

In both floral morphs the functional megaspore enlarges and its nucleus undergoes a first mitotic division unaccompanied by wall formation. The resulting nuclei are pushed to opposite poles by a central vacuole. Two further mitotic divisions give rise to an eight-nucleate female gametophyte, with four nuclei in each pole. Once cytokinesis has taken place, a seven-celled embryo-sac is formed. Megagametogenesis follows the Polygonum type (Figs 6*e*, 7*e*).

Mature chasmogamous flowers present a large egg cell, the nucleus of which is situated in the chalazal region owing to the presence of a vacuole at the micropylar pole (Fig. 7*e*). In contrast, the egg cell of the cleistogamous flowers is quite small and is laterally positioned with respect to the synergids and the secondary polar nucleus (Fig. 6*e*).

Synergids in the chasmogamous flowers show inverted polarity, whereas in the cleistogamous flowers vacuoles occupy the chalazal regions and the micropylar ends contain the nuclei (Fig. 6*e* cf. Fig. 7*e*).

In the mature embryo-sac the central cell produces a large vacuole that separates the two polar nuclei. These fuse to form a diploid secondary nucleus before fertilisation. Cleistogamous flowers show a much more conspicuous secondary nucleus than chasmogamous ones. In the latter, antipodals are still present at this stage, yet they have lost most of their cellular volume and their nuclei have started to degenerate. Antipodals are absent in cleistogamous flowers at such stage (Figs 6*e*, 7*e*).

Fertilisation and endospermogenesis

Chasmogamous flowers

The pollen tube grows into one of the synergids where both sperms are released. One male gamete fuses with the nucleus of the egg cell to form a zygote. The fusion of the second male gamete with the polar nuclei results in an endospermogenetic cell.

The primary endosperm nucleus divides to form two chambers where several free nuclear divisions take place. Cell-wall formation begins in the micropylar chamber, whereas the chalazal chamber remains coenocytic. The former has differentiated cells at torpedo embryo stage, whereas the latter functions as a haustorium (Fig. 5*c*).

Cleistogamous flowers

Neither has the discharge of the pollen tube nor fertilisation been observed in this floral morph.

The mature embryo-sac aborts while several cells of the inner integument of the ovule proliferate (Fig. 5*d*). Even though middle stages of seed development have not been observed, exalbuminous seeds have been found.

Discussion

This report brings new insights to the morphologic and functional characterisation of cleistogamous and chasmogamous flowers, especially in those species that produce both floral morphs during their reproductive cycle. Moreover, it contributes to the embryological knowledge of the family.

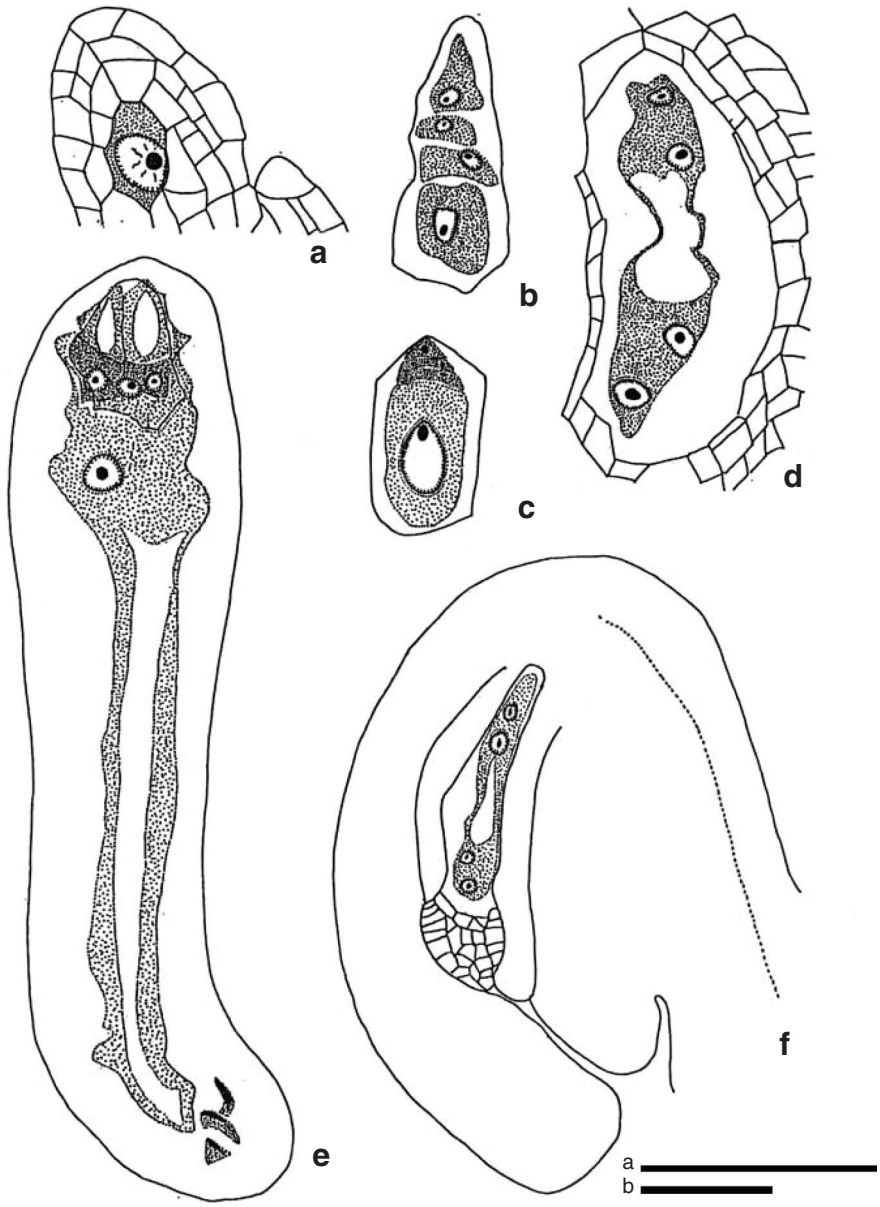

Fig. 7. Chasmogamous flowers. (*a–c*) Megasporogenesis. (*a*) Megaspore mother cell (scale bar a). (*b*) Lineal megaspore tetrad (scale bar a). (*c*) Functional megaspore and abortive micropylar megaspores (scale bar a). (*d*, *e*) Megagametogenesis. (*d*) Embryo-sac with four nuclei (scale bar a). (*e*) Mature embryo-sac, polar nuclei fused, synergids with inverted polarity and degenerating antipodals (scale bar a). (*f*) Longitudinal section of an ovule at four nuclei embryo-sac stage, epistase in the micropylar end (scale bar b). Scale bar a = 50 µm, scale bar b = 200 µm.

Anther-wall development in both floral morphs agrees with the dicotyledonous type described by Davis (1966), which has already been observed in the family (Lakshmi *et al*. 1987; Prakash 1987). *Macroptilium bracteatum* (Nees et Mart.) Maréchal et Baudet, *Phaseolus augusti* Harms, *P. vulgaris* var. *aborigineus* (Burkart) Baudet, and *Vigna adenantha* (G.Meyer) Maréchal, Mascherpa et Stainier show such wall ontogeny and present some cells of the middle layer related to the tapetum (Faigón Soverna 2002). Therefore, the dicotyledonous type of development could have derived from the basic type by suppression of a parietal layer.

Chasmogamous flowers of *M. arenarium* have uninucleate tapetal cells that quickly degenerate as well as *M. bracteatum* (Faigón Soverna 2002) whereas tapetal cells of *M. atropurpureum* (Sessé & Moc. ex DC) Urban.) (= *Phaseolus atropurpureus* DC.) are binucleate (Lakshmi *et al*. 1987). In cleistogamous flowers of *M. arenarium* the tapetum is still present at bicellular pollen grain stage.

In *M. arenarium* pollen grains are shed at bicellular stage as has been observed in *M. bracteatum* (Faigón Soverna 2002). According to Lakshmi *et al*. (1987) pollen grains of *M. atropurpureum* are three-celled when released.

Low pollen viability is not characteristic of the tribe. The presence of sterile pollen has only been mentioned in *M. atropurpureum* (Pritchard and Hutton 1972) and *P. vulgaris* L. (Johns *et al*. 1992). Great quantities of non-viable pollen in both floral morphs have been detected in species of *Viola* (Mayers and Lord 1984). Most pollen produced by *M. arenarium* is non-viable, pollen viability being slightly higher in chasmogamous flowers.

Research that shows palynological differences between the floral morphs has not been found. Similar descriptions on pollen grain morphology in chasmogamous flowers of *M. arenarium* have been given by Drewes (1996). Cleistogamous flowers of *M. arenarium* produce pollen grains that are apparently non-aperturate with a more irregular tectum surface than those of chasmogamous ones.

In several cleistogamous species pollen germinates within the anther and crosses through their apex where endothecial thickenings are only partly developed (Ritzerow 1908; Staedtler 1923; Madge 1929; West 1930; Hanson 1953; Daskalova and Genova 1996). If the endothecium is present, tubes grow from the open stomium after anther dehiscence. Pollen-grain germination on the stigmatic surface of cleistogamous *M. arenarium* flowers has not been observed, whereas in chasmogamous flowers pollen grains germinating between the stigmatic papillae and the fertilisation process have been recorded in pre- and post-anthesis flowers. This evidence suggests that chasmogamous flowers could be pre-anthesis cleistogamic according to the classification system of Lord (1981), as has been also observed by Drewes and Hoc (2000).

Most previous studies have described a zig-zag micropyle, with an exostome, mesostome and endostome constituted by both integuments. A true endostome was not formed in the four species studied by Faigón Soverna *et al*. (2003). In such species, as well as in *M. arenarium*, the inner integument never reaches the micropylar end of the nucellus. Therefore, the endostome channel is formed by the inner integument at the funicular side and by the internal face of the outer integument at the opposite side.

In *M. arenarium* an epistase is formed by the nucellar epidermis above the embryo-sac. This tissue, which in cleistogamous flowers extends and surrounds most of the megagametophyte, presents cell walls mainly composed by hemicellulose and peptic substances. The presence of epistase has been reported only in *M. bracteatum* (Faigón Soverna *et al*. 2003) for the Leguminosae. It has also been mentioned for other families such as Zingiberaceae and Nymphaeaceae (Bouman 1984).

Although cleistogamous flowers of *M. arenarium* may contain several ovules with two MMC, a single embryo-sac develops. Ovules with two MMC have also been observed in *Phaseolus vulgaris* (Weinstein 1926) and in *P. aureus* (George *et al*. 1979).

Conspicuous nuclei with two nucleoli resembling restitution nuclei have been observed in MMC of cleistogamous flowers of *M. arenarium*. The MMC enlarges and is still present at free microspore stage. Thus, there is a delay in the megasporogenesis. Finally, the MMC starts to divide meiotically but only Meiosis I takes place since dyads are formed, but not tetrads as in the chasmogamous flowers. According to these observations, megasporogenesis in the cleistogamous flowers is incomplete.

Female gametophyte development follows the Polygonum type (Maheshwari 1950), which seems to be a common feature in the Leguminosae. Only members of the Mirbeliinae subtribe exhibit unique patterns of embryo-sac development (Cameron and Prakash 1994).

The inverted polarity of the synergids in chasmogamous flowers of *M. arenarium* has not been observed in other species of the subtribe (Faigón Soverna *et al*. 2003).

The stigma surface of *M. arenarium* is composed of papillae with a thick cuticle and is surrounded by an incomplete crown of longer trichomes as is known for other species of the family (Faigón Soverna *et al*. 2003; Prenner 2004).

Drewes and Hoc (2000) concluded that there is an evolutionary tendency towards cleistogamy in *Macroptilium fraternum*. Differential features between both floral morphs suggest such tendency is present in *M. arenarium*. In cleistogamous flowers, the tapetum is persistent and more vacuolated, young microspores contain a higher number of vacuoles in the cytoplasm and pollen viability is slightly lower. Moreover, ovaries are smaller, contain less ovules, and more than one MMC with nuclei that resemble restitution nuclei have been observed. The MMC stage is prolonged to further stages of microsporogenesis, megaspore dyads instead of tetrads have been found, antipodals are ephemeral and the epistase extends and surrounds two-thirds of the embryo-sac. On the other hand, chasmogamous flowers are characterised by synergids with inverted polarity, a larger egg cell, a more inconspicuous polar nucleus and antipodals present at advanced stages of megagametogenesis. In this floral morph pollen tube discharges into one of the synergids, whereas in cleistogamous flowers the cuticle that coats the stigma is persistent and pollen-grain germination on the stigma has not been observed. Nevertheless, autogamy on cleistogamous flowers cannot be excluded since no manual attempts to self-pollinate the flowers have been made. In this floral morph, the mature embryo-sac aborts so the cells from the inner integument of the ovule proliferate. Both floral morphs develop into exalbuminous seeds. Regarding all these observations, and taking into account the embryological differences between cleistogamous and chasmogamous flowers, apomixis in the former is suggested as a possible developmental pathway of the embryo. If so, adventive embryos would arise directly from

somatic cells of the ovule, more specifically from the inner integument cells.

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