Development of the flower and inflorescence of *Arum italicum* (Araceae)

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Abstract: The spadix of *Arum italicum* Miller consists of two main parts: a clavate sterile portion (appendix) and a cylindroid fertile portion. In the fertile portion with both male and female zones, there are two zones of sterile flowers (bristles). The basal portion of bristles is surrounded by a verrucose structure consisting of a mass of tissular excrescences. During early stages of development, there is no free space between the different zones of the inflorescence. The elongation of the inflorescence axis is what eventually separates the different zones from each other. There are no atypical flowers that are morphologically intermediate between male and female flowers as is the case in other genera of Aroideae (e.g., *Cercestis*, *Philodendron*, *Schismatoglottis*). The structure of the bristles in the inflorescences of *Arum* does not correspond to any type of atypical flower (unisexual or bisexual) that has been analysed previously in the Araceae. From a developmental point of view, it is not possible to determine if the bristles correspond to aborted or modified female or male flowers. In the early stages of development, the stamens, staminodes, and appendix are covered by globular masses of extracellular calcium oxalate crystals.

Key words: development, unisexual flowers, gradient, calcium oxalate crystals.

Résumé : Le spadice de l'*Arum italicum* comporte deux parties principales : une portion claviforme stérile (appendice) et une portion cylindroïde fertile. Dans la portion fertile avec zones mâles et femelles, il y a deux zones de fleurs stériles (soies). La portion basales des soies est entourée par une structure verruqueuse, constituée d'une masse d'excroissances tissulaires. Aux premiers stades du développement, il n'y a pas d'espace libre entre les différentes zones de l'inflorescence. L'élongation de l'axe de l'inflorescence est ce qui sépare éventuellement les différentes zones l'une de l'autre. Il n'y pas de fleurs atypiques intermédiaires entre des fleurs mâles et des fleurs femelles, comme c'est le cas dans d'autres genres d'Aroideae (p. ex. *Cercestis*, *Philodendron*, *Schismataglottis*). La structure des soies chez les inflorescences de l'*Arum* ne correspond à aucune des fleurs atypiques (unisexuées ou bisexuées) qui ont précédemment été analysées chez les Araceae. Considérant le développement, il n'est pas possible de déterminer si les soies correspondent à des modifications de fleurs femelles ou mâles avortées. Aux premiers stades du développement, les étamines, les staminodes et les appendices sont couverts de masses globuleuses constituées de cristaux extracellulaires d'oxalate de calcium.

Mots clés : développement, fleurs unisexuées, gradient, cristaux d'oxalate de calcium.

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Introduction

Within the Araceae, the subfamily Aroideae (sensu Mayo et al. 1997) consists of 74 genera and is characterized by the presence of unisexual flowers on the spadix. The female flowers are located in the lower portion of the inflorescence and male flowers (sterile and fertile) are found directly above them. Related species with unisexual flowers representing a number of genera have been investigated by a variety of authors from the perspective of floral anatomy, developmental morphology, and phylogeny (e.g., Barabé and Forget 1988; Barabé et al. 2002*a*, 2002*b*; Barahona Carvajal 1977; Boubes and Barabé 1996; Buzgó 1994; Carvell 1989; Eckardt 1937; Engler and Krause 1912; Eyde et al. 1967; French 1985*a*, 1985*b*, 1986*a*, 1986*b*; Hotta 1971; Mayo 1986, 1989; Uhlarz 1982, 1986).

Until recently, very few genera have been examined from a developmental point of view to determine the number of types of flowers in the subfamily Aroideae. The developmental morphology of flowers has been well documented in genera where there is a sterile male zone of flowers between the typical male and the female zones (*Philodendron*, *Caladium*) or where such a zone is absent (*Culcasia*, *Cercestis*). Atypical flowers with male and female characteristics are often found in the intermediate zone of aroid inflorescences, more specifically between the male and female zones in genera with unisexual flowers, referred to as "monströsen

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Blüten" by Engler and Krause (1912). To date, two developmental patterns of atypical bisexual flowers have been recognized: the *Philodendron* type and the *Cercestis* type (Barabé and Lacroix 1999, fig. 45). These two types of flowers seem to correspond to two different evolutionary trends (Barabé et al. 2002*a*). In the *Philodendron* type, atypical bisexual flowers generally consist of functional carpels and staminodes inserted on the same whorl. In the *Cercestis* type, the atypical bisexual flowers are characterized by a functional or nonfunctional gynoecium surrounded by a few (one to five) vestigial stamens on a separate whorl (Barabé and Bertrand 1996, fig. 30). There are no studies, however, that have examined what happens developmentally in taxa where there is a sterile zone between the male and female flowers and another one above the male zone, as occurs, for example, in the genus *Arum*. The lack of floral developmental studies in the Araceae is due in great part to the difficulty in obtaining enough material to document the range of early stages of development (Barabé and Lacroix 2001). However, we were recently able to obtain enough samples of *Arum italicum* Miller at different stages of development to extend our survey of the group.

Studies dealing with thermogenesis, pollination, sexual mass allocation, or flowering dynamics of *A. italicum* have recently been published (e.g., Méndez 1998, 1999, 2001; Méndez and Diaz 2001; Albre et al. 2003). However, there are few publications that provide information on anatomy or developmental morphology of flowers and inflorescences in the genus *Arum* (Eckardt 1937; Nougarède and Rondet 1981). Eyde et al. (1967) and Hotta (1971) did not address this problem in their anatomical survey of the flowers of different genera of Araceae. In their study of vegetative development in *A. italicum*, Nougarède and Rondet (1981) presented a few photographs of young inflorescences without elaborating on floral development. Although the floral morphology of different species of *Arum* was described in detail in a thorough taxonomic study (Boyce 1993), an analysis of floral development is still lacking and is needed to determine the exact nature of different types of sterile organs present in the inflorescence.

In the present study, we will assess whether the different floral types on the inflorescence of *Arum* can be integrated into the developmental patterns recognized in the subfamily Aroideae.

Developmental studies often reveal particular features that are not visible on fully developed organs. This shortcoming was the case in a previous study when the accumulation of extracellular calcium oxalate crystals on anthers in *Philodendron* was observed (Barabé and Lacroix 2001). We present additional evidence for the presence of extracellular calcium oxalate crystals, this time in *Arum*. The mode of release of oxalate crystals is also compared with that of *Philodendron* from a developmental perspective.

The specific goals of this study are to (*i*) to compare the development of flowers of *Arum* with that of other genera to further characterize the range of floral developmental morphology in the subfamily Aroideae and (*ii*) further document poorly known developmental features in the genus *Arum* such as the release of calcium oxalate crystals by floral organs.

Materials and methods

Plant material

Specimens of *A. italicum* used for this study were collected in France (Campus of the Université Paul Sabatier, Toulouse) in October 2001 (voucher specimen deposited at MT: *Barabé 181*). Inflorescences at various stages of development were collected, dissected under a stereomicroscope to expose the spadix, and fixed in formalin – acetic acid – alcohol (1:1:9 by volume) and later transferred and stored in 70% ethanol.

Scanning electron microscopy

Thirty-seven samples of *A. italicum* were dehydrated in a graded ethanol series to absolute ethanol. They were then dried in a LADD model 28000 critical point dryer using CO_2, mounted on metal stubs, and grounded with conductive silver paint. Specimens were sputter-coated with gold–palladium to approximately 30 nm using a Denton Vacuum Desk II sputter-coater and viewed with a Cambridge S604 scanning electron microscope with digital imaging capabilities (SEMICAPS®).

For a description of the different morphological features, we follow the terminology of Boyce (1993).

Results

Mature structures

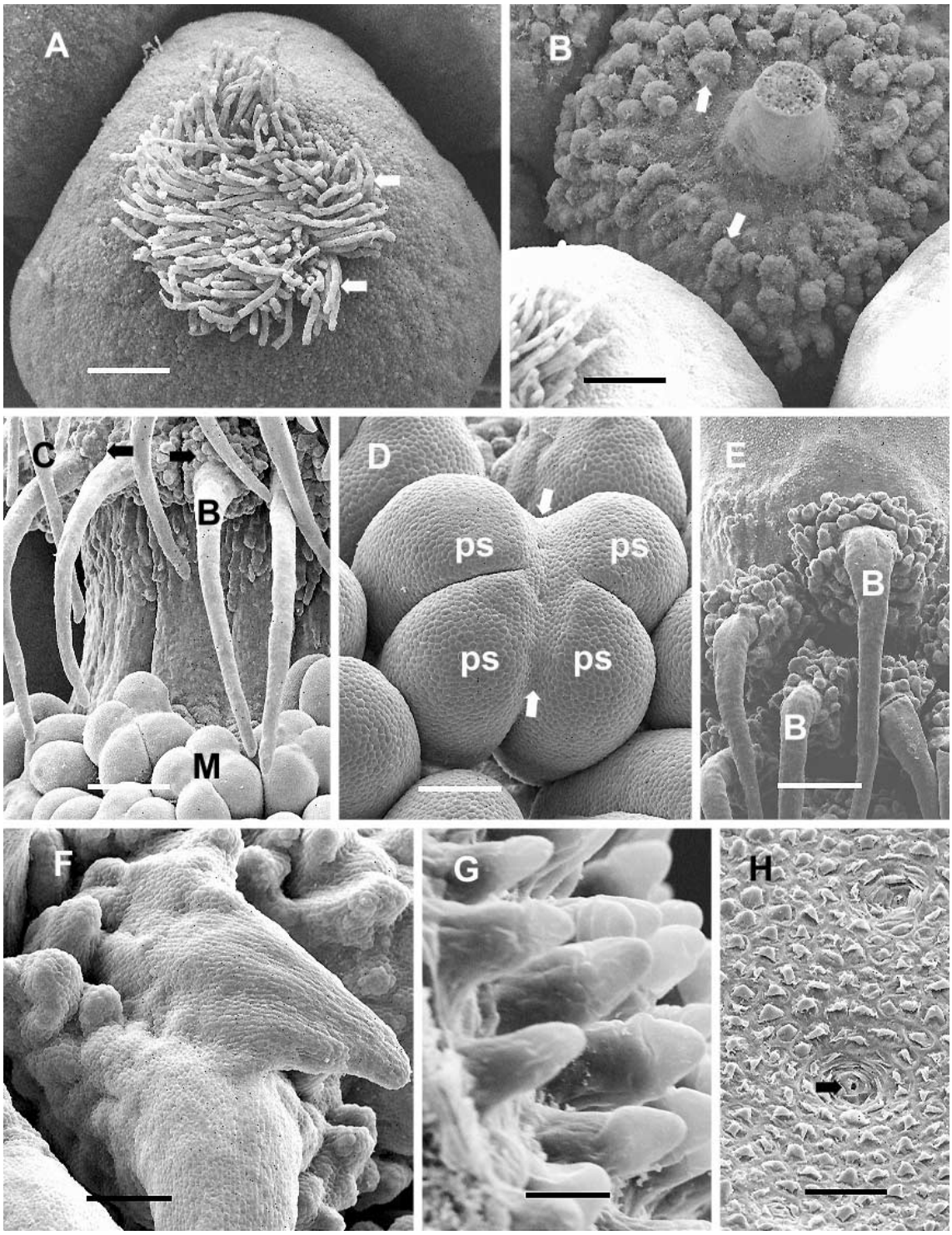
The spadix of *A. italicum* has a length ranging from 5.2 to 18 cm. It consists of two main parts: a clavate sterile portion (appendix) measuring 3–14 cm in length and a fertile portion representing approximately 1–4 cm of the length of the inflorescence. The base of the appendix (the stipe) is elongated and thinner than the rest.

The zone of female flowers is located at the base of the spadix. Each pistillate flower consists of a naked, sessile, oblong–ovoid gynoecium with a unilocular multiovulate ovary. The discoid stigma (Fig. 1A) is covered by elongated papillae.

In the fertile portion, there are two zones of sterile cream-coloured flowers (bristles) (Figs. 1B and 1C). The upper zone (0.2–0.5 cm long), consisting of two to five rows of bristle-like staminodes, is located above staminate flowers. The staminodes are 3.5–5 mm long, cream coloured, filiform, and flexuous (Fig. 1C). The basal portion of each staminode is surrounded by a verrucose structure consisting of a mass of tissular excrescences (Figs. 1E and 1F). Each of these excrescences looks like a small undifferentiated callus. The lower zone of bristles (0.1–0.3 cm long), termed "bristle-like pistillodes", consists of one or two rows of atypical flowers and is located between the female zone (0.2–3.3 cm long) and the male zone (0.3–1 cm long). Like the staminodes, the surface of the basal portion of each pistillode is covered by tissular excrescences (Fig. 1B).

In *A. italicum*, early stages of development (see section on development) show that a single staminate flower consists of three fused anthers. The thecae are joined by a very short slender connective (Fig. 1D). Anthers and connective are yellowish in colour and dehiscence is achieved through a longitudinal slit (Fig. 1D).

Fig. 1. Mature structures on inflorescence of *Arum italicum*. (A) Stigmatic surface of a mature female flower. Arrows indicate filiform papillae. Bar = 300 μm. (B) Atypical flower located between the male (above) and female (below) zones. Note the verrucose surface of the basal portion of structure (arrows). Bar = 300 μm. (C) Intermediate zone between bristles (B) and the male (M) floral zone. Arrows point to the verrucose surface of the basal portion of the bristles. Bar = 75 μm. (D) Mature stamen with four pollen sacs (ps) prior to dehiscence. Arrows indicate the location of the zone of dehiscence. Bar = 300 μm. (E) General view of the zone of bristles. Bar = 750 μm. (F) Closeup of the basal portion of the bristles showing the callus-like appearance of the tissue. Bar = 150 μm. (G) High magnification of the surface of the epidermis of the mature appendix. Note the peg-like appearance of the cells. Bar = 75 μm. (H) Top view of a stomate (arrow) on the surface of the mature appendix. Bar = 75 μm.



Development

During early stages of development, the inflorescence consists of a more or less cylindrical fertile portion in the lower half and an elongated conical sterile portion in the upper half (Fig. 2A). The upper portion corresponds to the sterile appendix that will develop subsequently. At the developmental stage represented in Fig. 2A, the female zone, located at the very base of the inflorescence, and the male zone represent approximately 45% of the total length of the inflorescence. The zone of bristle-like staminodes, located above the male zone, occupies only 5% of the total length of the inflorescence (Fig. 2B). The staminate floral primordia are arranged irregularly on the surface of the inflorescence. The zone of bristle-like pistillodes is not visible at this stage. The female primordia, on the other hand, form a more or less regular lattice on the surface of the inflorescence (Fig. 2B). The rest of the inflorescence (approximately 50%) consists exclusively of a sterile appendix. The lower portion of the structure is more tapered just above (arrow in Figs. 2A and 5A) the sterile zone. This narrowing corresponds to the location of a stipe that will develop subsequently. During the development of the inflorescence, the area occupied by the sterile appendix (including stipe) will increase considerably (i.e., 65% of the total length of the inflorescence). During early stages of development, there is no free space between the different zones of the inflorescence (Figs. 2A and 2B). The elongation of the inflorescence axis is what eventually separates the different zones from each other (Fig. 1C).

Female flowers

During early stages of development, the female floral primordia have a more or less cylindrical shape (Fig. 2C). The development of the ovarian cavity results from the growth of the meristematic tissue located at the periphery of the floral primordium (Figs. 2A and 2C). The growth of the ovary wall subsequently closes up the ovarian cavity (Figs. 2B and 2D). During later stages of development, the floral primordia like the stamens come in contact with each other as they expand. They eventually occupy all of the available space between flowers (Fig. 2B). The hole that is visible in the upper part of the ovary (Fig. 2B) corresponds to the location of the stigma that will form at a later stage of development (Fig. 1A).

Staminate flowers

The number of stamens per flower is very difficult to determine with certainty. However, some flowers consisting of three stamen primordia (Fig. 3A) were observed. The stamens look like prominent protuberances during early stages of development (Fig. 3A) and quickly come into contact with each other as they expand (Fig. 3B). They eventually occupy all of the available space between the flowers (Fig. 3C). During these later stages of development, the location of each of the pollen sacs is visible (Figs. 3C and 3D). On mature stamens, the future location of the dehiscence pores becomes recognizable (Fig. 1D). The surface of the epidermal cells is smooth (Figs. 1D and 3E).

On young stamens, the connective is often topped by a mass of extracellular calcium oxalate crystals (Fig. 3E), as described by Barabé and Lacroix (2001). The release of the oxalate crystals occurs before the stamens and stigmas are fully mature. During early stages of development, the calcium oxalate package appears to be covered by the cuticle, and the growth of the oxalate package eventually breaks through that cuticular cover (Fig. 3F).

Bristle-like staminodes

During early stages of development, a continuous rim of meristematic tissue forms just above the zone of male flowers (Figs. 4A and 4B). Structures that correspond to stamen primordia are often united to the rim on the side of the male zone (Fig. 4B). Following this stage, one or two rows of staminodal primordia will be initiated just above the meristematic rim (Figs. 4C and 4D). After the initiation of the first row of staminodes (Fig. 4C), a series of staminodes will emerge from the meristematic rim (Fig. 4E). During early stages of development, the staminodal primordia have a conical shape (Figs. 4E and 4F). During subsequent stages, they elongate, become flattened, and rapidly acquire their filiform and flexuous morphology (Fig. 4F).

Bristle-like pistillodes

In the intermediate zone located between the female zone and the male zone, structures that look like pistillodes can be observed. During early stages of development, the pistillodes have a conical shape (Figs. 2B and 2E) that will persist in mature stages. The morphological nature of these structures remains very difficult to determine.

Appendix

The sterile appendix becomes elongated and cylindrical in shape early in development (Fig. 5A). In young inflorescences, the stipe is separated from the upper portion of the sterile appendix where the structure is narrowest (Fig. 5A). During early stages of development, the surface of the sterile appendix is more or less verrucose (Fig. 5B). At the base of the appendix (Fig. 5C), extracellular oxalate crystals can be observed (Figs. 5C and 5D). These exudates form globular masses of hemispherical shape on the surface of the epidermis (Fig. 5C).

The release of the oxalate crystals occurs before the appendix reaches maturity. In *A. italicum*, the accumulation of extracellular calcium oxalate crystals occurs in the very early stages of development of stamens, staminodes, and the sterile appendix, when an inflorescence is approximately 6% of its final size. When the cuticle splits, crystals are liberated and become widespread on the surface of the male and sterile portions of the inflorescence. In the female zone, few crystals were observed. They probably originated from the upper zones.

The presence of elongated pegged cells and stomata can be observed on the surface of the sterile appendix (Fig. 1H).

Discussion

Extracellular calcium oxalate crystals

Extracellular crystal deposition is a characteristic feature of many lichens (Garty et al. 2002) and gymnospermous species (Pennisi et al. 2001; Oladele 1982; Fink 1991*a*). In angiosperms, the presence of extracellular calcium oxalate crystals was reported for Casuarinaceae (Berg 1994),

Fig. 2. General view of inflorescence and early stages of development of *Arum italicum* female flowers. (A) Early stage of development of the inflorescence showing the appendix (smooth upper portion) and the floral zone (lower portion with floral primordia). The arrow indicates the separation between the appendix proper and the basal stipe portion of the inflorescence. Bar = 750 µm. (B) General view of the floral portion of the inflorescence showing the different types of flowers: B, bristle; M, male flower; F, female flower; arrows, atypical flowers; asterisk, nearly enclosed ovary. Bar = 300 µm. (C) Early stage of initiation of female flowers. Bar = 75 µm. (D) Development of the ovary wall (O) of female flowers. Arrows point to atypical flowers. Bar = 150 µm. (E) Closeup of two atypical flowers (arrows). Bar = 75 µm.

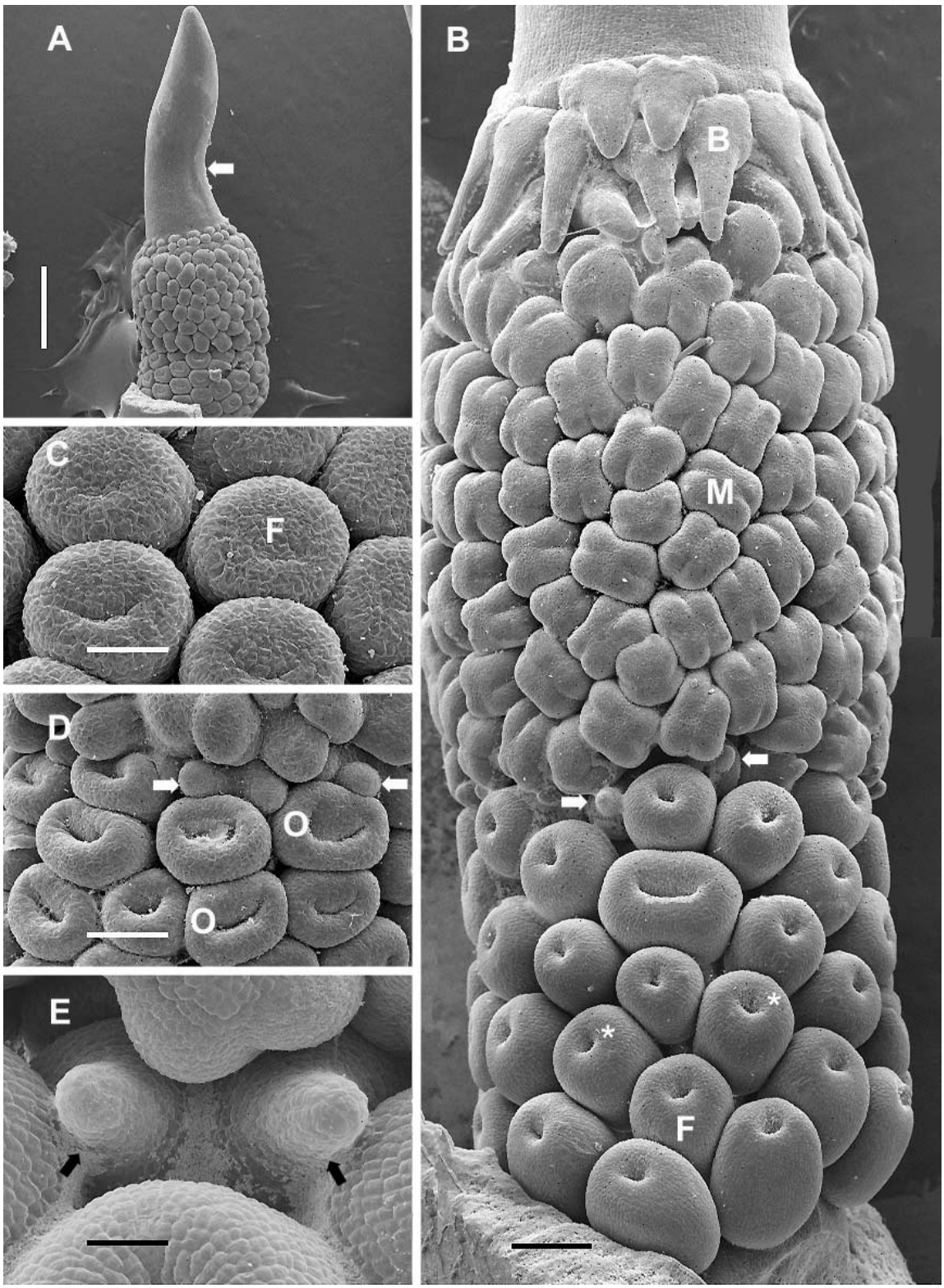


Fig. 3. Stages of development of *Arum italicum* stamens. (A) Early initiation of stamens (St). Bar = 75 µm. (B) Dense arrangement of stamens (St) at a later stage of development; individual male flowers cannot be distinguished. Bar = 75 µm. (C) Early stage of development of pollen sacs (arrows). Bar = 150 µm. (D) Nearly mature stamens with well-developed pollen sacs (arrows). Bar = 300 µm. (E) Formation of globular mass of calcium oxalate crystals (arrow) on the surface of young stamens. Bar = 75 µm. (F) High magnification of the epidermal surface of a stamen showing a closeup view of the mass of crystals (arrows) before their release. Bar = 15 µm.

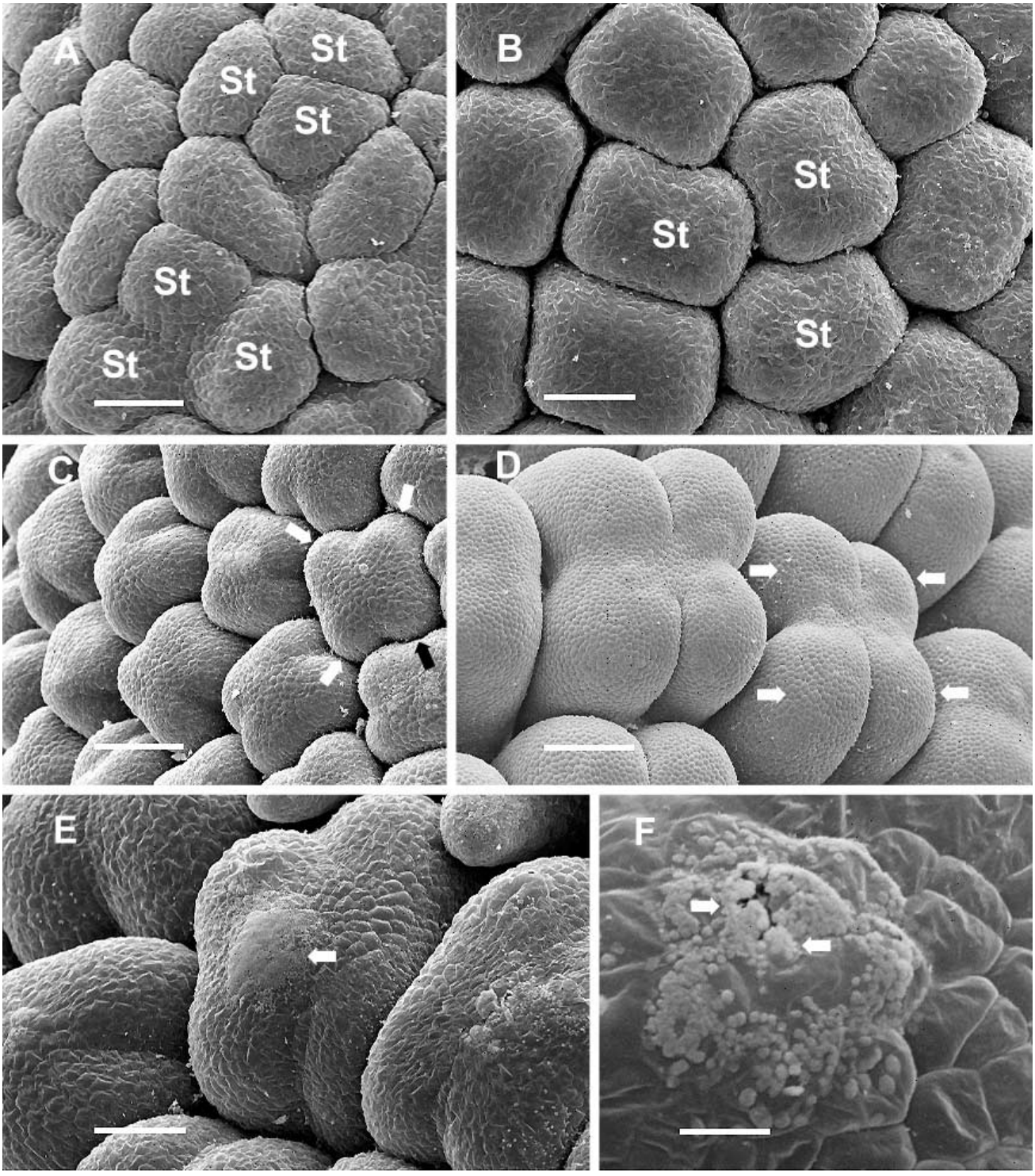
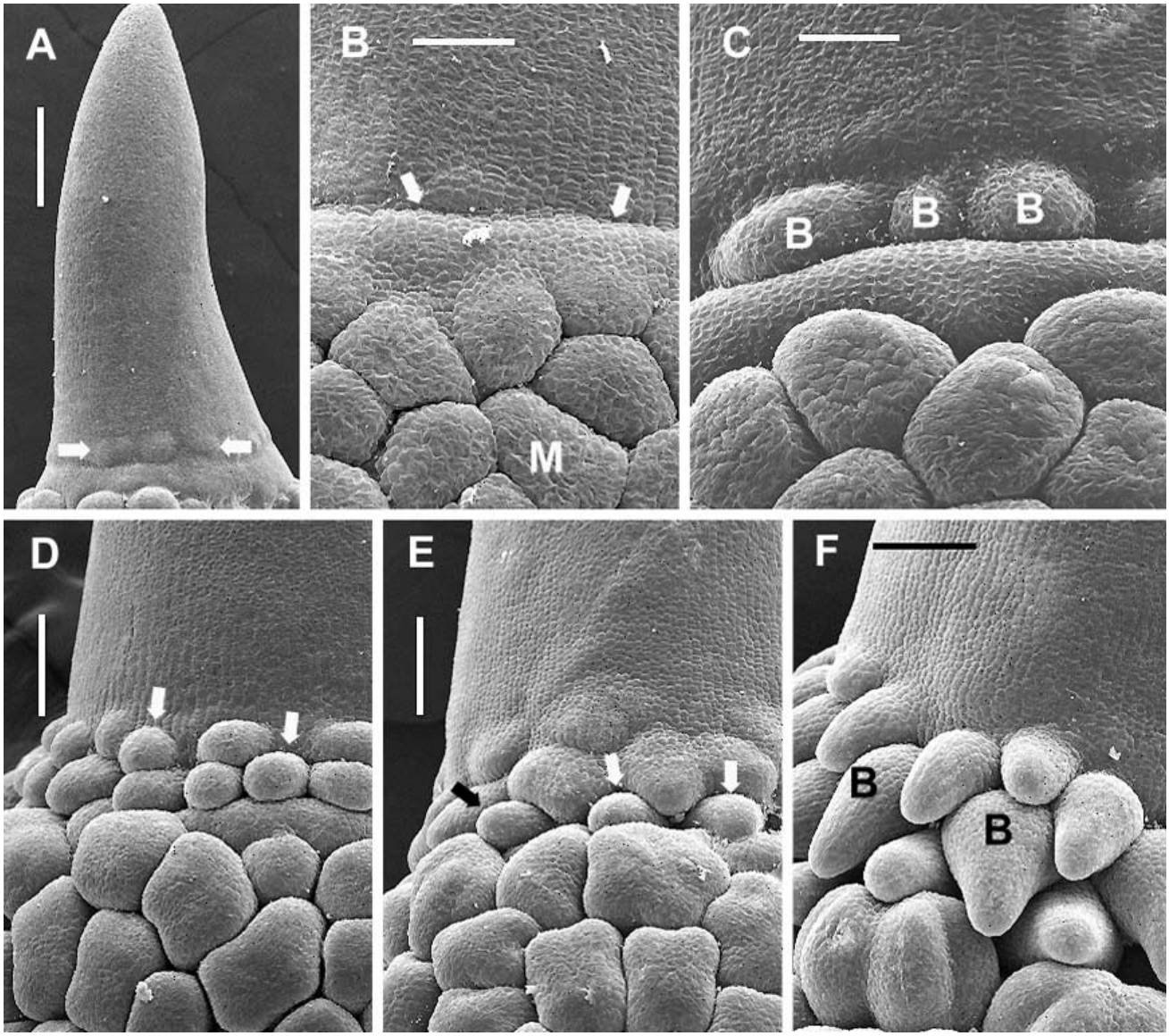


Fig. 4. Stages of development of *Arum italicum* bristles. (A) Tip of a young inflorescence showing the location of the zone of bristles (arrows). Bar = 300 µm. (B) Initiation of the rim (arrows) above the male zone (M). Bar = 150 µm. (C) Initiation of the first row of bristles (B) above the rim. Bar = 75 µm. (D) Initiation of another whorl of bristles (arrows) above the rim. Bar = 150 µm. (E) Early stage of differentiation of the rim into bristles (arrows). Bar = 150 µm. (F) Elongation stage of bristles. Bar = 150 µm.

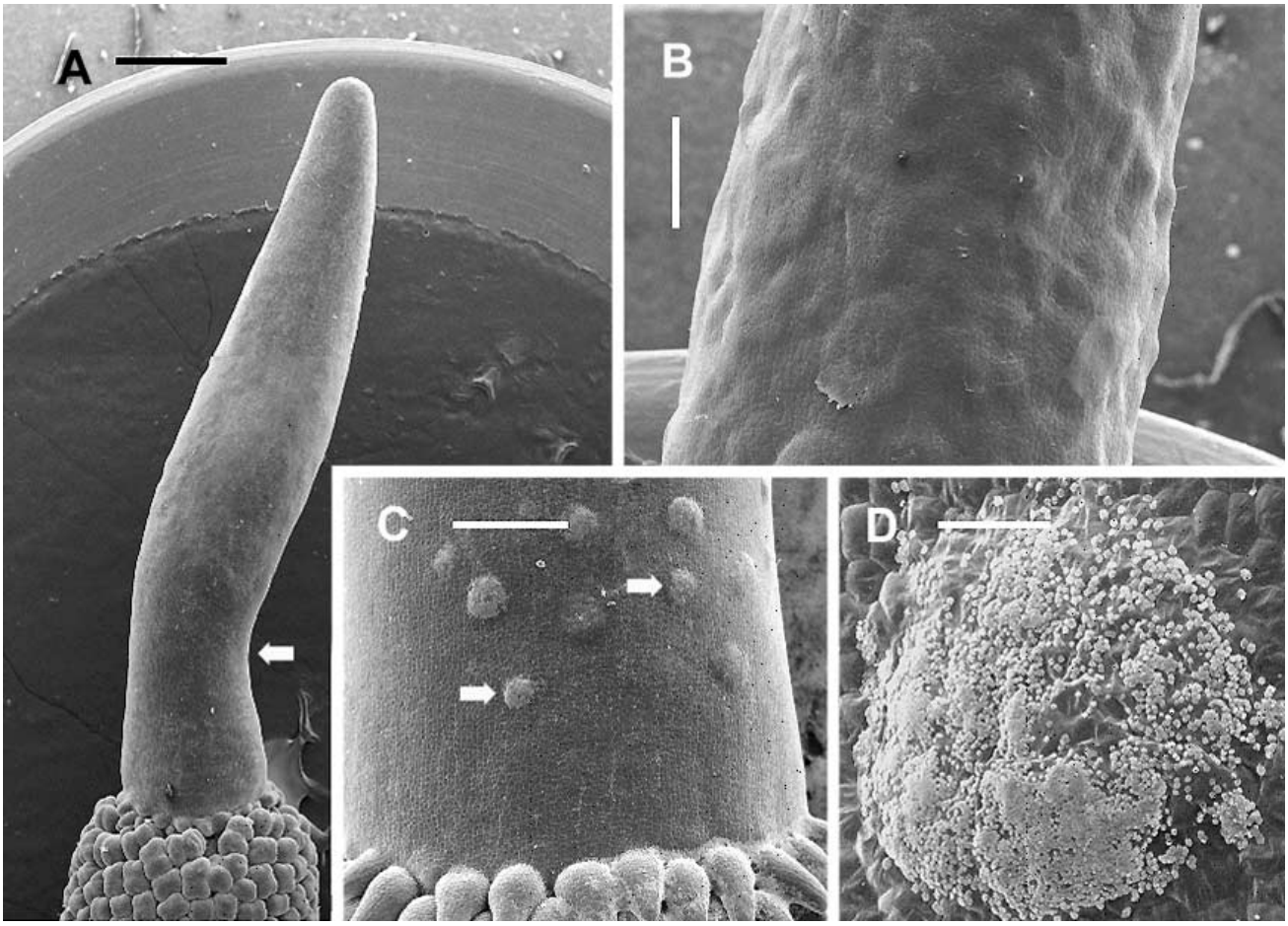


Gleditsia (Borchert 1984), *Nymphaea* (Franceschi and Horner 1980; Kuo-Huang 1992), *Draceana* (Fink 1991*b*; Pennisi et al. 2001), and *Sempervivum* (Fink 1991*b*; Vladimirova 1996).

D'Arcy et al. (1996) reported the presence of extracellular calcium oxalate crystals (referred to as oxalate packages) mixed with pollen in some members of Araceae, such as *Anthurium*, *Calla*, and *Zantedeschia*. However, there is no description in this report as to where the oxalate packages are produced, nor is there any indication of the mode of liberation of crystals.

Recent developmental studies have shown that extracellular calcium oxalate crystals are visible on the surface of the apical portion of nearly mature stamens of selected species of *Philodendron* (Barabé and Lacroix 2001). These exudates form a more or less globular mass on the epidermal surface. In all species of *Philodendron* examined to date, the accumulation of extracellular calcium oxalate crystals on the surface of stamens takes place during young stages of development, before the formation of the stigma on female flowers and before the release of pollen. In *Philodendron*, the presence of oxalate packages was not observed on mature stamens when the spathe opened. However, free crystals were observed on the stigmatic surface in *P. melinonii* and *P. ornatum* (Barabé and Lacroix 2000). In *A. italicum*, the accumulation of extracellular calcium oxalate crystals also occurs during early stages of development of stamens (Fig. 3E), staminodes, and the sterile appendix (Fig. 5C). As in *Philodendron*, the oxalate packages accumulate under the cuticular surface and form a flattened globular mass (Fig. 3E). The extracellular calcium oxalate crystals observed in these genera are similar to those reported for *Wel-*

Fig. 5. Morphology of a young *Arum italicum* appendix. (A) Arrow showing the separation between the appendix proper and the basal stipe portion. Bar = 750 µm. (B) Verrucose surface of the appendix. Bar = 300 µm. (C) Globular masses of calcium oxalate crystals (arrows) on the surface of a young appendix. Bar = 300 µm. (D) High magnification of a mass of crystals. Bar = 30 µm.



witschia (Scurfield et al. 1973) and *Tsuga* (Gambles and Dengler 1974). However, in the latter two cases, the crystals do not seem to be released in clusters.

In the genus *Stelis* (Orchidaceae), extracellular crystals have been interpreted as the product of pseudonectaries (Chase and Peacor 1987). Daumann (1930, 1970) reported the production of nectariferous fluid droplets by scales on the spadix of *A. italicum*. Based on our observations, we have not been able to relate the presence of extracellular crystals to a pseudonectary or stomata in *A. italicum*.

As noted by D'Arcy et al. (1996, p. 179), "...oxalate package bearing anthers are found in plants living under a wide range of environments...displaying a variety of forms...and facing a wide range of interactions...". This occurrence is also true of plants within the family Araceae where calcium oxalate packages are found in epiphytic (e.g., *Anthurium*), hemiepiphytic (e.g., *Philodendron*), or terrestrial plants (*Arum*, *Schismatoglottis*). Additionally, the production of extracellular calcium oxalate crystals in Araceae is not restricted to stamens. It also occurs, depending on the genera, on staminodes (e.g., *Schismatoglottis*, data not shown) or sterile appendices (*Arum*).

It is plausible to think that in Araceae, as is the case for other families, the oxalate packages are a physiological consequence of the removal of oxalate that may otherwise accumulate in toxic quantities in the plant (Franceschi and Horner 1980). The calcium oxalate packages may also play a biotic role in inhibiting herbivory (Franceschi and Horner 1980) or enhancing pollination (D'Arcy et al. 1996). On the other hand, we know that aroid tissues contain large amounts of druses or raphides. In that context, the advantage of producing extracellular oxalate packages is not evident. In cases where extracellular crystals are produced at anther dehiscence (*Schismatoglottis*, unpublished results), we can hypothesize that oxalate crystals could be related to pollination by providing a visual signal or a scent interesting to insects (D'Arcy et al. 1996).

In *Arum* and *Philodendron*, the presence of extracellular calcium oxalate crystals does not appear to be related to a dehiscence mechanism. The crystals are exuded on the epidermal surface and are released during early stages of development, long before anther dehiscence and the formation of the stigma occur. The production of crystals therefore appears to be morphologically independent of anther dehiscence. However, it is also possible that released crystals could remain enclosed in the spathe until the dispersal of

pollen. Therefore, without testing for the presence of crystals mixed in pollen samples, an effective presence of oxalate packages during pollination cannot be dismissed.

Development

The development of sterile flowers in *Arum* is very different from that of female and male flowers. There are no atypical flowers that are morphologically intermediate between male and female flowers as in other genera of the subfamily Aroideae (e.g., *Cercestis*, *Philodendron*, *Schismatoglottis*). From a morphological point of view, the structure of the bristles in the inflorescences of *Arum* does not correspond to any type of atypical flowers (unisexual or bisexual) that have been analysed previously (Barabé et al. 2002*a*, 2002*b*). The particular developmental pathway of bristles in *Arum* appears to be already determined at the time of their initiation.

Floral development in *Arum* begs the following question: what is the morphological nature of the sterile flowers (bristles)? Boyce (1993) considered bristles located between the male and the female zone as pistillodes and those located above the male zone as staminodes. In different species of *Arum* (Boyce 1993), a morphological transition between typical female flowers and pistillodes can be observed. The overall morphology of transitional forms of bristles indicates that the sterile flowers located between the female and male zones could correspond to underdeveloped female flowers. However, we did not observe intermediate structures between typical stamens and staminodes or pistillodes. This is very different from what occurs in other genera with unisexual flowers (e.g., *Cercestis*, *Philodendron*, *Schismatoglottis*) where intermediate forms between typical stamens and female flowers occur frequently. This indicates that developmental constraints (genetic or physiological) experienced by the floral primordia in the intermediate zones are not exactly the same for all genera of Aroideae. Additionally, we did not observe any residual ovary or stigma on these structures as in atypical flowers of *Schismatoglottis* (D. Barabé et al., data not shown). The basal portion of both types of sterile flower in *Arum* is covered by meristematic structures that look like callus. The function of these particular structures is not known. However, this indicates a similar type of developmental potentiality between the bristle-like staminodes and the bristles-like pistillodes. Based on the corresponding morphological structure of the bristles, we can formulate the hypothesis that those located above and beyond the male zone have the same morphological nature. However, based on developmental morphology, it is not possible to determine with certainty in both cases if these atypical flowers correspond to aborted or modified female or male flowers.

A solution to this problem is to examine the anatomy of sterile appendices. In *Theriophonum infaustum* N.E. Br., another Aroideae, the bristles designated as neutral flowers located between the male zone and female zones are vascularized by a single unbranched vascular bundle, as in stamens (Sivadasan and Wilson 1997). Based on this similarity, Sivadasan and Wilson (1997) postulated that the neutral flowers represented aborted male flowers. If we apply this methodology to the sterile flowers of *Arum*, we can come up with clues as to the nature of the bristles. French (1986*b*) stated that in the seven *Arum* species he studied, including *A. italicum*, two to five bundles typically enter a stamen, but some fusion occurs close to the base, leaving only one to three bundles unbranched. Based on photographs published by Eckardt (1937, end plate 16), we can estimate that the ovary wall is vascularized by 11 or 12 vascular bundles. If the bristles correspond to underdeveloped female flowers, we should expect to count a similar number of bundles in them. The observation of cleared material (D. Barabé, data not shown) shows that the bristle-like staminodes of *A. italicum* are vascularized by one or two bundles ($n = 4$), and two or three bundles enter a bristle-like pistillode ($n = 2$). This indicates that the vascular pattern of bristle-like pistillodes is much closer to that of stamens than to that of pistils.

The hypothesis of a hormonal gradient was formulated to explain the presence of atypical bisexual flowers in the inflorescences of *Philodendron* (Barabé and Lacroix 2000; Barabé et al. 2000), *Cercestis* (Barabé and Bertrand 1996), and *Schismatoglottis* (D. Barabé et al., unpublished results). In *Arum*, however, there is no apparent gradient in the sterile zone located above the male zone. There is a discontinuous transition between the male zone, the sterile zone, and the appendix. The study of early stages of development of the inflorescence of *Arum* shows that the upper rows of bristles are completely separated from the male zone by a rim of tissue. At these stages, the floral zone of the inflorescence of *Arum* can be divided temporally and spatially into two morphogenetic portions: a basal part consisting of the female zone, the intermediate zone, and the male zone and an upper portion, which develops subsequently, with the sterile flowers located above the male zone. In the early stages of development (Fig. 2A), the transition between the different zones of the inflorescence is more or less abrupt and all the zones of the inflorescence appear to be in contact with each other (Fig. 2B). In fact, the visible morphological gradient between the male and female zones is related to the elongation of the intermediate zone during growth. It is plausible to assume that the presence of sterile flowers between the female and male zones is related to the existence of a morphogenetic gradient between the typical female flowers and male flowers (Barabé and Lacroix 2000; Barabé et al. 2000). The hormonal gradient in place during early stages of development will result in the appearance of a visible intermediate zone on mature structures.

The unique morphology of the inflorescence of *A. italicum* widens our knowledge of floral structures in the subfamily Aroideae. This study, in combination with previous work, shows that Araceae in general and the subfamily Aroideae in particular present a great diversity of developmental features relating to floral biology and a unique system for studying the transition of different floral types along the same inflorescence.

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