

DEVELOPMENT OF ANTHERS AND POLLEN IN BRAZILIAN HETEROSTYLIC SPECIES OF *ERYTHROXYLUM* (ERYTHROXYLACEAE): AN ECOLOGICAL APPROACH

LUCIANA DA SILVA^{1*}, CAROLINNY LARA FERNANDES², EDSON SIMÃO³
AND ADRIANA TIEMI NAKAMURA³

¹Post-graduation in Applied Botany, Department of Biology, Federal University of Lavras,
Postal code: 37200-000 Lavras, Minas Gerais, Brazil

²Graduation in Agronomy, Department of Biology, Federal University of Lavras,
Postal code: 37200-000 Lavras, Minas Gerais, Brazil

³Instituto de Ciências Agrárias, Universidade Federal de Uberlândia, Avenida Amazonas s/n, Bloco 2E, Umuarama,
Postal code: 38400-902, Uberlândia, Minas Gerais, Brazil

*Corresponding author's email: bio.luciana@gmail.com

Abstract

This study describes the development of anthers and pollen in heterostylic flowers of two species of *Erythroxylum*. The features of anthers and their developmental pattern, which is of the dicotyledonous type, do not vary according to morphs or species. The pollen grains of both morphs show differences in their morphology, development, and content stored as a reserve source. In both species, thrum (short-styled) flowers presented bicellular pollen grains, while pin (long-styled) ones yielded bi- and tricellular pollen grains. The presence of inviable pollen grains in the two flower morphs of both species and the malformation of one of the four microsporangies in the thrum flowers of *E. campestre* are noteworthy. We verified that features found both in the anther wall and in the pollen grains could be related to the reproductive success and adaptation of the species to the *Brazilian savanna* biome.

Key words: Tapetum, Distyly, Microsporogenesis, Microgametogenesis.

Introduction

Erythroxylaceae comprises four genera: *Aneulophus*, *Nectaropetalum*, *Pinacopodium*, and *Erythroxylum*, totaling nearly 250 species. *Erythroxylum* is the only genus whose representatives grows in Brazil, with nearly 100 species (Souza & Lorenzi, 2012), 25 of which are native to this country (Barros, 1998) and commonly found in Brazilian savanna (*cerrado*) biomes (Souza & Lorenzi, 2012). The species of this genus stand out by their heterostylic flowers. Heterostyly is a type of genetically controlled floral polymorphism in which hermaphrodite flowers present different style and filament lengths and can be distylic (thrum morph - short-styled and pin morph - long-styled), such as *Erythroxylum* species, or tristylis (short-, mid-, and long-styled morphs). Plants with heterostylic flowers present a diallelic, sporophytic self-incompatibility system that impedes intra-morph fertilization and promotes cross-pollination (Ganders, 1979b).

Ganders (1979a) reported heterostyly in *Erythroxylum coca* (from which cocaine is extracted) and verified differences between morphs, mainly in terms of quantity and size of the pollen grains produced. The floral dimorphism can be detected in the exine sculpture, in the pollen starch, in pollen color, in stigma papillae, and in corolla morphology and size (Ganders, 1979b).

Plants whose flowers show heterostyly can break the incompatibility system and evolve towards the emergence of individuals of different sex (Gibbs, 1990; Barret, 2002). A good example of this is *Erythroxylum havanense* Jacq. Some researchers have reported male sterility in thrum flowers, demonstrating that this species is probably evolving towards dioecy (Dominguez *et al.*, 1997; Ávila-Sakar & Dominguez, 2000; Cuevas *et al.*, 2005; Rosas *et al.*, 2005; Rosas & Dominguez, 2009). On the other hand, according to level of male sterility, distyly is stable in *Erythroxylum suberosum* A.

St.-Hil., a species from Brazilian savanna (Del-Carlo & Buzato, 2006).

The species of *Erythroxylum* are among the most representative of the Brazilian *cerrado* (Felfili *et al.*, 2008). Thus, assuming that heterostylic flowers present differences in pollen morphology, the aim of this study is to compare the development of anthers and pollen grains in *Erythroxylum campestre* A. St.-Hil. and *E. suberosum* A. St.-Hil. to highlight similarities and differences between floral morphs and species. The following question is addressed: can the anther and pollen grain morphological features influence the reproductive success and the adaptation of these species of *Erythroxylum* to the *cerrado* environment?

Material and Methods

Plant material was collected in fragments of *campos rupestres* (rocky outcrops) in areas close to the Parque Florestal Quedas do Rio Bonito, county of Lavras, in the mesoregion of Campo das Vertentes, state of Minas Gerais, Brazil. Vouchers are housed in the ESAL (Herbarium of the Department of Biology of the Federal University of Lavras) under the following codes: *E. campestre* 27328 and *E. suberosum* 27324.

Flower buds at various stages of development and flowers at anthesis and post-anthesis were fixed in Karnovsky's solution (Karnovsky, 1965) and conserved in ethanol 70%. To prepare permanent slides, samples were dehydrated in a series of ethanol, infiltrated and embedded in hydroxyethyl-methacrylate Leica®, cut to 3-5 µm serial longitudinal and cross sections using a rotary microtome, stained with Toluidine Blue (O'Brien *et al.*, 1964) and mounted in Permount®. The slides were then examined under light microscopy (LM) and images were acquired with a trinocular microscope (Zeiss Lab. A1) coupled to an image capture software (AxioCam ERc5s).

To characterize pollen shape at presentation time and detect the presence of “pollenkitt”, pollen grains were removed from dehiscent anthers and submitted, immediately after collection, to tests with immersion oil, water, Lugol, and Sudan III (Pacini & Hesse, 2005) and observed under light microscopy (LM), following the methodology described by Pacini *et al.* (2006). The terminology adopted to describe pollen grains with regard to their hydric status follows Pacini *et al.* (2006). To verify pollen viability, smashed anthers were stained with Alexander’s stain (Alexander, 1980) and observed under light microscopy (LM). Purple- and green-stained (aborted pollen, lacking protoplasm) pollen grains were considered as viable and inviable, respectively.

Results

Flower morphology: The species of *Erythroxylum* (*E. campestre* – Figs. 1A, B; and *E. suberosum* – Figs. 1C, D) present thrum (Fig. 1E) and pin (Fig. 1F) hermaphrodite flowers, which are cyclic, pentamerous, and complete. The five sepals and petals are free from one another and from whorls. The androecium is composed of five antisepalous and five antipetalous stamens (Fig. 1E, F), whose filaments are only basally connate. All stamens of thrum flowers have the same length (Figs. 1A, C, E), while those of pin ones form two levels (Figs. 1B, D, F), since the antisepalous stamens have shorter filaments than the antipetalous ones. The gynoecium is tricarpetar, trilocular, with free styles and stigmas (Figs. 1E, F). Ovaries present only one locule with a single ovule of parietal placentation. The others loculi do not house any ovule.

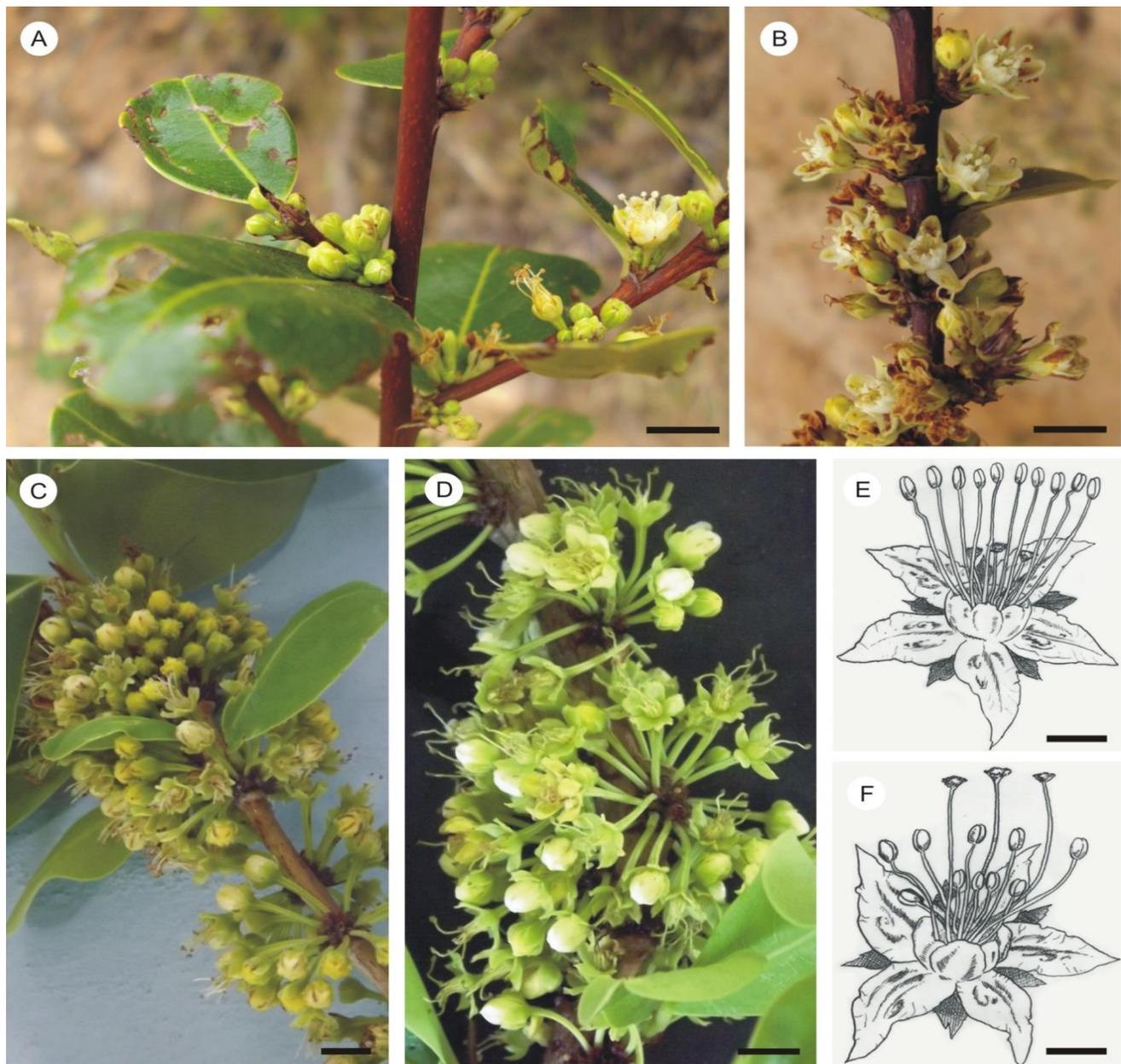


Fig. 1. Morphology of the two species of *Erythroxylum* studied. A and B: *E. campestre*, branches bearing thrum (short-styled) and pin (long-styled) flowers, respectively. C: Branch of *E. suberosum* with thrum flowers. D: Branches of *E. suberosum* with pin flowers. E: Thrum flower of *E. campestre* with all the stamens at the same height. F: Pin flower of *E. campestre* with stamens on two levels. E, F: Flowers illustrated by Alexandre Reis. Bar: 1 cm (A, B, C, D); 0.25 cm (E, F).

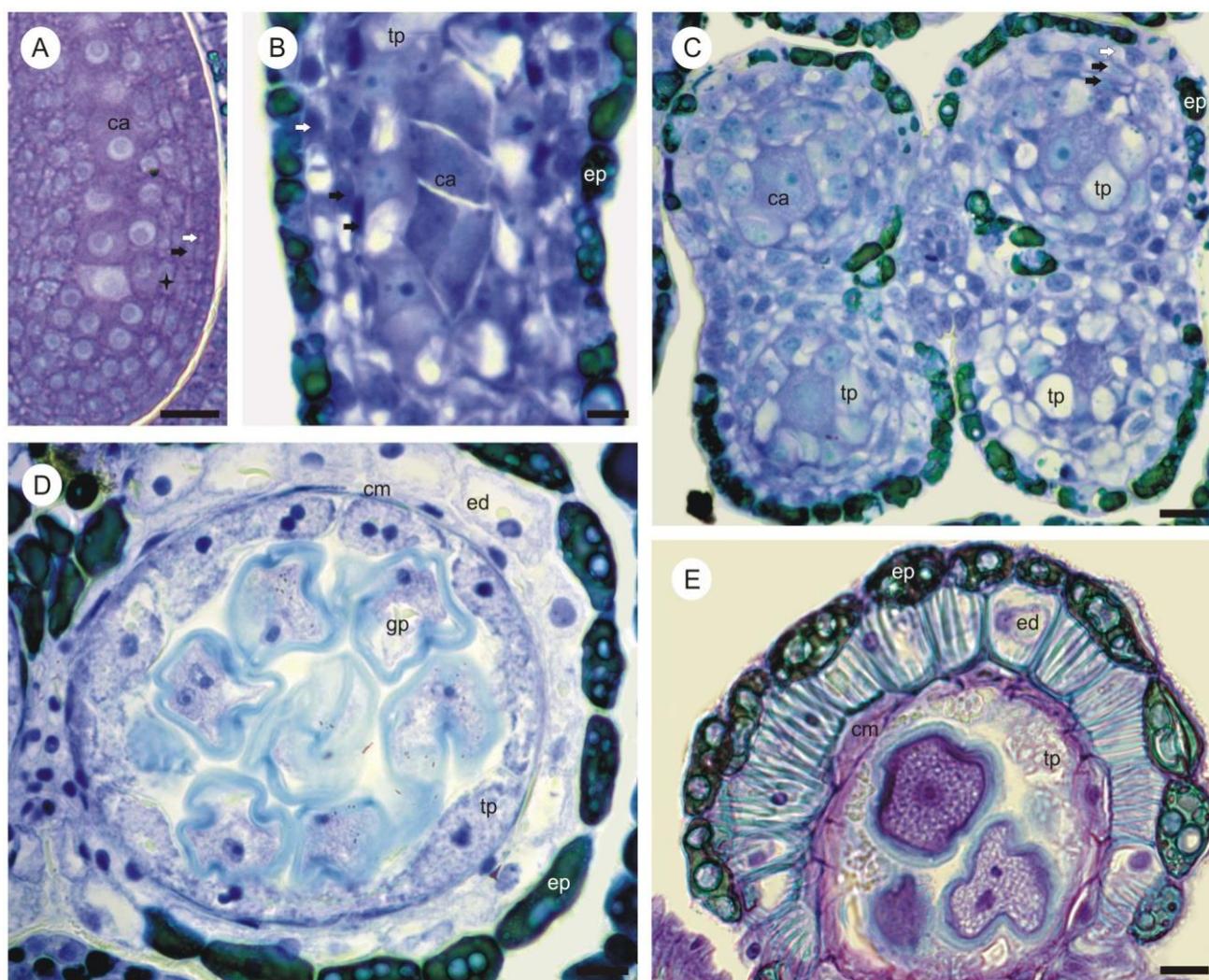


Fig. 2. Anther wall development in *Erythroxylum*. Longitudinal (A, B) and cross sections (C, D, E). A: Stratification of anther presenting four parietal layers in *E. suberosum*. B: Anther of *E. campestre* with five parietal layers and archesporial cells. C: Bitheous, tetrasporangiate anther of *E. campestre*, with five parietal layers and archesporial cells. D: Detail of the microsporangium of *E. campestre* with binucleate secretory tapetum. E: Endothecium thickening in *E. campestre*. (ca: archesporial cell; cm: middle layer; ed: endothecium; ep: epidermis; gp: pollen grain; tp: tapetum; star: tapetum; white arrow: endothecium; black arrow: middle layer). Bars: 10 μ m (B), 20 μ m (A, C, D, E).

Anther wall: Anther wall development is of the dicotyledonous type. Below the protoderm, the primary parietal layer divides periclinally resulting in an outer and an inner secondary parietal layers. The outer secondary parietal layer divides to form the endothecium and the middle layer (Fig. 2A). The latter divides again and forms two strata (Figs. 2B, C). The secondary inner stratum directly differentiates into the tapetum (Fig. 2B). The meristematic tissue subjacent to the inner secondary parietal layer forms the archesporium (Figs. 2A, B, C). Flower buds of both species show young stamen with bithecal, tetrasporangiate anthers (Fig. 2C), with five parietal layers already differentiated: epidermis, endothecium, two middle layers, and tapetum (Figs. 2B, C).

In young buds, the epidermis is uniseriate composed of different-sized, slightly convex papilla cells with phenolic content (Figs. 2B, C). The endothecium is composed of vacuolated, tangentially elongated cells with evident nuclei (Figs. 2C, D, E). Internally the endothecium, differ two strata of middle layers (Figs. 2B,

C) with thin-walled, tangentially elongated cells. The tapetum consists of one layer of thin-walled, differently shaped cells, a dense, sometimes hyaline, cytoplasm and a large vacuole, and a centrally placed, conspicuous nucleus (Fig. 2C). The tapetum is secretory (Figs. 2B, C).

When the buds differentiate and the flowers reach pre-anthesis stages and anthesis, the epidermis maintains its features while the endothecium cells become cuboid to slightly radially elongated and acquire secondary thickenings (Fig. 2D, E). Endothecial cells have only one nucleus and develop a central vacuole that confers their hyaline aspect (Fig. 2E). The middle layers degenerate at the end of anther development. During the phase of tetrad formation, the tapetum cells become binucleate. Later, their volume diminishes and they become tangentially elongated to cuboid, the cytoplasm continues dense and binuclear, and the nuclei reduce in size (Fig. 2D). Later, when pollen grains are already completely differentiated, tapetum cells are no longer observed. No orbicules were observed during tapetum differentiation.

Microsporogenesis and microgametogenesis: Longitudinal sections of young anthers show that archesporial cells show dense cytoplasm and conspicuous nuclei (Fig. 3A). They divide once through mitosis to give rise to microsporocytes, or microspore mother cells, which undergo meiosis to produce a dyad (Fig. 3B) and, later, a tetrahedral microspore tetrad (Fig. 3C). As there is no wall formation after each nuclear division, microsporogenesis is simultaneous. After this phase, cytokinesis occurs and callose deposition separates microspores from each other (Fig. 3D). At this stage, the sporoderm already begins to develop (Fig. 3E). Later, callosic walls dissolved thus releasing the microspores.

Microgametogenesis begins and cells undergo asymmetric mitosis to yield bicellular pollen grains (Fig. 3F). At this point, gametogenesis begins to vary according to morphs.

In the thrum morph, cytokinesis produces a large vegetative cell and a small generative one. The former has a spherical, central nucleus and it occupies most of the young gametophyte, while the latter is found at the periphery of the vegetative one. Later, the generative cell migrates towards the central area, getting closer to the vegetative cell nucleus. The generative cell presents a dense cytoplasm and an evident nucleus (Fig. 3F).

In the pin morph, this process also occurs, but in some cases, the generative cell divides to give rise to two spermatid cells thus producing tricellular pollen grains (Fig. 3G).

Therefore, bi- and tricellular pollen grains are observed in the mature anther of pin flowers, while only bicellular pollen grains were seen in those of thrum flowers.

Pollen grains: Both pin and thrum flowers have pollen grains released in monads (Fig. 4) but can form agglomerates of two, three, or more pollen grains (Figs. 4C, E).

The pollen grains are harmomegathic, partially dehydrated or orthodox because in immersion oil, they are sub-spheroidal and polarly elongated (Figs. 4A, B), when immersed in water they hydrate and become rounded (Figs. 4C, D) and when observed in oil/water their shape changes.

The pollen of both morphs react positively to Sudan III, proving the presence of pollenkit (Fig. 4E, F). In pin flowers (Figs. 4G, I), positive reactions to Lugol show that the carbohydrate reserve of pollen grains is amylaceous. In the thrum flowers, the vast majority of pollen grains had no starch reserve (Figs. 4H, J).

The pin flowers produce a greater quantity of well-formed, circular pollen grains with a dense cytoplasm (Figs. 5A, C) than thrum flowers, that are visually smaller in both *E. campestre* and *E. suberosum* (compare Fig. 4 G and H, I and J).

The thrum flowers of *E. campestre* (Fig. 5B) and *E. suberosum* (Figs. 5D, E) produce a greater quantity of inviable pollen grains than the pin flowers. Out of 250 randomly chosen grains of the thrum morph of *E. suberosum*, 177 (71%) were unviable and 73 (29%) were viable, while, in the pin morph, 210 (84%) were viable and 40 (16%) unviable.

Furthermore, in the thrum morph of *E. campestre*, one of the four microsporangies can atrophy, and one of the anther locules sometimes has a hypertrophied tapetum occupying the whole locule, where, therefore, no pollen grains can fit (Fig. 5F). On the other hand, in *E. suberosum*, no malformations of anthers were observed.

Discussion

Adaptations to Brazilian savanna (cerrado): The convex to papillate shape of the epidermal cells of the anthers of both floral morphs in both species suggests an increase in light absorption (Vogelmann, 1993). In Brazilian savanna environments, papillate epidermis can maximize water loss, contributing to anther opening and pollen grain presentation, thus to the maturation of the anther. The dehydration mechanism usually involves the evaporation of the water contained in the anthers or its migration towards other flower parts (Heslop-Harrison *et al.*, 1987; Pacini, 1990), and water loss causes anthers to open in the hottest hours of the day (Pacini & Hesse, 2004).

The flowers of *E. campestre*, *E. suberosum* and *E. tortuosum* of Brasília cerrado open between 6:00 and 18:00 and are visited by insects often from 9:30 to 11:30, when it is hot (Barros, 1998). Therefore, papillae can play a key role in the reproduction of these species of *Erythroxylum*. In addition, cuticle thickness influences the mechanism of anther opening and pollen presentation (Pacini & Hesse, 2004). Thus, the thin cuticle observed in the anthers of species of *Erythroxylum* can favor water loss into the environment and pollen maturation.

The great quantity of phenolic compounds stored in the anther epidermis of the two studied species of *Erythroxylum* and also in several plant organs (Bieras & Sajo, 2004a; 2004b) is also noteworthy. The accumulation of such substances in vegetation growing on poor soils, as those of Brazilian savannas, is due to the high cost to replace the material lost through the action of phytophagous animals, which provokes a deviation in the biosynthetic pathways to produce defense compounds (Varanda *et al.*, 1998). Phenolic compounds are also considered as a type of chemical defense against herbivory (Swain, 1979), especially in the Brazilian savanna.

Another important character observed in the anther of the studied species of *Erythroxylum* is the secretory tapetum, which is common in eudicotyledons, and degenerates during the free microspore and pollen grain development stages (Furness & Rudall, 2001). In the studied species of *Erythroxylum*, the persistence of this tissue in almost ripe anthers may be explained by the presence of pollenkit that is deposited at the end of pollen grain development (Pacini & Hesse, 2005).

Pollenkit can have many functions and it is deposited on and between the exine cavities (Pacini & Hesse, 2005). In the studied species of *Erythroxylum*, it presumably helps maintaining the sporophytic proteins, which are responsible for pollen-stigma recognition (Pacini & Hesse, 2005). In distylic plants, self-incompatibility is sporophytic and pollen specificity is determined by the diploid genotype of the sporophyte (mother plant), a function exerted by pollenkit (Ganders, 1979a).

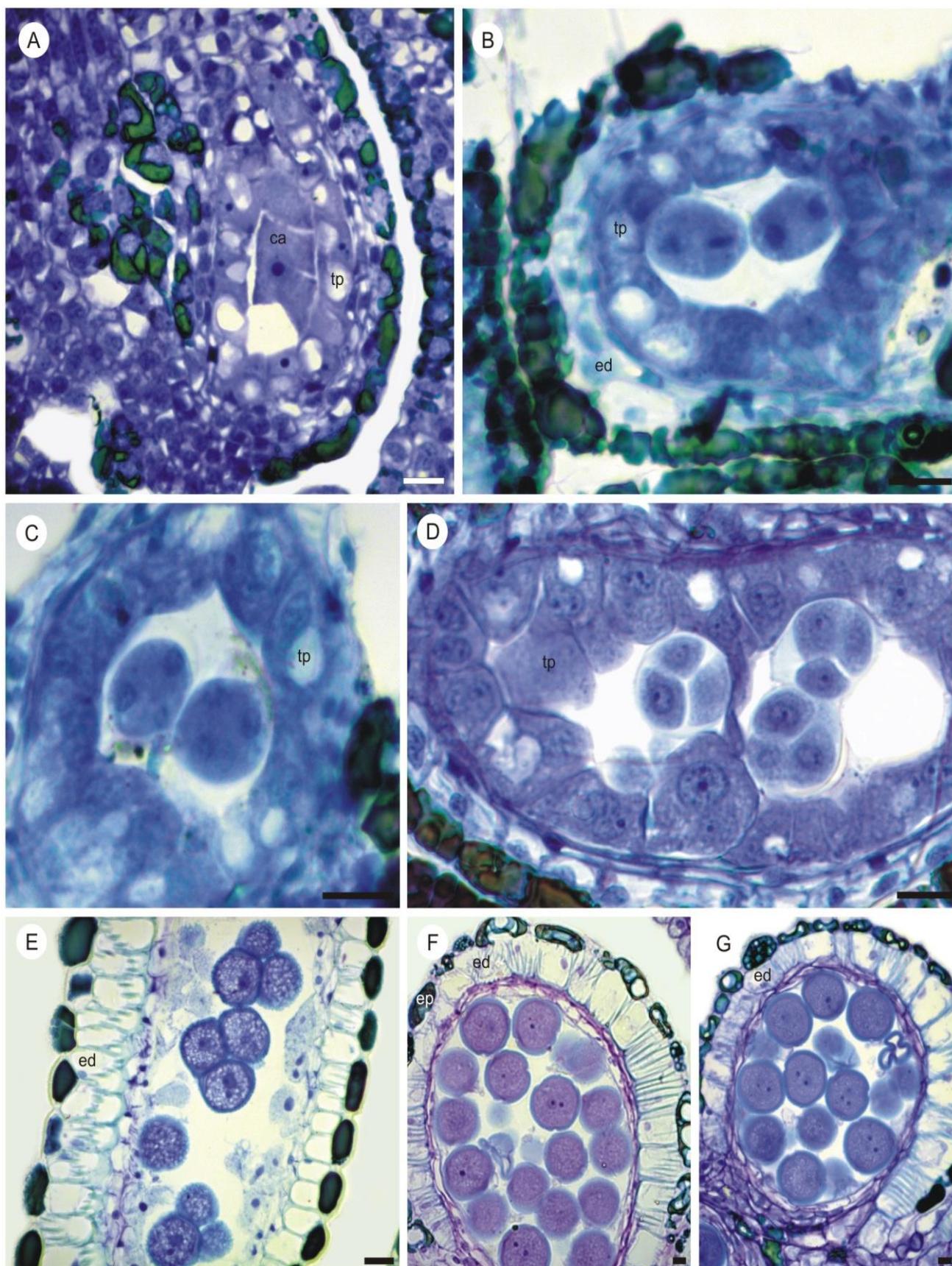


Fig. 3. Microsporogenesis and microgametogenesis in *Erythroxyllum*. Cross (B, C, D, F, G) and longitudinal (A, E) sections of anthers. A: Archesporial cells in *E. campestre*. B: Microspore dyads in *E. campestre*. C: Tetrahedral microspore tetrad in *E. suberosum*. D: Tetrad cytokinesis and detail of binucleate secretory tapetum in *E. campestre*. E: Microspores about to separate in *E. suberosum*. F: Bicellular pollen grains in thrum flower of *E. suberosum*. G: Bi- and tricellular pollen grains in pin flower of *E. suberosum*. Bars: 20 μ m (A, B, C, D and E); 10 μ m (F and G).

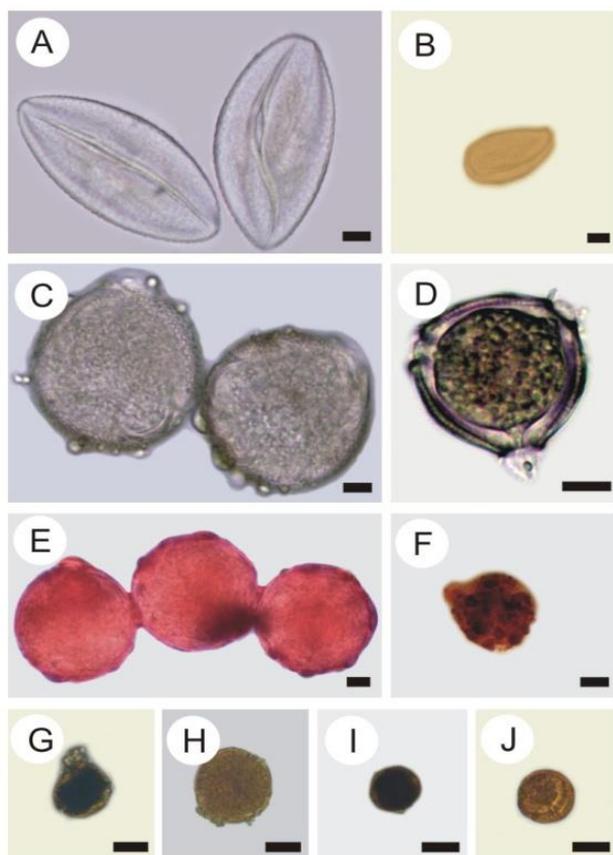


Fig. 4. Pollen grains of *E. campestre* (A, C, E, G, H) and *E. suberosum* (B, D, F, I, J). Immersed in oil (A, B). Immersed in water (C, D). Stained with Sudan III (E, F). Stained with Lugol (G, H, I, J). Pin flower (G, I). Thrum flower (H, J). Bars: 10 µm (A, B, C, D, E, F); 20 µm (G, H, I, J).

The pollenkitt found in the pollen grains of both flower morphs of *E. campestre* and *E. suberosum* must be related to the entomophily in the group, as already reported by Barros (1998), who established that flowers of *Erythroxyllum* species are visited by wasps, bees, and flies, independently of morphs. The absence of orbicules in the species of *Erythroxyllum* studied here corroborates that pollinators are insects, but not wind (Pacini & Franchi, 1993). An advantage of the pollen grains of entomophilous plants is their longevity, since they can last from days to months (Ackerman, 2000). Although we do not know the exact longevity of the pollen grains of *Erythroxyllum*, the entomophily (Barros, 1998; Barbosa & Sazima, 2008) suggests that the pollen grains of its species have a very long viability, which favors cross-pollination.

The presence of pollenkitt also suggests that the pollen grains of *Erythroxyllum* are probably scattered in agglomerates, since pollen grains dispersed individually do not show pollenkitt. Dispersal in agglomerates can affect the distance covered by pollen grains (Pacini & Hesse, 2005) - the higher the number of grains in the agglomerate, the smaller the distance from the mother plant and vice-versa - in addition to allow a higher protection against desiccation and damages caused by UV light (Eisikovitch *et al.*, 1987).

The natural landscape of the studied species of *Erythroxyllum* includes only small trees, so that canopies were open and the individuals of *E. campestre* and *E.*

suberosum received direct solar radiation, common features of Brazilian savannas (Gurevitch *et al.*, 2009). In addition, plants of *Erythroxyllum* species can form dense groupings (this study, Barros, 1998). The pollen dispersion in agglomerates could be an efficient reproductive strategy because of the smaller distance traveled by a great number of pollen grains, which would be protected from desiccation and the influence of solar rays.

The difference in pollen grain morphology observed in oil and in water allows to classify them as orthodox (Pacini *et al.*, 2006). The water content is related with ecological and physiological strategies, and pollen behaves differently to the relative humidity of the environment (Franchi *et al.*, 2011). Water balance is critical to pollen grain viability since usually the lower the water content, the less active the pollen metabolism. This characteristic confers greater resistance to plants in hostile environments, with high temperature and low relative humidity (Guarnieri *et al.*, 2006), as Brazilian savannas, where the studied species of *Erythroxyllum* grow.

Heterostyly: In both *E. campestre* and *E. suberosum*, the pollen grains of thrum flowers are visually bigger than those of pin ones (Ganders, 1979b; Barros, 1998; present work), as found in the distylic species *Vismia guianensis* (Clusiaceae) (Santos & Machado, 1998). This fact was attributed to the lower stigmatic surface of thrum flowers (Santos & Machado, 1998). Additional studies are needed in *Erythroxyllum* species to show a relation between pollen size and stigmatic surface.

The presence of starch grains in pollen varies among the floral morphs of *Erythroxyllum campestre*, *E. suberosum* (present study), and *E. havanense* (Vázquez-Santana *et al.*, 1996). This carbohydrate was not detected in a vast majority of pollen grains housed in the anthers of thrum flowers, whereas in pin flowers, most pollen grains had starch as a reserve source. Starch is totally or partially converted into other carbohydrates before anther opening, and these can modify the internal turgor pressure of the microgametophyte, allowing pollen to be more resistant in hostile environments (Pacini *et al.*, 2006), as previously discussed. As for the pollen grains of pin flowers, they may remain viable in the environment for a longer period, because of their harmomegathy provided by the starch they contain.

Our results show that the studied species present bi- (pin and thrum flowers) or tricellular (pin morph only) pollen. Narayana (1960) reported that the pollen grains of family Erythroxyllaceae, in their dispersal phase, are bicellular and Vázquez-Santana *et al.* (1996) corroborated this information in *E. havanense*. On the other hand, Narayana (1964) and Rao (1968) described tricellular, mature pollen grains. This divergence in the literature may be due to the fact that only one of the morphs was observed, or to a lack of distinction between floral morphs in the studies. Furthermore, the pollen polymorphy (bi- and tricellular pollen grains in the same anther) was reported by Furness (2011) for Euphorbiaceae, Passifloraceae, and Salicaceae, all of which pertain to order Malpighiales. Since Erythroxyllaceae also belongs to this order, it would not be surprising if this family showed this feature. Nevertheless, this is the first report of pollen polymorphy in this family, although it was only verified in pin flowers.

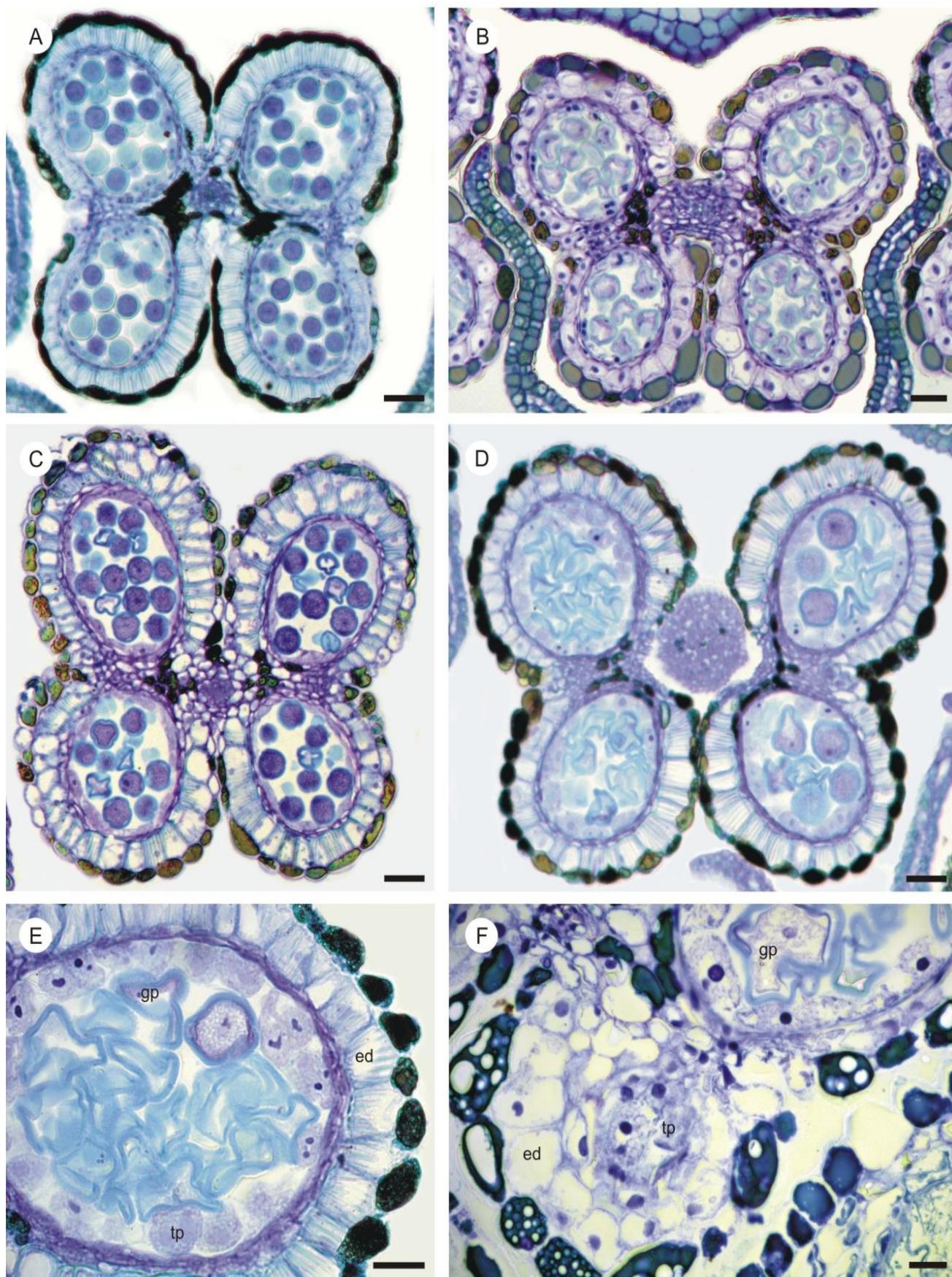


Fig. 5. Anatomical differences between the anthers of both morphs of *Erythroxyllum*. A: *E. campestre*, pin flower. B: *E. campestre*, thrum flower. C: *E. suberosum*, pin flower. D: *E. suberosum*, thrum flower. E: Microsporangium with malformed pollen grains in *E. suberosum*, pin flower. F: Malformed microsporangium in *E. campestre*, thrum flower. (ed: endothecium, gp: pollen grain, tp: tapetum). Bar: 20 μ m (E, F); 50 μ m (A, B, C, D).

We suggest that the pin flowers of the studied species undergo late, continuous dehydration until anther opening, so that tricellular pollen can be produced. In thrum flowers, water loss is more precocious and it fixes pollen development at the bicellular stage, as Lora *et al.* (2009) reported for *Annona cherimola*, a species that presents bi- and tricellular pollen grains in a same anther.

The presence of bi- and tricellular pollen grains can be a reproductive strategy for species. Tricellular pollen grains germinate quicker, so that a competition for the fastest genotype (Dafni & Firmage, 2000) to fecundate the only ovule present in the tricarpelar gynoecium takes place, whereas, bicellular grains remain viable longer although their germination is slower (Hanna & Towil, 1995).

This makes sense when compared to the anthesis in individuals. During collection, we verified that the flowers of thrum plants, which bear bicellular pollen, open before those of pin individuals. Since thrum individuals present pollen grains with longer viability, even though pin individuals open later, their pollen should remain viable. Similarly, when the flowers of the pin morph open, their pollen grains must quickly reach the stigma of already opened flowers. Therefore, the shorter the distance, the shorter the time and the quicker the germination. The dispersal of these pollen grains in agglomerates intensifies the competition for the fastest genotype even more, as already discussed. On the other hand, the bicellular pollen grains of the pin morph could reach individuals at longer distances. Although the difference in anthesis time between pin and thrum individuals in species of *Erythroxylum* has not been previously reported, the earlier opening of flowers in one of the morphs was mentioned by Darwin (1877) in plants of *Primula veris*. However, in this species, thrum morphs begin to open before pin ones. In species of *Erythroxylum*, our observations were incidental and this fact still needs to be carefully studied.

With regard to the occurrence of inviable pollen grains in both morphs of these two species, Del-Carlo & Buzato (2006) reported this characteristic in *E. suberosum*. Maybe because she did not have these data, Barros (1998) considered that the pollen/ovule (P/O) ratio in both morphs of the species of *E. campestre* and *E. suberosum* was high. After taking into account all the pollen grains of anthers of the former species, she reported 12,000/1 and 9,000/1 ratios for pin and thrum flowers in *E. campestre*, respectively. In *E. suberosum*, ratios were 24,000/1 and 18,000/1, respectively. As a comparison, in xenogamous species, Cruden (1977) established a P/O ratio of 5,859/1. Barros (1998) also ratified that the low ratio found in *E. campestre*, when compared to *E. suberosum*, was due to its partial self-compatibility.

The low production of pollen grains in the thrum morph of *E. campestre* may also be explained by the malformation of the anther microsporangium. We observed that some pollen grains are not formed, in addition to tapetum hypertrophy. Changes in the development of flower anther of this morph were also observed by Domínguez *et al.* (1997) in *Erythroxylumhavanense*, although they presented different features. These authors affirmed that short-styled flowers failed to develop fertile androecia because of the abnormal formation of the

sporogenous tissue, absence of tapetum, and malformed microspores. In *E. havanense*, flowers specialized to differentiate male parts in pin morphs and female ones in thrum ones (Ávila-Sakar & Domínguez, 2000), which led these authors to affirm that this species tended to dioecy.

Cuevas *et al.* (2005) described the general patterns of male sterility in *E. havanense* and considered that it can be one of the first steps in the evolution of genus specialization. In the Brazilian savannas, this genus is well represented and adapted, and its heterostyly is stable, as observed by Del-Carlo & Buzato (2006). When they evaluated the degree of male sterility of *E. suberosum*, these authors found no difference in reproductive success between morphs. Barros (1998) verified that the number of fruits yielded by pin and thrum flowers did not vary between species in *E. campestre*, *E. suberosum*, and *E. tortuosum*.

Del-Carlo & Buzato (2006) affirmed that, based on the data they produced, male sterility is apparently well spread among specimens of *Erythroxylum*. However, the species of *Erythroxylum* present tricarpelar, trilocular, and uniovulate gynoecia, i.e. only one of the carpels differentiates into a fertile ovule, while the other two are infertile and no primordia of differentiating ovules have ever been observed (Narayana, 1960; 1964; Rao, 1965; 1968). This indicates that resource allocation in the female parts of flowers is retarded at some moment and for some reason and we observed male sterility, which may be a way to balance the equation to maintain hermaphrodite flowers of this distylic reproduction system in Brazilian savanna species. On the other hand, in the coastal species *E. havanense*, this balance tends to dioecy (Ávila-Sakar & Domínguez, 2000; Rosas *et al.*, 2005; Rosas & Domínguez, 2009). Therefore, wider studies integrating other areas of knowledge as genetics, ecology, physiology, and morphology are needed to enlighten this issue.

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