Floral glands in asclepiads: structure, diversity and evolution

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ABSTRACT

Species of Apocynaceae stand out among angiosperms in having very complex flowers, especially those of asclepiads, which belong to the most derived subfamily (Asclepiadoideae). These flowers are known to represent the highest degree of floral synorganization of the eudicots, and are comparable only to orchids. This morphological complexity may also be understood by observing their glands. Asclepiads have several protective and nuptial secretory structures. Their highly specific and specialized pollination systems are associated with the great diversity of glands found in their flowers. This review gathers data regarding all types of floral glands described for asclepiads and adds three new types (glandular trichome, secretory idioblast and obturator), for a total of 13 types of glands. Some of the species reported here may have dozens of glands of up to 11 types on a single flower, corresponding to the largest diversity of glands recorded to date for a single structure.

Keywords: anatomy, Apocynaceae, Asclepiadoideae, diversity, evolution, flower, secretory structures

Introduction

Apocynaceae is an extremely diverse family in morphological terms, represented by trees, shrubs, herbs and climbers, with single leaves usually opposite, rarely alternate or whorled, with stipules modified in colleters in several species (Endress & Bruyns 2000; Capelli et al. 2017) and with various secretory structures in vegetative and reproductive organs of recognized importance in taxonomy, phylogeny and/or ecology (Thomas & Dave 1991; Demarco 2008). Due to their highly elaborate flowers, the family stands out among the eudicotyledons, especially when considering its most derived subfamily Asclepiadoideae.

The close relationship between the former families Apocynaceae and Asclepiadaceae has always been recognized since its establishment as “Apocineae” by Jussieu (1789). Although Brown (1810) divided it into two families and this separation had been maintained in the subsequent taxonomic studies until recently (Cronquist 1981), many researchers have found a gradation in the morphology of the complex reproductive organs between the two families. Phylogenetic studies carried out mainly during the 1990s have shown that the two families form a monophyletic group, thus constituting a single family (Judd et al. 1994;
Asclepiads have received the attention of researchers for centuries because of their complex pollination system, but few anatomical studies have attempted to unravel the complex floral morphology of the group. The first comprehensive anatomical studies were made by Brown (1810), who described the formation of the translator in *Asclepias syriaca* L., and Corry (1883), Gager (1902) and Frye (1902), all of which described many flower characteristics of *Asclepias* species, especially the gynostegium. Despite the great diversity of species and the long period of study, there is little information available on the anatomy of the species of this group.

The complex floral pollination mechanism of Asclepiadoideae is only comparable to orchids (Endress 2016). These two families present a series of evolutionary convergences that allowed the production and dispersion of pollen aggregate into pollinia. Apparently, a high degree of synorganization of the floral organs seems to have been necessary to allow the evolution of pollinia. In Apocynaceae, the presence of corona has greatly increased the morphological complexity of the flowers (Fig. 1). In addition, the highly complex pollination mechanism seems to have influenced mainly the diversity of clades bearing pollinia in Apocynaceae and Orchidaceae since these clades represent more than half of the species of both families (Endress 2016). The relation of at least some glands with pollination resulted in a large diversity of floral secretory structures and, theoretically, the greater the complexity and/or the specificity of the pollination mechanism, the greater the number of glands that provide this interaction.

### Secretory structures

Among the anatomical characters reported for Apocynaceae, only three are present in Asclepiadoideae and all other members of the family: amphiphloic siphonostele, laticifers and style head. Of these three, the latter two are secretory, and Metcalfe & Chalk (1950) considered the occurrence of laticifers as one of the most important characteristics demonstrating the close relationship between the former Apocynaceae and Asclepiadaceae. In addition, one of the diagnostic features of the family is the style head, which has a secretory epidermis (Judd et al. 2002).

The floral secretory structures found in this group are extremely diverse and distinguish asclepiads as the group with the largest number of glands in a single flower among the angiosperms, which is related to a large extent to the complex reproductive system of this group. The glands reported up to now added to those described in this review are the following: colleters, glandular trichomes, laticifers, secretory idioblasts, nectaries (primary and secondary), osmophores, style head, tapetum, staminal wing gland, extragynoecial compitum, stylar canal and obturator. These structures are detailed later.

### Floral glands in asclepiads

The secretory structures of asclepiads occur in vegetative and/or reproductive organs and are involved in the production of different compounds of the secondary metabolism. They may be classified as protective glands, which play a defensive function, or nuptial glands, associated with pollination.

The protective function is performed by external and internal glands of the flowers, which are also frequently found in the stem and/or leaves; the defensive function is also necessary for vegetative organs. On the other hand, the nuptial glands of asclepiads are exclusive to flowers and serve to attract or provide nutritional resources for the pollinator. In some cases, they are also related to pollen removal and/or pollen adhesion to the stigma, as a stimulus for pollen germination, a guide and nourisher for pollen tubes, etc. All these functions and others are found in the flowers of asclepiads.
Figure 1. Flowers of Asclepiadoideae. (A) Asclepias curassavica L. (B) Peplonia axillaris (Vell.) Fontella & Rapini. (C) Matelea denticulata (Vahl) Fontella & E.A. Schwarz. (D) Oxypetalum banksii subsp. banksii Roem. & Schult. (E-F) Blepharodon bicuspidatum E. Fourn. (F) Longitudinal section of the flower. Abbreviations: C, corona; GR, guide rail; P, petal; S, stigma; SC, stigmatic chamber; SH, style head; arrow, translator.
**Colleters (Fig. 2)**

Colleters are widespread in Apocynaceae (Endress & Bruyns 2000) and occur in flowers of all Asclepiadoideae. In this family, they are calycine emergences (Fig. 2) that produce a viscous secretion which protects the meristems against desiccation (Thomas 1991) and can also protect the flowers against fungal proliferation (Ribeiro et al. 2017).

The position of colleters may be variable (Woodson & Moore 1938), but the asclepiads have colleters alternating with the sepals (Frye 1902; Rao & Ganguli 1963; Tiagi & Dixit 1965; Valente et al. 1973; Silva et al. 1975; Valente 1983; 1984; 1995; Pereira & Schwarz 1983; Endress & Bruyns 2000; Valente & Costa 2005; Demarco 2008), except in Oxytelson esculentum R.Br., which have opposite colleters (Rao & Ganguli 1963). Although alternisepalous colleters have been considered a plesiomorphic feature in Apocynaceae (Woodson & Moore 1938), they occur in almost all members of Asclepiadoideae, which is the most derived subfamily.

Morphologically, the calycine colleters are much more constant than those in the leaf (Demarco 2005). In general, they are classified as the standard type in the family (Thomas 1991), being cylindrical or dorso-ventrally flattened, persistent (Thomas et al. 1989; Thomas & Dave 1989a; b; c; 1991; Thomas 1991; Appezzato-da-Glória & Estelita 2000; Schwarz & Furlan 2002; Demarco 2005; Simões et al. 2006; Martins et al. 2010; Martins 2012) (Fig. 2C), and may be found at the base of fruits (Thomas 1991; Thomas & Dave 1991; 1994). The most frequent variations observed are the presence or absence of peduncles (Fig. 2D) and the number of colleters per flower (Rao & Ganguli 1963; Ramayya & Bahadur 1968; Silva et al. 1975; Stevens 1975; 1988; Pereira & Schwarz 1983; Thomas & Dave 1989a; Schwarz & Furlan 2002; Demarco 2005; Rio et al. 2005; Simões et al. 2006; Martins et al. 2010; Martins 2012). Colleters have taxonomic significance for the family (Woodson & Moore 1938; Thomas 1991; Simões et al. 2006) and their occurrence, type and/or position have been used as diagnostic characters in identification keys at the genus and species level (Barroso 1986; Rio & Kinoshita 2005; Rio et al. 2005).

Colleters are formed early in the ontogeny of sepals, originating from the adaxial side of the connate portion of the calyx (Fig. 2A-B), just below the sinus. Immediately after their formation in the floral meristem, the colleters begin secreting. The secretory portion is composed of a uniseriate palisade epidermis covering a non-secretory parenchyma (Demarco 2005; 2008) (Fig. 2C-D). More than one layer of secretory epidermis has been observed in a few species of other subfamilies of Apocynaceae (Ramayya & Bahadur 1968; Thomas et al. 1989). Secretory cells have dense cytoplasm, and the secretion is accumulated in a periplasmic space before it is released to the outside through the cell wall and cuticle (Ribeiro et al. 2017). According to Fahn (1990), secretion release in colleters usually occurs due to cuticle rupture, but this was not observed in my study nor in several species recently investigated (Appezzato-da-Glória & Estelita 2000; Rio et al. 2002; Demarco 2005; 2008; Simões et al. 2006; Martins et al. 2010; Martins 2012; Canaveze & Machado 2015).

Calycine colleters are always avascularized in asclepiads (Woodson & Moore 1938), but vascularized colleters have already been recorded in flowers of other subfamilies (Woodson & Moore 1938; Rao & Ganguli 1963; Dave et al. 1987; Thomas & Dave 1989c) and crystalliferous idioblasts and laticifers are often found in many species (Ramayya & Bahadur 1968; Arekal & Ramakrishna 1980; Fjell 1983; Murugan & Inamdar 1987a; b; Thomas & Dave 1989a; b; Subramanian et al. 1989; Thomas et al. 1989; Thomas & Dave 1991; Appezzato-da-Glória & Estelita 1997; 2000; Schwarz & Furlan 2002; Demarco 2005; 2008; Martins et al. 2010).

Calycine colleters remain in secretory activity during the entire floral development and maintain their shape during the post-secretory phase in post-anthetic flowers, unlike the leaf colleters (Demarco 2005; 2008). Among the asclepiads analyzed histochemically to date, the production of a heterogeneous secretion composed of mucilage and lipidic compounds seems to be predominant (Fig. 2E-H), with the occurrence of exclusively mucilaginous secretion found only in Peplonia (Ribeiro et al. 2017). Proteins, phenolic compounds and fatty acids have been detected in the secretion of calycine colleters, as well as several alkanes. The distinct components of secretion confer different functions to the colleters. While the mucilage protects against desiccation, the lipophilic compounds provide an antifungal property (Ribeiro et al. 2017).

**Glandular trichomes (Fig. 3)**

Glandular trichomes have a restricted occurrence in Apocynaceae and have been reported for only eight genera of Asclepiadoideae: Araujia, Dischidia, Fischeria, Gongronema, Gonolobus, Marsdenia, Matelea and sarcostemma (Solareder 1908; Woodson 1941; Metcalfe & Chalk 1950; Stevens 1975; 1988; Murphy 1986; Morillo 1998). Among these genera, the presence of mixed indumentum composed of long tector trichomes and short glandular trichomes in Fischeria and Matelea is unique and shows the relation of these genera (Woodson 1941), both grouped in the subtribe Gonolobinae (Endress et al. 2014).

These glandular trichomes have never been studied anatomically and are described for the first time in the present work for Matelea denticulata. In this genus, glandular trichomes are present on the pedicel and abaxial side of the sepals (Stevens 1975; 1988) (Fig. 3A-B). In M. denticulata, they are multicellular, uniseriate with lignified peduncle (Fig. 3C-D) and an apical secretory cell with a dilated base.
Figure 2. Calycine colleters in Asclepiadoideae. (A) Matelea denticulata (Vahl) Fontella & E.A. Schwarz. (B, F) Oxypetalum banksii subsp. banksii Roem. & Schult. (C, E, G-H) Asclepias curassavica L. (D) Blepharodon bicuspidatum E. Fourn. (A-B) Colletor initiation in floral buds (asterisk). (C-D) Mature colleters formed by palisade secretory epidermis and a parenchyma axis. (C) Colletor with peduncle (standard type). (D) Sessile colletor. (E) Detection of acidic mucilage with ruthenium red. (F) Identification of starch grains using safranin, astra blue and iodine-potassium iodide. (G-H) Lipids detected with Sudan black B (G) and Nile blue (H). Abbreviations: P, petal; Pe, peduncle; S, sepal.
Figure 3. Glandular trichomes in flowers of *Matelea denticulata* (Vahl) Fontella & E.A. Schwarz. (A-B) General view of the glandular trichomes in light microscopy (A) and scanning electron microscopy (B). (C, E) Mature trichomes. (D, F) Identification of secondary cell walls. Polarization microscopy of 3C and 3E respectively. (D) Secondary walls in the peduncle cells. (F) Crystal in the apex of the glandular cell (arrow). (G) Detection of proteins with aniline blue black. Abbreviations: Pd, pedicel; S, sepal.
and an elongated, acuminate upper portion (Fig. 3C, E). This cell has a rounded tip with a constriction just below it where crystals are located, providing a mechanical rupture (Fig. 3F). The secretion is composed exclusively of amino acids and/or proteins (Fig. 3G). The morphology of the trichome, composition of the secretion and its mechanism of release to the outside resemble those of stinging trichomes (Thurston & Lersten 1969; Thurston 1974; 1976; Fahn 1979).

Internal protective glands

Laticifer (Fig. 4)

Laticifers are ubiquitous in Apocynaceae (Metcalfe & Chalk 1950) and are found in all vegetative and floral organs of asclepiads, absent only in the ovules (Demarco et al. 2006). Although those laticifers are generally interpreted as non-articulated type in the family (Chauveaud 1891; Solereder 1908; Metcalfe 1967; Mahlberg 1993), recent developmental studies of laticifers indicate that possibly all the vegetative and floral laticifers of Apocynaceae are articulated anastomosing (Fig. 4A-C) with early dissolution of the terminal walls, a fact that led many authors to misclassify them (Demarco et al. 2006; Demarco & Castro 2008; Gama et al. 2017, and references therein).

Laticifers branch by lateral fusion in the meristematic regions, forming a system that likely interconnects most laticifers of the adult plant (Demarco et al. 2006; Demarco & Castro 2008; Lopes et al. 2009; Canaveze & Machado 2016; Gama et al. 2017) (Fig. 4D). Cell walls are dissolved from the center to periphery, followed by the fusion of protoplasts, resulting in a continuous multinucleated protoplast throughout the laticifer system (Gama et al. 2017). They are found in the fundamental and vascular systems of all organs (Groom 1889; Blaser 1945; Milanez 1960/1961; 1966; 1977; Mahlberg 1963; Valente 1977; 1984; 1995; 1996; Murugan & Inamdar 1987a; b; Appezzato-da-Glória & Estelita 1997; Sacchetti et al. 1999; Valente & Costa 2005; Demarco et al. 2006; Demarco & Castro 2008) (Fig. 4E-F) and ultrastructural analyses have demonstrated the impossibility of intrusive growth of these laticifers (Gama et al. 2017).

The laticifer cell walls are exclusively primary and highly hydrated, especially in the young portion, where their acidic characteristic (Fig. 4G) makes them more flexible, allowing the increase of cell diameter (Demarco et al. 2006). Immunocytochemical studies of laticifers in Asclepias speciosa Torr. (Serpe et al. 2001; 2002) have shown that the pectin composition of the cell wall in the mature portions of the laticifers is different from that of the younger portions.

Latex is observed from the younger region of the laticifer and corresponds to its protoplast (Demarco 2015). Some vesicles and small vacuoles with secretion fuse to the central vacuole, transferring their contents and increasing its volume, restricting the cytoplasm to a thin parietal layer (Gama et al. 2017). According to Giordani (1978), Fahn (1979) and Fineran (1983), the protoplast can remain intact or degenerate at maturity. However, the protoplast disarrangement is apparently due to an artifact during the plant collection and fixation caused by the destabilization of the turgor pressure, modifying all laticifer content.

The latex of Apocynaceae may have different colors (Solereder 1908), but the few laticifers described for flowers to date have all been milky-white (Appezzato-da-Glória & Estelita 1997; Demarco et al. 2006; Demarco & Castro 2008; Demarco 2015). While the latex is generally described as having predominantly lipids (Fig. 4H-I), especially terpenes (Die 1955; Warnaar 1982; Giordani 1996), many other compounds have been detected in the latex of the family, such as triterpenes and polysacoprenes, steroids, fatty and aromatic acids, polysaccharides (Fig. 4J), cardenolides and proteins (Fig. 4K), including enzymes, phenolic compounds and alkaloids (Die 1955; Rao & Malaviya 1966; Wilson et al. 1976; Yoder & Mahlberg 1976; Baas et al. 1981; Groeneveld & Made 1982; Warnaar 1982; Allen & Nessler 1984; Eliet et al. 1985; Murugan & Inamdar 1987b; Giordani & Lafon 1993; Giordani 1996; Appezzato-da-Glória & Estelita 1997; Sacchetti et al. 1999; Giordani et al. 2000; Castro & Demarco 2008; Demarco 2015). The various compounds protect the plant against herbivores and microorganisms as well as seal wounds (Fahn 1979; 1990; Farrel et al. 1991; Hunter 1994; Demarco 2015).

Secretory idioblasts (Fig. 5)

There have been few reports of secretory idioblasts in Apocynaceae, and almost all are restricted to vegetative organs (Solereder 1908; Metcalfe & Chalk 1950; Baas & Gregory 1985; Endress & Bruyns 2000; Demarco 2005). In Asclepiadoideae, secretory idioblasts have been reported for the tribes Ceropogieae and Asclepiadeae (Solereder 1908; Metcalfe & Chalk 1950; Endress & Bruyns 2000), but their presence varies, even in the same subtribe. All of these reports referring to vegetative organs and floral secretory idioblasts are described in asclepiads for the first time in this review.

Oil idioblasts have been identified in flowers of Peplonia axillaris (Fig. 5A). The production of oil by idioblasts has been reported for 12 genera of Apocynaceae, but none of them belong to the subfamily Asclepiadoideae (Metcalfe & Chalk 1950). The idioblasts of P. axillaris occupy the most outer region of the pedicel cortex (Fig. 5B) and are found beneath the epidermis of sepals (Fig. 5C) and petals (Fig. 5D-E). Their shape varies from cubic to elongated and have trilamellar walls with a median suberin lamella between two cellulosic portions of the cell wall (Fig. 5F-G), as is normally observed in oil idioblasts (Postek & Tucker 1983) with the oil occurring as droplets in the periphery of the vacuole (Fig. 5H-I).
Figure 5. Oil idioblasts in flowers of *Peplonia axillaris* (Vell.) Fontella & Rapini. (A) General view of the flower in longitudinal section. (B-D) Oil idioblasts in the pedicel (B), sepal (C) and petal (D) with the secretion stained red. (E-F) Detection of cellulose with calcofluor white under UV. (F) Idioblast trilamellar wall. Note the absence of cellulose in a median lamella inside the cell wall (arrow). (G) Presence of suberin in the median lamella inside the cell wall (arrow) detected with Sudan IV. (H-I) Detection of oil using Sudan black B (H) and Sudan IV (I). Abbreviations: Id, oil idioblast; P, petal; St, stamen.
Nuptial glands

Nectary (Figs. 6-7)

Nectaries occur exclusively in flowers of Apocynaceae. Although there have been reports of extrafloral nectaries in the group, in actuality, these reports misinterpreted the colleters (Thomas 1991, and references therein).

The position of the nectaries is controversial in asclepiads, often due to the terminology applied in the description of flowers and to the inaccuracies in relation to the complex floral morphology. However, all species of this subfamily have primary nectaries in the filament tube (Galil & Zeroni 1965; Christ & Schnepf 1985; Kunze 1991; 1995; 1997; Kunze & Liede 1991; Endress & Bruyns 2000; Vieira & Shepherd 2002; Demarco 2005; Monteiro & Demarco 2017) (Fig. 6A), which corresponds to the secretory epidermis of the stigmatic chamber (Galil & Zeroni 1965; Valente 1977; 1984; 1995; Schnepf & Christ 1980; Valente & Silva 1984; Kunze 1991; 1995; 1999; Kunze & Liede 1991; Vieira & Shepherd 2002; Demarco 2005; Valente & Costa 2005; Monteiro & Demarco 2017) (Fig. 6B-E). In general, it is assumed that only the primary nectary is secretory and the nectar flows through an intricate capillary system to the nectar holder (Galil & Zeroni 1965; Kunze 1997). However, nectariferous tissue has been described in the corona of some genera (Rao & Ganguli 1963; Fallen 1986; Endress 1994; Swarupanandan et al. 1996; Endress & Bruyns 2000). The main reports have been the description of the scent in taxonomic studies and a few chemical analyses (Stevens 1988; Vogel 1990; Rohrbeck et al. 2006; Jürgens et al. 2008; 2010; Setzer 2014). In Ceropogia, the osmophore was found at the tip of petals, consisting of secretory epidermis and subepidermal layers, as well as in Ditassa (Fig. 8A-F) and Boucerosia, but Orbea has two types of secretory epidermal cells with distinct structural characteristics (Vogel 1990; Plachno et al. 2010).

The main components of the scents are terpenes and phenolic compounds of low molecular weight (Jürgens et al. 2010), which produce a sweet aroma in some species and a fetid aroma in others (Stevens 1988; Vogel 1990; Wolff et al. 2008). The different types of scents are often associated with the corolla color, as in the sapromyophilic flowers of Ceropegieae, which have dark brown, red or yellow corollas and release a characteristically putrid aroma (Meve & Liede 1994). In general, sweet scents are related to white corollas (Vogel 1990), as is the case with Ditassa gracilis (present study).

Style head (Fig. 9)

The style head is present in all Apocynaceae and corresponds to the upper portion of the styles (Figs. 1E-F, 9A), which fuses postgenitally and dilates. With the exception of Rauvolfioideae, in all other members of the family the style head is adnate to the anthers through the retinaculum, forming the gynostegium (Rao & Ganguli 1963; Fallen 1986; Endress 1994; Swarupanandan et al. 1996; Endress & Bruyns 2000). The style head in the family is covered by a secretory epidermis (Walker 1975; Fallen 1986; Kunze 1993; 1994; Galetto 1997; Lin & Bernardello 1999; Demarco 2014) (Fig. 9A-B), and its secretion is related to the process of pollen transportation. Initially, it helps adhere the pollen to the pollinator and then assists in the capture of pollen by the stigma or the guide rail/stigmatic chamber of another flower. The secretory surface is present only on the lateral side of the style head and alternate with the anthers in asclepiads (Fig. 9B-C), but in Rauvolfioideae and Apocynoideae it covers almost the entire surface of this dilated portion of the style apex. This is one of the differences that led Fallen (1986) to describe four basic types of style head and define a morphological progression of this structure for the family.
Figure 6. Primary nectaries in flowers of *Blepharodon bicuspidatum* E. Fourn. (A) General view of the primary nectaries (stigmatic chambers) behind the guide rail in transversal section. (B) Detail of the nectary which is formed by the nectariferous epidermis of the stigmatic chamber. (C) Detail of the nectariferous epidermis. (D) Longitudinal view of the stigmatic chamber and its opening at the stigma level. (E) Pollinium inserted into the guide rail and germinated due to the presence of nectar in the stigmatic chamber. Note the entrance of pollen tubes into the stigma (section stained with PAS reaction). Abbreviations: GR, guide rail; Po, pollinium; S, stigma; SC, stigmatic chamber.
Figure 7. Secondary nectaries in flowers of *Blepharodon bicuspidatum* E. Fourn. (A) General view of the secondary nectary in the staminal corona. (B) Longitudinal view of the nectariferous epidermis of the corona. (C) Detail of B. (D) Nectariferous tissue composed exclusively of epidermis. Abbreviation: Cs, staminal corona.
In Asclepiadoideae, the formation of the style head occurs during the beginning of floral development and is an indication of its complexity and importance in the later stages (Fallen 1986; Endress 1994; Swarupanandan et al. 1996; Demarco 2014). The secretory tissue is responsible for the secretion of the translator in Periplocoideae, Secamonoideae and Asclepiadoideae (Brown 1810; Corry 1883; Rao & Ganguli 1963; Vijayaraghavan & Cheema 1977; Dicko-Zafimahova 1980; Valente & Silva 1984; Endress 1994; Kunze 1994; Valente 1995; Endress & Bruyns 2000;
Figure 9. Style head of *Blepharodon bicuspidatum* E. Fourn. flowers. (A) General view of the style head in longitudinal section. (B) Style head is pentagonal in transverse section and present five secretory regions alternate to anthers. (C) Secretory portion of the style head constituted of a palisade epidermis. (D) Translator composed of corpusculum and two caudicles produced by the style head. (E) Morphology of the pollinarium formed by the translator and two pollinia. Abbreviations: A, anther; Ca, caudicle; Co, corpusculum; Po, pollinium; SH, style head; TI, translator.

Valente & Costa 2005; Demarco 2014) (Fig. 9D).

In asclepiads, the translator is a thick secretion composed of a corpusculum and two caudicles which attach to two pollinia of adjacent anthers, forming the pollinarium (Brown 1810; Corry 1883; Schumann 1895; Valente 1977; 1984; 1995; Valente & Silva 1984; Kunze 1993; 1994; Endress 1994; Swarupanandan et al. 1996; Valente & Costa 2005; Demarco 2014) (Fig. 9D-E). The caudicles are absent only in *Fockea* and *Cibirhiza* (Kunze 1993; 1994; Swarupanandan et al. 1996), genera placed in Marsdenieae, the most basal
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the pellicle (hyaline crest) in a sterile portion of the anther (Vijayaraghavan & Cheema 1977; Schnepf et al. 1979). In
et al. (2014), the secretion produced by tapetum may help the corpusculum and caudicles differ from each other in
role in the formation of pollinia and pollinarium as a whole (Woodson 1954; Linskens & Suren 1969; Demarco
1977; Schnepf et al. 1979; Demarco 2014). Fatty acids, phenolic compounds, mucilage and proteins have been
detected in the corpusculum and only neutral lipids and mucilage in the caudicles (Vijayaraghavan & Cheema
1977; Demarco 2014). This difference in the composition of the translator parts is related to their different functions.
The corpusculum must adhere to the pollinator’s body, while the caudicles dehydrate after pollinarium removal
from the flower and often contracts, moving the pollinia to the correct position for their insertion in the stigmatic
chamber (Vijayaraghavan & Cheema 1977; Kunze 1991; Demarco 2014). Although there is a high probability that the
corpusculum and caudicles differ from each other in relation to the chemical composition in the majority of
asclepiads, the shape of some pollinia does not change after dehydration of the caudicles (Wiemer et al. 2012).

Tapetum (Fig. 10)

Although the tapetum is usually not considered a secretory structure, all Apocynaceae have tapetum of the
secretory type (Pacini et al. 1985), which plays an important role in the formation of pollinia and pollinarium as a whole in
all asclepiads (Woodson 1954; Linskens & Suren 1969; Schnepf et al. 1979; Demarco 2014) (Fig. 10A-H).

The pollinium corresponds to the aggregation of all pollen grains from a pollen sac, and this grain aggregation
is increased by the secretion by the tapetum cells of a secretory type (Pacini et al. 1985), which secretes mucilage and lipids. These glands senesce and necrose before pre-anthesis (Demarco 2017) (Fig. 11F). During pollination, the secretion exuded by the staminal wing gland in the floral bud is present inside the guide rail and might play an important role as lubricant, facilitating the entrance of the insect’s proboscis or leg in this slit and/or assisting in the removal of pollinium or part of the pollinarium adhering to the insect. The disintegration of the gland before anthesis is also related to the introduction of proboscis and/or pollinium into the guide rail due to the increase of the chamber area without the glands (Demarco 2017).

Extragynoecial compitum (Fig. 12)

All asclepiads have an apocarpic bicarpelar gynoecium with partially free styles, united only in the apical region
where the style head and a subterminal stigma are formed (Fellen 1986; Endress & Bruyns 2000) (Fig. 1F). Since the
stigma is located below the style head, it has been proposed that one of the functions of this connate region may be to act as a compitum (Fellen 1986). However, the compitum is formed by the union of the transmitting tissue tract from

Staminal wing gland (Fig. 11)

In most Apocynaceae, there is a transfer of the pollen capture area from the gynoecium to the androecium, which
seems to have also occurred in Apocynoideae (Fellen 1986). In Asclepiadoideae, the capture function is performed by
the guide rail (Figs. 6E; 11A-B), which guides the insect to the nectar holder in an interstaminal position at the base of
the flower (Fig. 1E) and retains the pollinium brought by the pollinator inside the stigmatic chamber (primary
nectary). The nectar present in this chamber induces pollen germination (Bookman 1981; Kunze 1991; Endress
1994; Vieira & Shepherd 2002; Demarco 2017; Monteiro & Demarco 2017) (Fig. 6E). The upward movement of
the pollinator, directed by the guide rail, also leads the proboscis or leg of the pollinator to the corpusculum of the translator (Fig. 1E), promoting the removal of the whole pollinarium (Kunze 1991; Wiemer et al. 2012; Demarco 2014).

Recently, an ontogenetic study of Asclepiadaceae flowers has shown that the origin of the wings, which compose the
guide rail, is variable. In Asclepias, Oxytetrum and Peplonia, they are formed by lateral projections of the anther
and filament and, therefore, should be designated staminal wings (Demarco 2017) and not anther wings, as reported by several authors (Frye 1902; Rao & Ganguli 1963; Valente 1977; 1980; 1983; 1995; Valente & Silva 1984; Swarupanandan et al. 1996; Endress & Bruyns 2000; Vieira & Shepherd 2002; Valente & Costa 2005). In the intermediate stages of the floral bud development, two glands are formed along the staminal wings: one at the outer margin and the other at the inner margin of the guide rail (Fig. 11C-E). The secretory tissue is composed exclusively of the epidermis which secretes mucilage and lipids. These glands senesce and necrose before pre-anthesis (Demarco 2017) (Fig. 11F).

tribe of Asclepiadoideae (Endress & Bruyns 2000). The
morphogenesis of the translator begins in the early stages of floral development, and its specific shape is mainly due to
the differential secretory activity of the cells and the undulated outline of the secretory surface of the style
head (Kunze 1994; Demarco 2014). Serbanescu-Jitariu & Tarnavsci (1976) observed that the structure of the pollinarium provides useful characters for the identification and classification of this subfamily, which is still used in taxonomic and phylogenetic studies today (Endress & Bruyns 2000; Rapini et al. 2003; Rapini 2012).

Secretory cells produce different amounts of secretion
and distinct types of compounds in each region of the
style head. The translator is composed mainly of lipids,
but the composition of the corpusculum and caudicles is
different (Woodson 1954; Safwat 1962; Vijayaraghavan &
Cheema 1977; Schnepf et al. 1979; Demarco 2014). In
et al. (2014), the secretion produced by tapetum may help
the corpusculum and caudicles differ from each other in
relation to the chemical composition in the majority of
asclepiads, the shape of some pollinia does not change
after dehydration of the caudicles (Wiemer et al. 2012).

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Figure 10. Tapetum in flowers of Blepharodon bicuspidatum E. Fourn. (A–F) Floral buds. (G–H) Mature flower. (A) Bithecal, bisporangiate anther with initial projection of the staminal wing from its dorsolateral side (asterisk). (B) Longitudinal section of the young anther. (C) Secretory tapetum surrounding elongated microspore mother cells. (D) Presence of secretory globules in the tapetum cells. (E) Tapetum in secretory activity around the microspores. (F) Detail of the tapetum with secretory globules and vacuoles with heterogeneous content (arrow). (G) Mature anther without tapetum containing pollinia covered by a pellicle (arrowhead) secreted by tapetum. (H) Pollinarium formed by translator and two pollinia from adjacent anthers. Abbreviations: A, anther; Po, pollinium; TI, translator.
Figure 11. Staminal wing gland of *Ditassa gracilis* Hand.-Mazz. flowers. (A-B, F) Anthetic flowers. (C-E) Floral buds. (A) General view of anthers in transverse section. (B) Anther with two pollen sacs and two lateral wings. Staminal wings of two adjacent anthers form the guide rail. (C) Guide rail with glands in front of the stigmatic chamber. (D) Staminal wing glands in the outer and inner margins of the guide rail. Note staminal wings completely lignified, except in the glandular areas. (E) Detail of the glands with palisade secretory epidermis. Note the presence of lignified trichomes in the middle region of the guide rail. (F) Necrotic wing glands in a mature flower. Abbreviations: A, anther; GR, guide rail; Po, pollinium; SC, stigmatic chamber; Ws, staminal wing; arrow, wing gland.
Figure 12. Extragynoecial compitum in flowers of Oxypetalum banksii subsp. banksii Roem. & Schult. (A) Longitudinal section. (B-F) Transverse sections. (A-B) Extragynoecial compitum formed by the secretion produced by the inner epidermis of the filament tube in its upper portion. (C) Secretory epidermis around the dry stigma. (D) Detail of C. (E) Continuity between the secretory epidermis of the stigmatic chamber and extragynoecial compitum at stigma level. (F) Secretory portion of the extragynoecial compitum composed exclusively of epidermis. Abbreviations: EC, extragynoecial compitum; FT, filament tube; S, stigma; SC, stigmatic chamber; St, stamen; arrow, epidermis of the extragynoecial compitum.
Floral glands in asclepiads: structure, diversity and evolution

Andrea Demarco


Each carpel at the level of style, which allows pollen tubes to reach the ovules of different carpels (Carr & Carr 1961) independently from where they entered the stigma. The analysis of the connate region of the style below the stigma in asclepiads shows that the two strands of transmitting tissue are independent in most species and the pollination by a single pollinium generally forms one single follicle (Kunze 1991). Among the asclepiads, compitum has been identified only in *Tylophora* and *Matelea* (Kunze 1991; Demarco 2008).

In addition to this type of gynoecial compitum, some species have a secretion involving the stigma (or stigmata) that allows the entrance of pollen tubes through different regions in order to reach all the free ovaries (Endress 1980). This secretion functions as an extragynoecial compitum. In Apocynaceae, the production of twin follicles from a single pollinium demonstrates the presence of a compitum in the asclepiad *Oxypetalum banksii*, and the anatomical study revealed the presence of an extragynoecial compitum formed by the mucilage produced by epidermal cells of the inner surface of the filament tube around the stigma (Vieira & Shepherd 2002) (Fig. 12A-F). This is the only report for the family.

In addition, the mucilage of some Monimiaceae flowers not only acts as an extragynoecial compitum but also serves as a primary pollen receptor and has been given the name hyperstigma (Endress 1979; 1980; 1982). Although the mucilage present in the primary nectar within the stigmatic chamber (Monteiro & Demarco 2017) does not have the function of pollinium capture in Asclepiadoideae and the concept may not be applied in the same sense, this secretion has previously been considered a hyperstigma in *Oxypetalum* (Vieira & Shepherd 2002).

**Stylar canal (Fig. 13)**

The general description of the pollen tube paths through the gynoecium in asclepiads begins with the germination of pollen grains in the stigmatic chamber promoted by the nectar (Eisikowitch 1986; Kevan *et al.* 1989; Valente 1994; Wyatt & Broyles 1994; Sage & Williams 1995; Monteiro & Demarco 2017). Pollen tubes penetrate the dry stigma and grow through a non-secretory transmitting tissue, reaching a canal lined with a secretory epidermis (Sage *et al.* 1990; Kunze 1991; Sage & Williams 1995; Demarco 2008) (Fig. 13A-F). The pollen tubes grow through the canal and obliterate it, as occurs with the adjacent parenchyma cells (Sage *et al.* 1990; Sage & Williams 1995; Vieira & Shepherd 2002). In the ovary, pollen tubes grow in the ovarian locule on the surface of the placenta to the ovule micropyle (Sage & Williams 1995; Vieira & Shepherd 2002).

Along the pathway through the style, pollen tubes grow, digesting the cells of the transmitting tissue strand at first but then grow immersed in the secretion of the stylar canal at a later stage (Sage & Williams 1995; Vieira & Shepherd 2002; Demarco 2008). Stylar canals, occurring in all asclepiads (Kunze 1991), are very narrow (Fig. 13B, F) and promote a place of strong pollen tube competition, increasing the male gametophyte selection (Kunze & Liede 1991). The secretory activity starts in pre-anthetic flowers, and the secretion is composed of mucilage and lipids, which will nourish and direct the pollen tubes towards the ovarian locule (Demarco 2008) (Fig. 13B).

**Obturator (Fig. 14)**

Upon reaching the ovary, the pollen tubes are directed through the locule to the micropyle of the ovules by an obturator (Fig. 14A-B). The obturator was first reported and described for Apocynaceae in *Aspidosperma* (Rauvolfoideae; Demarco 2005); later, its presence was also confirmed for Asclepiadoideae (Demarco 2008), and recently, the obturator was also identified in another species of Rauvolfoideae (Morokawa *et al.* 2015). It is possible that it is present in all Apocynaceae. In some Rauvolfoideae, the obturator is composed of secretory placental trichomes (Demarco 2005; Morokawa *et al.* 2015), but in asclepiads it is formed by secretory cubic cells on the surface of placenta and at the base of funiculus (Fig. 14C-F), which is described for the first time for asclepiads in this review.

The aperture of the stylar canal is continuous with the ovary locule (Fig. 14A), and the secretory epidermis of this canal is continuous with the secretory epidermis of the placenta and funiculus (Fig. 14B). The secretion produced by the obturator fills the entire locule and has the same components as the secretion of the stylar canal: mucilage and lipids. Therefore, the pollen tubes grow inside a continuous layer of secretion from the style to the ovary until they fertilize the ovules (Demarco 2008).

**Evolution and ecological importance of the glands**

Some secretory structures found in the flowers of asclepiads reveal their relationship with other members of Apocynaceae due to their conservative nature, such as the presence of the style head and articulated anastomosing laticifers in the entire family (Tab. 1). On the other hand, the huge diversity of floral glands in Asclepiadoideae and their much more elaborate and synorganized flowers emphasize their derived condition in the family and highlight the asclepiads as the group with the largest diversity of floral glands among the angiosperms. In the 13 types of glands described in this review, *Matelea* (Gonolobinae, Asclepiadeae; Endress *et al.* 2014) has 11 in the same flower (colleters, glandular trichomes, laticifers, primary nectaries, secondary nectaries, osmophores, style head, tapetum, staminal wing gland, stylar canal and obturator; Demarco 2008). The two remaining secretory structures have restricted occurrence. Secretory idioblasts have been observed only in *Peplonia* (Metastelmatinae, Asclepiadeae) and extragynoecial...
Figure 13. Stylar canal in flowers of *Blepharodon bicuspidatum* E. Fourn. (A) General view of the gynoecium with two free ovaries and the free portion of the styles in longitudinal section. (B) Secretory stylar canal with opening in the ovarian locule. Note the presence of secretion (arrow). (C) Stylar canals in the free portion of the styles surrounded by the filament tube. (D) Stylar canal occurs in the adaxial side of the style at the suture line. (E) Stylar canal originated from the adaxial epidermis of the folded style. (F) Stylar canal with a very narrow lumen lined by a secretory epidermis. Abbreviations: FT, filament tube; Sc, stylar canal; Su, suture; O, ovary; Sl, style.
Figure 14. Placental-funicular obturator in flowers of Asclepiadoideae. (A-B, E-F) *Ditassa gracilis* Hand.-Mazz. (C-D) *Blepharodon bicuspidatum* E. Fourn. (A-B) Longitudinal sections. (C-F) Transverse sections. (A) Continuity between the secretory epidermis of the stylar canal and obturator. (B) Detail of A. (C) General view of the ovaries. (D-E) Obturator composed of the secretory epidermis of placenta and funiculus base. (F) Detail of the obturator. Abbreviations: Fu, funiculus; Ov, ovule; Pl, placenta.
When the position of these genera is analyzed in the distinct functions in so many parts of the flower. Tissues which exude completely different compounds with needed to better understand the evolution of epidermal evolution of the group. Studies focusing on protoderm are fusion of protodermal surfaces and related to the floral development in this group is also due to the number of postgenital connations and adnations occurring from the protoderm or mainly from this meristem. The large in the flowers have secretory tissue originating exclusively glands, we noticed that 10 of the 13 gland types present to their dispersion of pollen grains in pollinia. The complexity of pollination types in asclepiads, mainly due types per flower in the family is directly related to the greater 2016). The exponential increase in the number of gland. Among the Apocynaceae already studied, the genus with the lowest number of floral glands is Aspidosperma (Aspidospermateae, Rauvolfioideae; Endress et al. 2014) with three types of glands (laticifers, style head and tapetum; Demarco 2005), the one with the largest number being Matelea (Gonolobinae, Asclepiadeae, Asclepiadoideae). When the position of these genera is analyzed in the phylogeny, Aspidospermateae is the most basal tribe of Aspidospermanae, Asclepiadeae, Asclepiadoideae). The exponential increase in the number of gland types per flower in the family is directly related to the greater complexity of pollination types in asclepiads, mainly due to their dispersion of pollen grains in polinia. If we consider the origin of the secretory tissues in the glands, we noticed that 10 of the 13 gland types present in the flowers have secretory tissue originating exclusively from the protoderm or mainly from this meristem. The large number of postgenital connations and adnations occurring during flower development in this group is also due to the fusion of protodermal surfaces and related to the floral evolution of the group. Studies focusing on protoderm are needed to better understand the evolution of epidermal tissues which exude completely different compounds with distinct functions in so many parts of the flower.

Two evolutionary trends may be noted within asclepiads in relation to the glands: 1) redundancy in protection with external and internal glands protecting the flowers against herbivory, microorganism proliferation, meristem desiccation, etc. (e.g., colleters, trichomes, laticifers and idioblasts) and 2) functional division between glands (e.g., primary and secondary nectaries) or between cells of a same secretory tissue (e.g., style head).

The redundancy in relation to the protection of glands is reflected in the low predation rate of these plants, but the division of functions between glands or between cells of the same gland is related to the evolution of the secretory structures in the family. When we analyze the secretory epidermis of the style head from the most basal genera, all cells produce the same type of secretion. On the other hand, in Periplocoideae, Secamonoideae and Asclepiadoideae the thick secretion (translator) produced by this tissue has specific morphology and distinct chemical composition in each part, demonstrating differences in the secretory activity of the cells of the style head.

In spite of the great diversity of glands in the flowers of asclepiads and the occurrence of some specific secretory structures in some genera, the glands related to pollination are relatively constant throughout the group (Tab. 1), making the general description of the pollination more uniform. The pollinator is often attracted by the scent produced by osmophores or by the accumulation of nectar in cups formed by staminal corona. When collecting the nectar in the corona or in the interstaminal position,

### Table 1. Distribution of floral glands in the tribes and subtribes of Asclepiadoideae (sensu Endress et al. 2014).

<table>
<thead>
<tr>
<th>Tribe</th>
<th>Subtribe</th>
<th>Glands already registered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fockeeae</td>
<td></td>
<td>L, PN, Os (2), SH, T</td>
</tr>
<tr>
<td>Eustegieae</td>
<td></td>
<td>L, PN, SH, T</td>
</tr>
<tr>
<td>Marsdenieae</td>
<td></td>
<td>C, GT (3), L, PN, SN (1), Os (5), SH, T, SC</td>
</tr>
<tr>
<td>Ceropgeieae</td>
<td>Heterostemmini</td>
<td>C, L, PN, SH, Os (1), T, SC</td>
</tr>
<tr>
<td></td>
<td>Leptadeniinae</td>
<td>C, L, PN, SH, T, SC</td>
</tr>
<tr>
<td></td>
<td>Anisotominae</td>
<td>C, L, PN, SH, T, SC</td>
</tr>
<tr>
<td></td>
<td>Stapelinae</td>
<td>C, L, PN, SN (3), Os (13), SH, T, SC</td>
</tr>
<tr>
<td></td>
<td>Astephaniniae</td>
<td>C, L, PN, SH, T, SC</td>
</tr>
<tr>
<td></td>
<td>Asclepiadinae</td>
<td>C, L, PN, SN (2), SH, T, WG (1), SC, Ob (1)</td>
</tr>
<tr>
<td></td>
<td>Cynanchinae</td>
<td>C, GT (1), L, PN, SN (2), Os (1), SH, T, SC</td>
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<tr>
<td></td>
<td>Stylorrhinae</td>
<td>C, L, PN, SN (1), SH, T, SC</td>
</tr>
<tr>
<td></td>
<td>Pentacynphinae</td>
<td>C, L, PN, SH, T, SC</td>
</tr>
<tr>
<td></td>
<td>Dipolepinae</td>
<td>C, L, PN, SH, T, SC</td>
</tr>
<tr>
<td></td>
<td>Orthosinae</td>
<td>C, L, PN, Os (2), SH, T, SC</td>
</tr>
<tr>
<td></td>
<td>Metastelmatinae</td>
<td>C, L, SI (1), PN, SN (3), Os (2), SH, T, WG (3), SC, Ob (3)</td>
</tr>
<tr>
<td></td>
<td>Tassadiinae</td>
<td>C, L, PN, SH, T, SC</td>
</tr>
<tr>
<td></td>
<td>Oxypetalinae</td>
<td>C, GT (1), L, PN, Os (2), SH, T, WG (1), EC (1), SC, Ob (1)</td>
</tr>
<tr>
<td></td>
<td>Gonolobinae</td>
<td>C, GT (3), L, PN, SN (1), Os (2), SH, T, WG (1), SC, Ob (1)</td>
</tr>
</tbody>
</table>

**Note.** The number in parenthesis represents the quantity of genera where the gland has already been registered and its absence indicates the ubiquitous occurrence of the gland in the group. The references for this data survey are found in the description of each gland in this review. C = colleter; GT = glandular trichome; L = laticifer; SI = secretory idobiast; PN = primary nectary; SN = secondary nectary; Os = osmophore; SH = style head; T = tapetum; WG = staminal wing gland; EC = extragynoecial compitum; SC = stylar canal; Ob = obturator.
the insect introduces the proboscis or leg in the guide rail and can only withdraw it by making a movement forward and upward. The corpusculum secreted by the style head and located above the guide rail adheres to the part of the pollinator’s body, thus removing the entire pollinium from the flower. When collecting nectar from another flower, the insect is again caught by the guide rail and, by making the movement forward and upward, introduces the pollinium into the guide rail or the stigmatic chamber by its basal aperture. The insertion of the pollinium or part of the pollinium into the guide rail is facilitated by the secretion of the wing gland and the primary nectar, present in the stigmatic chamber, stimulating the germination of the pollen grains, which will grow through the nectar of the chamber to the stigma located below the style head. When the pollen tubes penetrate the gynoecium, they are directed by transmitting tissues to the stylar canal, where they grow immersed in the secretion of the canal until the ovary and then grow immersed in the secretion of the placental-funicular obturator, fertilizing the ovules.

Future perspectives

The floral glands of asclepiads have been poorly studied in structural terms and, despite their simple tissue composition often containing only a secretory epidermis, recent studies have shown that their secretion may be much more complex and heterogeneous than previously thought, demonstrating a high metabolic complexity of their cells. Therefore, new studies are still necessary to verify the actual composition of some secretions, how the exudates are produced by the organelles, the process of secretion release to the outside and the ontogenetic factors related to the formation of the different glands in an evolutionary perspective.

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Floral glands in asclepiads: structure, diversity and evolution


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