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Stigma, pollen tube transmitting tract, and epidermal micromorphology of the style of *Sarracenia purpurea* (Sarraceniaceae)

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Abstract: Entomophilous flowers of the genus *Sarracenia* have a unique umbrella-shaped style, which consists of a broadened and flattened umbrella canopy and a thin cylindrical umbrella stalk. Anatomical and micromorphological features of the style of *Sarracenia purpurea* were studied using light microscopy and scanning electron microscopy. This study found that the pollen tube transmitting tracts (PTTTs) start as a semi-solid canal filled with endotrophic conducting tissue and run from the peripheral to the center of the canopy where the PTTT becomes a hollow canal supported by ectotrophic conducting tissue. The presence of stomata on the epidermis of the canopy and chloroplasts in its ground parenchyma indicate photosynthetic activities. Convex epidermal cells with intense cuticular striations on the canopy that are similar yet different from those on various regions of the sepal and petals indicate that it may provide contrasting visual cues for pollinators. Multicellular secretory glands and trichomes, which may provide olfactory cues and tactical cues respectively, are also found on the canopy. Thus, the stylar umbrella not only serves as a region for pollen grain capture, pollen germination, and pollen tube transmission but may also play an important role during pollinator-flower interactions.

Key words: carpel, North American Pitcher Plants, Northern Pitcher Plant, tannins, vascular tissue.
Introduction

The genus *Sarracenia* L. is one of the three genera of Sarraceniaceae Dumort., a family of rhizomatous carnivorous herbs with pitcher-like leaves (Kubitzki 2004; McPherson and Schnell 2011), within which the monophyletic North America *Sarracenia* is sister to the northern South America genus *Heliamphora* Benth., which together are sister to the monotypic western North America genus *Darlingtonia* Torrey based on phylogenetic data (Ellison et al. 2012). The genus *Sarracenia*, endemic to fens, acidic *Sphagnum* bogs, savannas, alkaline marls, and seepage slopes (McPherson and Schnell 2011; Stephens et al. 2015), consists of 8–11 species and as many as 41 infraspecific taxa, all of which are distributed in eastern North America except *Sarracenia purpurea* L. subsp. *purpurea* (Raf.) Wherry that extends from the northeastern regions of North America and the Great Lakes region to east of British Columbia across southern Canada (Ellison et al. 2012; Stephens et al. 2015). Even though *Sarracenia* has undergone a recent radiation within the last three million years and the phylogenetic relationships of the infraspecific taxa are not fully resolved (Ellison et al. 2012; Stephens et al. 2015), the floral morphology of the genus is fairly conserved except for some variations in terms of overall size, coloration, sepal recurvature, and petal shape (Schnell 1978; Burr 1979).

The axillary, solitary, bisexual, hypogynous and actinomorphic flower of *Sarracenia* has three spiral prophylls (bracts), pentamerous perianth with completely free petaloid sepals and petals, 60–100 tetrasporangiate stamens with dorsifixed and dithecate anthers, and a pentamerous syncarpous gynoecium, consisting of an ovary with axile placentation and anatropous unitegmic ovules and an umbrella-shaped style, which is unique within Sarraceniaceae (Figs. 1A, 1B, and 1D; Shreve 1906; Löfstrand and Schönenberger 2015; Löfstrand et al. 2016). The umbrella-shaped style consists of a thin cylindrical umbrella stalk and a flattened and broadened umbrella
canopy with five notched lobes, each of which is terminated with a stigma at the inner distal region (Figs. 1C and 1D). At anthesis, the flower is pendulous because of the bending of the distal region of the scape, and the floral center, consisting of the ovary, stamens, and the umbrella stalk, is concealed by the five lobes of the style and the five alternating petals, forming a chamber or compartment (Figs. 1D and 1E).

Flowers of *Sarracenia* are entomophilous (Mandossian 1965; Burr 1979; Ellison and Gotelli 2001; Schnell 2002). Bees of the genus *Bombus* were reported as the most important pollinators of *Sarracenia* spp. (Jones 1908; Mandossian 1965; Burr 1979; Schnell 1983; Horner 2014). *Bombus* spp. were observed collecting pollen and nectar and are considered to be the principal pollinators of *S. purpurea* (Mandossian 1965; Burr 1979), while small solitary bees of Halictidae and Megachilidae are considered as effective pollinators (Mandossian 1965; Burr 1979). Flies (*Fletcherimyia fletcheri* and *Sarcophaga sarraceniae*), which were observed taking shelter in the flower (Jones 1908; Krawchuk and Taylor 1999), are considered as potential pollinators of *S. purpurea* (Jones 1908; Burr 1979; Ne’eman et al. 2006). The floral structure of *Sarracenia* is believed to encourage cross-pollination (Burr 1979; Schnell 1983, 2002). The sepals and proximal region of the petals serve as the roof of the pollination chamber while the relatively flat region of the stylar umbrella canopy serves as the floor (Fig. 1E; Schnell 1983); the nectar-bearing ovary (Vogel 1998) and the pollen-bearing anthers serve as the reward center of the pollination chamber; the five upward projecting stigma lobes as well as the alternating and hanging middle region of the five petals provide the wall (Schnell 1983); the only doors to the pollination chamber are the five openings above each stigma lobe, below each sepal, and between the edges of adjacent petals (Figs. 1B and 1E). Insects were observed landing on the petals of *S. purpurea*, then turning to one of the five openings, and forcing their way through the
door while scraping on the stigma (Jones 1908; Mandossian 1965; Burr 1979); insects were also observed emerging through the doors while scraping the stigma during departure (Jones 1908) or emerging from other regions of the flower (Burr 1979). Pollen grains of *S. purpurea*, which are shed within 24 hours after the beginning of anthesis, are caught and retained by the stylar umbrella canopy (Burr 1979; Jones 1908). Thus, the flattened and broadened umbrella canopy of the style of *Sarracenia* plays an important role in this distinct and specialized pollination system within Sarraceniacae.

The stylar umbrella not only provides the platform for insects to collect pollen, forage nectar (Jones 1908; Burr 1979), and roost and take shelter (Burr 1979; Krawchuk and Taylor 1999) during pollinator-flower interactions, but also serves as a part of the gynoecium for pollen grain capture, pollen germination, and pollen tube transmission. Even though some detailed data are available on the micromorphology and anatomy of the immature style (within the flower bud) of *Sarracenia* (Löfstrand and Schönenberger 2015), very limited data are available for the mature (during anthesis) style (Shreve 1906). For pollen capture and germination, the stigma of *Sarracenia* is classified as a type of “dry” stigma on which stigmatic papillae have non-flowing secretions (Heslop-Harrison and Shivanna 1977). Shreve (1906) provided a drawing of a partial view of the stigmatic surface of a mature style of *S. purpurea* and found that pollen tubes always grow between the stigmatic papillae. However, the spatial distribution of the stigmatic papillae and the internal histology under the stigmatic papillae are still unknown. For pollen tube transmission, the five rays of the umbrella canopy (Fig. 1C) are found to contain the pollen tube transmitting tracts (PTTTs), one of which was depicted by Shreve (1906) in a drawing of a transverse view: the conducting canal is sealed by the epidermis, filled with aerenchymatous conducting tissue that consists of conducting cells and intercellular spaces, and flanked by
vascular tissue. Whether there are variations of these anatomical features along the entire length of the umbrella canopy is unclear. Most importantly, Shreve (1906) mentioned that at the center of the umbrella canopy (Fig. 1C), there is a cavity that “connects the interior of the capsule with the external air,” a conclusion that goes against the apomorphy of angiosperms for which ovules are completely sealed by the carpel(s) (Endress 2015). Thus, the histology of the center of the umbrella canopy transitioning to the umbrella stalk needs to be investigated. Moreover, anatomical features of the epidermal cells, ground parenchyma, and vascular bundles both along and in between the PTTTs from the periphery to the center of the umbrella canopy (Fig. 1C) are still largely unknown, except that tannins were found in the epidermal and subepidermal layers of all floral organs of *S. purpurea* (Löfstrand and Schönenberger 2015), and epidermal trichomes, stomata, and multicellular glands were reported for both the outer and inner surfaces of the umbrella canopy (Shreve 1906; Schnell 1978) even though no data are available regarding their micromorphology, anatomy, and distribution patterns.

We chose *S. purpurea* because a substantial amount of data are available for its pollination ecology (Mandossian 1965; Burr 1979; Ne’eman et al. 2006). We sampled mature (at anthesis) and naturally pollinated styles to investigate the micromorphological and anatomical characteristics of the stigma region and the PTTT along its entire length in the style (Figs. 1C, 1D, and 1E). We also studied the micromorphological and anatomical features of the non-PTTT regions of the umbrella canopy from the periphery to its center, the transition zone between the umbrella canopy and the umbrella stalk, and the umbrella stalk (Figs. 1C and 1D) to facilitate further studies on the development of the stylar umbrella. In addition, the micromorphological and anatomical features of the sepal, petal, and the lid of the pitcher leaf were studied and compared to those of the broadened and flattened stylar umbrella canopy to gain insights into
flower-pollinator interactions. The aim of this study is to provide in-depth anatomical and micromorphological data on the natural history of the unique umbrella-shaped style of *Sarracenia* and its associated pollination chamber, which sheds light on an important floral innovation in eudicotyledons.

**Material and methods**

Flowers at anthesis (with or without pollination) and the lid of mature pitcher leaves of *S. purpurea* subsp. *purpurea* were collected at a privately-owned *Sphagnum* bog in Oswego County, New York, USA and immediately fixed in a formalin-propionic acid-alcohol (FPA) solution. After one week of fixation, styles, sepals, and petals were dissected from the flower. For micromorphological studies, two different pitcher leaf lids, and sepals and petals from two different flowers were dissected; eight different styles were dissected at the stigma, PTTT, and non-PTTT regions. For anatomical studies, two different pitcher leaf lids were dissected for cross sections at the distal, middle, and proximal regions; two sepals and two petals from two different flowers were dissected for cross sections in six different regions from distal to proximal; three replicates for the stigma region (Fig. 1D), different regions of the umbrella canopy (as shown in Fig. 1C), the center of the stylar umbrella (Fig. 1C), and the entire umbrella stalk (Fig. 1D) were dissected for cross and longitudinal sections. Dissected samples were kept individually in plastic cassettes and dehydrated through an ethanol (Fisher Scientific, Fair Lawn, NJ: https://www.fishersci.com) series: 50%, 60%, 70%, 80%, and 100% twice.

For micromorphology, dehydrated samples were dried in an EMS 850 Critical Point Dryer (Electron Microscopy Sciences, Hatfield, PA: http://www.emsdiasum.com) using liquid CO₂, coated with gold using a Denton Vacuum Desk V HP Sputter Coater (Denton Vacuum...
LLC, Moorestown, NJ: https://www.dentonvacuum.com), and observed and photographed using a JEOL JSM-6610LV Scanning Electron Microscope (JOEL Ltd., Tokyo, Japan: https://www.jeol.co.jp/en/). The average diameter of the pollen tubes and conducting cells, and the average length of the trichomes were calculated by measuring at least 10 randomly chosen individuals using images from scanning electron microscopy (SEM) and/or light microscopy (LM).

For anatomy, dehydrated samples were gradually infiltrated with Histo-Clear II (National Diagnostics, Atlanta, GA: https://www.nationaldiagnostics.com) and then liquid paraffin (Paramat Embedding Media, Electron Microscopy Sciences, Hatfield, PA: http://www.emsdiasum.com) at 60 °C, and then embedded in paraffin individually for cross or longitudinal sections. An AO 820 rotary microtome (American Optical, Rochester, NY) with a Tissue-Tek Accu-Edge high profile disposable blade (Sakura Finetek USA, Torrance, CA: https://www.tedpella.com) was used to obtain paraffin ribbons at 8–10 µm thickness, which were then mounted to glass slides (Globe Scientific, Mahwah, NJ: https://www.tedpella.com) using Sass’s adhesive (Sass 1940). Each slide was stained with 0.5 % solution of Safranin O (Fisher Scientific, Fair Lawn, NJ: https://www.fishersci.com) and counterstained with 0.5 % solution of Fast Green FCF (Fisher Scientific, Fair Lawn, NJ: https://www.fishersci.com) following Jensen (1962) and then covered with Omnimount (National Diagnostics, Atlanta, GA: https://www.nationaldiagnostics.com) and a Schott D263M Glass Coverslip (Schott North America, Elmsford, NY: https://www.tedpella.com) with 0.16–0.19 mm thickness. Stained slides were studied using LM and imaged using a ZEISS LSM 700 confocal microscope (Carl Zeiss Microscopy GmbH, Jena, Germany: https://www.zeiss.com). Figure plates were assembled using Adobe Illustrator CC Version 23.1.1 (Adobe Inc., San Jose, CA: https://www.adobe.com).
Results

Micromorphology and anatomy of the stigma region

The five stigmas projecting from the five stigma lobes of the umbrella canopy (Fig. 1D) represent the asymplicate zone of the gynoecium. The stigma consists of a receptive surface covered with stigma papillae for the capture and germination of pollen grains (Figs. 2A–2F) and non-receptive surfaces, which encloses the endotrophic conducting tissues at the beginning of the PTTT (Figs. 2G–2K). The conduplicate folding of the carpellary lamina forms a furrow (Figs. 2G–2K), and its lateral margins are densely covered with unicellular and unbranched stigma papillae with decreasing density from the distal to the proximal region (Figs. 2A–2C). All stigma papillae face the top and the outside of the pollination chamber (Figs. 1E, 2A, and 2B), have cuticular striations (Figs. 2E and 2F), and are darkly stained, indicating their secretory nature (Figs. 2G–2K). Pollen grains are mostly observed being captured and germinating on the distal region of the stigma (Figs. 2C and 2D), where pollen tubes grow between the stigma papillae (Figs. 2C and 2D) towards the endotrophic conducting tissues (Figs. 2D and 2I–2M). No conspicuous stigmatic secretions are observed on the stigma papillae.

The complex transmitting tissue consists of one or two layers of darkly stained and densely packed transmitting tract cells (equivalent to canal cells in a hollow style) forming a canal (Figs. 2I–2M) and loosely arranged endotrophic conducting cells (Fig. 2D) with intercellular spaces (Figs. 2I–2M). The transmitting tract cells are densely cytoplasmic, suggesting that they are metabolically active and secretory. Large intercellular spaces are observed for the conducting cells at the distal regions of the stigma (Fig. 2I), while the intercellular spaces decrease in size towards the middle (Fig. 2J) and proximal (Figs. 2K and 2L).
regions. Pollen tubes with an average diameter of 4.3 µm +/- 0.4 µm (with a sample size of 10) are observed growing along the distal-proximally elongated conducting cells. The conducting cells have big and darkly stained nucleus are connected with each other at their tapered ends forming a filament, and are distributed near the transmitting track cells (Figs. 2I–2M).

Vascular tissue is absent at the distal region of the stigma (Figs. 2G–2I), while four to six carpellary bundles are found in the ground parenchyma of the middle (Fig. 2J) and proximal (Figs. 2K and 2L) regions. The non-receptive surface of the stigma is covered by more or less flat cells that are distal-proximally elongated (Figs. 2B and 2C). The non-receptive epidermal cells are darkly stained and filled with tannins or condensed tannins and are especially darker for those near the lateral carpellary margins flanking the stigma papillae (Figs. 2I–2K) and lateral carpellary margins at the proximal end (Fig. 2L).

*Micromorphology and anatomy of the pollen tube transmitting tracts of the stylar umbrella canopy*

The PTTT starts at the stigma region, where endotrophic conducting cells connect the stigma papillae with the conducting cells of the PTTT within the ray of the umbrella canopy (Figs. 1C, 1D, and 3A). From the transverse sections of the ray at the stigma lobe (Figs. 1C and 3B), peripheral (Figs. 1C and 3C), middle (Figs. 1C and 3D), and near center (Figs. 1C and 3E) regions of the umbrella canopy, the complex transmitting tissue consists of loosely arranged conducting cells and one or two layers of tightly packed and densely cytoplasmic transmitting tract cells that form a canal, which is filled with secretions (Fig. 3F). From longitudinal sections of the ray, the pollen tubes are embedded in the secretions (Figs. 3F and 3H) and run along the elongated conducting cells (Figs. 3I and 3J) that connect with each other at their tapered ends.
and are bigger in diameter (8.4 µm +/- 0.7 µm with a sample size of 10) than the pollen tubes (Fig. 3G). The conducting cells gradually decrease in density from the stigma lobe region to the near center region of the umbrella canopy (Figs. 3B–3E).

The PTTT is surrounded by tightly packed ground parenchyma cells that have thicker cell walls than other ground parenchyma cells (Figs. 3B–3E). At the stigma lobe region, several minor vascular bundles are located between the tightly packed ground parenchyma and the aerenchymatous ground tissue surrounding the PTTT (Fig. 3B). At the peripheral (Fig. 3C), middle (Fig. 3D), and near center (Fig. 3E) regions of the umbrella canopy, accompanying the PTTT, one major collateral vascular bundle is found in which the xylem faces the outer epidermis, while the phloem faces the inner epidermis (Figs. 3C–3F), and the vascular bundle is also located between the tightly packed ground parenchyma and the aerenchymatous ground tissue (Figs. 3C–3E). The vessel elements of the xylem have annular or helical secondary thickening (Fig. 3G).

The lateral carpellary margins at the ray region of the outer epidermis (Figs. 3K–3N) are flanked by darkly stained tanniniferous epidermal cells (Figs. 3A–3E); these cells are elongated along the center-peripheral axis of the umbrella canopy (Figs. 3K–3N) accompanying the PTTT and are relatively flatly arranged at the stigma lobe region (Figs. 3B and 3K). They are arranged in a convex curve at the peripheral (Figs. 3C and 3L), middle (Figs. 3D and 3M), and near center (Figs. 3E and 3N) regions of the umbrella canopy. At the middle region, short trichomes that are similar to the stigma papillae also occur at the lateral carpellary margins pointing towards the peripheral of the umbrella canopy (Figs. 3D and 3M). The epidermal cells of the ray region on the inner epidermis of the umbrella canopy are also more elongated along the center-peripheral axis of the umbrella canopy compared with those at the region between the rays (Figs. 3O–3R);
the stigma lobe (Fig. 3O), peripheral (Fig. 3P), and middle (Fig. 3Q) regions are densely covered with trichomes, while the near center region (Fig. 3R) is absent of trichomes.

Anatomy of the center of the stylar umbrella canopy and its transition to the umbrella stalk

Both the umbrella canopy and the umbrella stalk (Fig. 1D) are part of the symplicate zone of the gynoecium. A small funnel-shaped cavity is found at the center of the umbrella canopy where the five rays converge (Fig. 1C). The epidermal cells that cover the entire surface of the funnel-shaped cavity (Figs. 4D–4F) are the same kind of darkly stained tanniniferous cells that cover the outer epidermis of the center region of the umbrella canopy and the epidermis of the umbrella stalk (Figs. 4A–4L), sealing the five PTTTs from the outside air. From longitudinal sections, at the center region of the umbrella canopy (Fig. 1C), the PTTT gradually increases in depth (Figs. 4A–4D) until it becomes vertical in the umbrella stalk (Figs. 4E and 4F). From transverse sections, the central cavity gradually decreases in size (Figs. 4G–4L), and the PTTT gradually increases in its relative width from the distal region (Fig. 4I) to the middle region (Fig. 4L) of the umbrella stalk. Each PTTT is originally sealed by the tanniniferous cells at the lateral carpellary margins (Figs. 4G–4L at arrowheads, 4M, 4N, and 4P), and when the pollen tubes go through, several PTTTs are opened to the outside (Figs. 4H–4L and 4O) because of the breakage at the site of the lateral carpellary margins by pollen tube growth. Conducting cells are present in the PTTTs at the transition region from the umbrella canopy to the umbrella stalk (Fig. 4M) and are absent in the PTTTs of the umbrella stalk where PTTTs are lined with two layers of transmitting tract cells and are only filled with secretions before pollen tube growth (Figs. 4N–4P).
At the middle region of the umbrella stalk, one carpellary vascular bundle is found accompanying each PTTT, and one intercarpellary vascular bundle is found between adjacent PTTTs (Fig. 4L); these carpellary and intercarpellary vascular bundles gradually branch towards the center region of the umbrella canopy (Figs. 4G–4K). All vascular bundles are collateral with xylem facing the center, and phloem facing the periphery (Figs. 4N–4P). The ground parenchyma that surrounds each PTTT at the center region of the umbrella canopy (Fig. 4G), the transition region (Fig. 4H), and at the intercarpellary region of the umbrella stalk (Figs. 4I–4L) is more tightly packed and has thicker primary cell walls than those of the ground parenchyma of other regions (Figs. 4M–4P). No obvious chloroplasts are observed in the ground parenchyma at the center of the umbrella canopy and in its transitional region to the umbrella stalk.

Micromorphology and anatomy of the proximal region of the stylar umbrella stalk

At approximately two-thirds of its length from the distal end of the umbrella stalk, the tanniniferous cells at the center of the umbrella stalk gradually decrease in number until they are absent (Fig. 5A), and the five PTTTs reach each other at the center where the carpel lateral margins meet (Fig. 5B at asterisk). There are two layers of transmitting tract cells lining the PTTT (which are the adaxial epidermis and subepidermis of the carpel), and they are darkly stained and densely cytoplasmic (Figs. 5C–5F). Before the pollen tubes enter, the transmitting tract cells produce secretions that open up a space between the left and right adaxial surfaces of the carpel (PTTT 1 in Figs. 5A and 5B; PTTT 3 and PTTT 4 in Fig. 5B; Fig. 5C), which grows bigger with increased amount of secretions (PTTT 2 in Figs. 5A and 5B; Fig. 5D); after pollen tube growth, the PTTT is filled with secretions covering the pollen tubes (PTTT 3 in Figs. 5A and 5B; Figs. 5E and 5F). For the PTTT distal to (towards the umbrella canopy) or at the region
of closure, numerous pollen tubes can be present in one PTTT (Fig. 5G) while absent in others (Fig. 5B) depending on whether the stigma receives pollen grains or not. Proximal to the region of closure (towards the ovary), the space at the center of the umbrella stalk opens up again without the presence of any tanniniferous cells lining this space, and each PTTT is open to the center forming a star-shaped compitum lined with two layers of darkly stained transmitting tract cells (Fig. 5H) where pollen tubes from a different PTTT could enter another PTTT, each of which is connected to a locule in the ovary (Fig. 5I).

At the proximal region of the umbrella stalk, ten major collateral vascular bundles are found, with a carpellary vascular bundle accompanying each PTTT and an intercarpellary vascular bundle between adjacent PTTTs, and with the xylem facing the center of the umbrella stalk and the phloem facing the periphery (Figs. 5A, 5B, and 5H). The epidermis and ground parenchyma at the proximal region (Figs. 5A and 5H) are similar to those of other regions of the umbrella stalk (Figs. 4I–4L). The epidermal cells of the umbrella stalk are more or less distal-proximally elongated (Figs. 5B), filled with condensed tannins (Figs. 5A, 5H, and 5J), and have uneven cuticular striations (Fig. 5J). The subepidermal cells are also darkly stained and filled with tannins (Figs. 5A, 5H, and 5J). No obvious chloroplasts are observed in the ground parenchyma of the umbrella stalk.

Micromorphology and anatomy of the umbrella canopy between the PTTTs

Elongated trichomes are highly abundant on the inner epidermis of the umbrella canopy excluding the periphery of the stigma lobe region, the margin of the indented area, and the center region (Figs. 1C, 1D, 6A, 6E, 6G, and 6I) and are sparsely distributed on the outer epidermis only at the stigma lobe region, the ray region, and the narrow regions flanking the ray (Figs. 1C
and 6B). Secretory glands are abundant on the outer epidermis of the umbrella canopy excluding the ray region (Figs. 6D–6F) and are sparse on the inner epidermis excluding the center region and the ray region, with a higher density (abundant) around the margins of the stigma lobe region (Fig. 6C). Stomata have very similar distribution patterns as the secretory glands on the inner and outer epidermis of the umbrella canopy, except that they do not occur in higher density around the margins of the stigma lobe region on the inner epidermis (Table 1).

The elongated and unbranched trichomes, ranging from 60 µm to 370 µm long with an average length of 280 µm +/- 107 µm (with a sample size of 13), have linear cuticular striations that are slighted intertwined (Fig. 6H), are heavily stained and unicellular (Figs. 6E and 6G) and are sometimes also found to hold chunks of pollen grains on the inner epidermis (Fig. 6J). The secretory glands are multicellular (Figs. 6E, 6F, 6I, 6K, 6M, and 6N), consisting of more than ten small densely cytoplasmic and darkly stained secretory cells without cuticular striations, which are surrounded by five to seven (usually six) much bigger subsidiary cells that are covered by mostly radial cuticular striations (Figs. 6K and 6M). For most of the secretory glands examined, the cuticular layer of the top secretory cells is broken and peeled off from the cell wall (Fig. 6K; the arrow indicates a piece of peeled cuticle that is partially attached at the base of the cell).

Stomata with darkly stained guard cells are found on both the inner and outer epidermis of the umbrella canopy (Figs. 6E, 6F, 6I, 6L, 6M, and 6O). The regular epidermal cells on both the inner and outer epidermis of the umbrella canopy have a convex outer surface (Figs. 6E–6G, 6N, and 6O) and intense irregular cuticular striations (Figs. 6P and 6Q), except small patches of the epidermal cells that often occur near the stomata and/or the secretory glands and have a more or less smooth outer surface (Figs. 6I and 6M).
The inner and outer epidermis and the subepidermal layer of the umbrella canopy are filled with tannins (Table 1) and are relatively darker stained compared to the ground parenchyma (Figs. 6E–6G, 6N, and 6O). The ground parenchyma is aerenchymatous (Figs. 6E–6G), and chloroplasts are found next to the membranes of its cells (Figs. 6N and 6O). Both major and minor vascular bundles are found within the ground parenchyma (Figs. 6E–6G). The collateral major vascular bundles are surrounded by one layer of small and thick-walled bundle sheath cells, within which the xylem faces the outer epidermis, and the phloem faces the inner epidermis (Figs. 6E and 6F). The major vascular bundles are more or less parallel to their adjacent PTTT at the peripheral and middle regions (Figs. 6E and 6F), while they start to converge at the near center region of the umbrella canopy (Fig. 6G).

Comparison of micromorphology and anatomy among the broadened and flattened stylar umbrella canopy, sepal, petal, and lid of the pitcher leaf

The umbrella canopy of the style shares micromorphological and anatomical similarities with the sepals, petals, and lid of the pitcher leaf, but also has its unique characteristics.

Even though secretory glands are present on the epidermal surfaces of the lateral organs examined (Table 1), the stylar umbrella canopy has a unique type of secretory gland with similar secretory cells at both the epidermal and subepidermal levels (Figs. 6K, 6M, and 6N), while the secretory gland of the sepal (Figs. 7K and 7L), petal (Figs. 8N and 8O), and lid of the pitcher leaf (Figs. 9M and 9N) have two distinctly stained apical cells that are flanked by four secretory cells at the epidermal level and surrounded by other secretory cells at the subepidermal level. Secretory glands on the sepal are sparse on the abaxial epidermis; decrease distal-proximally in density from sparse to absence on the adaxial epidermis (Table 1; Figs. 7A–7F and 7I). Secretory
glands of the petal are only present on adaxial and abaxial epidermal surfaces of the middle and proximal regions of the R1 segment (Table 1; Figs. 8A, 8F, and 8G), regions that overlap with the stylar stigma lobes (Figs. 1E and 8A). Secretory glands are sparse on both the abaxial and adaxial epidermis of the lid of the pitcher leaf (Table 1; Figs. 9A, 9C, 9E, 9F, and 9H).

The stylar umbrella canopy is similar to the lid of the pitcher leaf in terms of the presence of elongated trichomes (Table 1), which are present on both the adaxial and abaxial epidermis of the lid of the pitcher leaf (Figs. 9A–9C and 9H–9L), while absent from the sepals (Figs. 7B–7J) and petals (Figs. 8B–8M). On the lid of the pitcher leaf, the adaxial trichomes have almost perfectly parallel linear cuticular striations with a pointed tip (Figs. 9I and 9J), and the abaxial trichomes have a swollen base and irregular wavy cuticular striations (Figs. 9K and 9L), while the elongated trichomes of the stylar umbrella canopy have slightly intertwined linear cuticular striations (Fig. 6H). Trichomes on the inner epidermis of the stylar umbrella canopy and the adaxial epidermis of the lid of the pitcher leaf are both highly abundant (Table 1; Figs. 6A, 6I, 6J, and 9I).

The stylar umbrella canopy is similar to the sepal and lid of the pitcher leaf in that they all have abundant stomata and chloroplasts (Table 1; Figs. 7M, 7N, 9O, and 9P). Stomata are highly abundant on the abaxial epidermis and abundant on the adaxial epidermis of both the sepal (Figs. 7A–7I) and the lid of the pitcher leaf (Figs. 9A–9C, 9E, 9F, and 9H), except the adaxial epidermis of the R3 segment of the sepal (Fig. 7A) where they are absent (Table 1). Even though chloroplasts are not observed in petals, sparse stomata are still found on the abaxial epidermis of the distal and middle regions of the R1 segment and rarely found on the adaxial epidermis of the middle region of the R1 segment of the petal (Table 1; Figs. 8A, 8F, 8P, and 8Q).
The stylar umbrella canopy is similar to the petal in terms of the shape of normal epidermal cells and the presence of intense cuticular striations on them (Table 1; Figs. 6K, 6P, 6Q, 8C, 8D, 8I, 8J, 8L, and 8M). Epidermal cells are papillate and isodiametric at the distal R1 segment (Figs. 8A–8G) and gradually transition (Figs. 8H–8J) to convex or flat-convex and slightly elongated at the proximal R3 segment (Figs. 8K–8M) of the abaxial and adaxial epidermis of petals (Table 1). All epidermal cells, except those that cover the ray regions (Figs. 3K–3R) of the stylar umbrella canopy, are similar to the normal epidermal cells of the R2 segment of the petal (Fig. 8A) in terms of their isodiametric cell shape (Figs. 6P, 6Q, 8I, and 8J) and are similar to those of the R3 segment of the petal (Fig. 8A) in terms of their slightly convex profile (Figs. 6E–6G, 6O, and 8K). Epidermal cells on both the sepal and the lid of the pitcher leaf are irregularly-shaped (Figs. 7C, 7D, 7F, 7G, 9E–9H) except the R3 segment of the sepal (Fig. 7A), where they are more or less rectangular and slightly elongated (Figs. 7I and 7J), and they lack intense cuticular striations and are flatter (Figs. 7B–7J and 9A–9H).

Similar to the stylar umbrella canopy (Table 1), tannin deposits and/or condensed tannins are also found in the sepal (Figs. 7B, 7E, 7H, 7L, and 7N), petal (Figs. 8B, 8E, 8H, 8K, 8O, and 8Q), and the lid of the pitcher leaf (Figs. 9A–9D, 9N, and 9P). Xylem in the major vascular bundles faces the outer epidermis of the stylar umbrella canopy (Figs. 3B–3F, 6E, and 6F) and faces the adaxial epidermis of the sepal (Figs. 7B, 7E, and 7H), petal (Figs. 8B, 8E, 8H, and 8K), and the lid of the pitcher leaf (Figs. 9A, 9B, and 9D).

**Discussion**

*Functions as part of the female reproductive structure*

*Pollen grain capture and germination*
This is the first study that showed the detailed micromorphology and anatomy of the stigma region of a mature flower of *S. purpurea*. This study agreed with previous studies (Shreve 1906; Löfstrand and Schönenberger 2015) that the stigma papillae are unicellular, unbranched, and are of the “dry” type (Heslop-Harrison and Shivanna 1977). This study also agreed with Shreve’s (1906) study that pollen grains germinate after contact with the stigma papillae and pollen tubes grow between the cells of the stigma papillae. In addition, this study showed the pattern of cuticular striations on the densely cytoplasmic stigma papillae and the anatomical characteristics of the complex transmitting tissue of the stigma region.

The stigma of *S. purpurea* is found to be at least partially receptive even before anthesis (Burr 1979). Pollination studies on *Sarracenia flava* L. (Sheridan and Karowe 2000) and *S. purpurea* (Mandossian 1965; Burr 1979) showed that even though the flower is self-compatible, self-pollinated flowers produce significantly lower quantity (Burr 1979) and quality of offspring in terms of seed number, seed mass, germination rate, and survivorship (Sheridan and Karowe 2000). Thomas and Cameron (1986) observed that large pollinators of the genus *Bombus* (Hymenoptera, Apidae) both enter and exit the pollination chamber through the opening or doors above the stigma and based on this observation concluded that these pollinators potentially both contribute to self-pollination and cross-pollination of the flower of *S. purpurea*, which is in agreement with Burr’s (1979) observations. The data from this study showed that stigma papillae are only found facing the top and outside of the pollination chamber, thus pollen grains are much more likely captured when insects enter the pollination chamber rather than exit, encouraging cross-pollination. In fact, field studies (Mandossian 1965; Burr 1979; Thomas and Cameron 1986) indicated that self-pollination rarely happens in native populations of *S. purpurea.*
Pollen tube transmission

Shreve’s (1906) study found that pollen tubes grow within the rays of the umbrella canopy and through the continuous conducting tissue of the umbrella stalk of S. purpurea. This study agreed with Shreve’s (1906) study that the PTTT of the stylar umbrella canopy is semi-solid according to Hanf’s (1935) classification and is filled with aerenchymatous conducting tissue that has elongated conducting cells; the PTTT of the stylar umbrella stalk is of the hollow type according to Hanf (1935), and the transmitting track cells on both sides of the PTTT split at the time of pollination to form a canal. In addition, this study found that pollen tubes received from a certain stigma can only go through the same PTTT until they reach the proximal region of the umbrella stalk near the ovary where they have a chance to go through other PTTTs and eventually reach the ovules in another locule. Shreve (1906) also observed that the pollen tubes are at the juncture of adjacent placental outgrowths when they enter the ovary, which further supports the fact that even if only one of the five stigmas receives pollen grains, all ovules in the ovary could possibly receive the sperms from the mature microgametophytes.

Shreve (1906) concluded that at the center of the umbrella canopy of the mature flower where the five rays converge, the pentagonal cavity “connects the interior of the capsule with the external air,” based on his observations that there is a “central cavity” in the umbrella stalk and “in an ovary the cavity of which has direct communications with the external air.” However, this study showed that even though a funnel-shaped cavity is found at the center of the stylar umbrella stretching from the umbrella canopy to two-thirds of the length of the umbrella stalk, the entire surface of this cavity is covered by darkly stained tanniniferous cells, which are also found in the epidermis of the umbrella canopy and umbrella stalk, the sepal, petal, and lid of the pitcher leaf. Our developmental study demonstrated that the five PTTTs within the umbrella
stalk are originally sealed by the postgenitally fused lateral carpellary margins, ensuring that the ovules are completely enclosed by the carpels (angiospermy), and the internals of the PTTTs are not in contact with the environment except at the stigma region. This study showed that the presence of numerous pollen tubes co-occurred with an open PTTT. Thus, the “open gynoecium” that Shreve (1906) observed is the result of the breakage of postgenitally fused carpel lateral margins when the pollen tubes go through the PTTT in the umbrella stalk. When this breakage happens at approximately two thirds of its length at the proximal region of the umbrella stalk where the five PTTTs converge, it exposes the center cavity that is connected with the ovary. Since at anthesis the flower of *S. purpurea* is pendulous, rain cannot drop into the funnel-shaped cavity at the center of the stylar umbrella, thus protecting the growing pollen tubes within the PTTTs even when the PTTTs are open.

*Potential energy provider for pollen tube growth and secretory structures*

Consistent with the results from this study, chloroplasts are found in the epidermal cells of the carnivorous leaves of *S. purpurea* ssp. *purpurea* and have been demonstrated to have photosynthetic activities by tests using fluorescent and immunohistochemistry (Joel and Gepstein 1985). Photosynthesis of *S. purpurea* leaves is considered as a phenotypically plastic trait because manipulated fertilization experiments showed that elevated nitrogen availability resulted in the production of photosynthetic leaves with large keels and small carnivorous tubes, as well as enhanced photosynthetic rates (Ellison and Gotelli 2001). In addition, field studies found that even though carnivorous pitchers of *S. purpurea* benefit from additional nutrients by capturing insects, their overall efficiency of capture is only less than one capture per 100 of insect visits (Newell and Nastase 1998). Thus, during anthesis and pollination, the critical stage of the sexual
reproductive life cycle of *S. purpurea*, in conditions without enhanced level of photosynthates from the leaves, the photosynthetic stylar umbrella canopy and sepals could supplement the critical energy needed to provide for pollen tube growth as well as secretory structures of the flower that are essential for interacting with insects (discussed below). Our results agreed with Shreve’s (1906) results that the umbrella canopy of *S. purpurea* is leaf-like, with homogenous aerenchymatous ground tissue, numerous stomata on both inner and outer epidermis, are potentially photosynthetic, and possibly provide nutrition for pollen tube growth and other secretory epidermal structures. In agreement with this study, Löfstrand and Schönenberger (2015) also found that stomata are present on both the inner and outer epidermal surfaces of the stylar umbrella canopy and on the epidermis of the sepals and petals. However, in this study, chloroplasts are not observed in the ground parenchyma of the petal and are only found in the stylar umbrella canopy (excluding the center region), sepal, and lid of the pitcher leaf, all of which have abundant or highly abundant stomata.

Stomatal glands or ‘nectarostomata’ are found in pitcher leaves of carnivorous plants (Joel 1986; Juniper et al. 1989; Nepi 2007). Löfstrand and Schönenberger (2015) found that stomata that are present on the sepals, petals, and stylar umbrella canopy of *S. purpurea* exude viscous droplets that are carbohydrate-rich. This study showed that the guard cells of the stomata of the stylar umbrella canopy, sepal, and petal are densely cytoplasmic and darkly stained, further supporting their secretory nature. In addition to the secretory stomata, multicellular secretory glands are also found on the stylar umbrella canopy, sepals, and petals of *S. purpurea*. In this study, the cuticular layer of the top secretory cells of these glands is peeled off during anthesis, which we hypothesize to facilitate secretion. This is consistent with a previous study (Tilton and Horner 1980) which also found that the cuticular layer of the endothelium cells of the
style of *Ornithogalum caudatum* (Liliaceae) is intact before the secretory phase and is sloughed off when these cells enter the secretory phase. Furthermore, the ovary epidermis of *S. purpurea* is embedded with nectarioles that produce nectar containing saccharose, oligosaccharides, trisaccharides, and disaccharide raffinose (Vogel 1998), and nectar that contains about 39% of sugar was found at the base of the ovary and distributed to the base of stamen filaments in the flower of *S. flava* (Schnell 1983). These secretory structures play an important role during flower-pollinator interactions, as discussed below, and are very likely to consume photosynthates from the stylar umbrella canopy and sepal of the flower of *Sarracenia* in addition to those from the leaves.

**Significant impacts on the pollination system**

**Floor of the pollination chamber and pollen retainment**

The evolution of a stylar umbrella canopy not only gives rise to the spatial separation of the pollinator rewards (nectar and pollen) and the stigmas to encourage cross-pollination but also gives rise to a novel pollination chamber by providing the floor and part of the wall (Fig. 1E). Field studies of *S. purpurea* showed that pollen grains are collected by bees of *Bombus affinis* and *Augochlorella aurata*, and the fly of *Fletcherimyia fletcheri* (Burr 1979; Ne’eman et al. 2006). A *S. flava* flower produces 30.77 mg of pollen grains on average (Schnell 1983). Multiple authors have proposed that one of the functions of the stylar umbrella of the pendulous flower of *Sarracenia spp.* is to retain dehisced pollen grains from the anthers on top of the pollination chamber (Shreve 1906; Jones 1908; Burr 1979; Schnell 1983). During controlled pollinations, researchers (Sheridan and Karowe 2000) collected pollen grains by scraping them from the inner surface of the stylar umbrella, which showed that the pollen grains are still viable after landing
on the stylar umbrella. Both Shreve (1906) and Löfstrand and Schönenberger (2015) observed unicellular trichomes on both the inner and outer epidermal surfaces of the umbrella canopy; Burr (1979) proposed that these elongated trichomes on the inner epidermis could catch and retain pollen and Shreve (1906) observed that they do catch a considerable amount of pollen; all of which are consistent with the results from this study. Furthermore, the distribution pattern of the elongated trichomes on the inner epidermis of the umbrella canopy (Fig. 6A) matches the distribution pattern of anthers on the roof (Fig. 1E). Thus, the stylar umbrella canopy also provides pollen retainment to encourage pollinators for cross-pollination.

*Visual cues during pollinator-flower interactions*

Even though the inner and outer epidermal cells of the stylar umbrella canopy of *S. purpurea* are not as papillate as those of the distal region of the petal, they are similar to those of the middle and proximal regions of the petal by having convex epidermal cells with intense cuticular striations. Comprehensive studies found that papillate cells are the most common epidermal cells on petals and petal-like floral organs even though their epidermis is largely heterogeneous, and papillate cells cooccur with convex and flat cells (Kay et al. 1981; Christensen and Hansen 1998). Cell shape may affect visual stimuli to pollinators by affecting visible light penetration and appearance (Kay et al. 1981; Gorton and Vogelmann 1996). Papillate cells are shown to give rise to unique optical properties for petals during pollinator-flower interactions (Gorton and Vogelmann 1996; Christensen and Hansen 1998; Glover and Martin 1998; Whitney et al. 2009; Ojeda et al. 2012) because they provide a unique combination of strong light absorption, external reflection, refraction, and internal reflection compared to flat epidermal cells (Kay et al. 1981). Cuticular striations may act as light-trapping structures by
efficiently absorbing incident light, and striated convex cells may provide similar optical properties as papillate epidermal cells (Kay et al. 1981).

Field studies on pollinator behaviors indicated that flowers of *Sarracenia* spp. provide both visual and olfactory cues or stimuli for pollinators (Burr 1979; Schnell 1983). Detailed observations showed that at a distance above one meter, *Bombus* spp. approach the flower of *S. flava* in a straight flight pattern (Schnell 1983), indicating visual cues for pollinators because straight approaches generally indicate visual cues while irregular approaches indicate olfactory cues (Fægri and van der Pijl 1979). Burr (1979) placed glass cylinders over flowers of *S. purpurea* to block olfactory cues and observed that a *Bombus* bee responded to the visual stimulus by circumnavigating at the level of the flower. When insects approach a pendulous flower of *Sarracenia*, in which the five stigma lobes of the stylar umbrella canopy alternate with the five hanging petals (Figs. 1B and 1E), the outside of the pollination chamber is not visually uniform to pollinators because the distal regions (R1 segment) of the abaxial (outer) epidermis of the petals are coated with papillate cells, and the outer and inner surfaces of the stigma lobes of the stylar umbrella canopy are coated with convex cells, so they together may form an alternating optical pattern for pollinators to easily find the door above each of the five stigma lobes to enter the pollination chamber. In addition, Burr (1979) noted that the distinct color difference between the distal and proximal regions of the same petal may provide a visual guide for foraging bees, which is also supported by the micromorphological data from this study that epidermal cells at the proximal (R3) region of the petal are flatter and have less intense cuticular striations.

*Olfactory cues or stimuli during pollinator-flower interactions*
Floral odors or fragrances of different degrees are recorded for eight species of *Sarracenia*, including *S. purpurea* subsp. *purpurea* (Schnell 1978), even though the degree of the fragrance of *Sarracenia* flowers is reported to be stronger towards the evening (Jones 1908). Observations on *Bombus* bee’s approach to flowers of *S. flava* showed that at a distance around one meter, *Bombus* spp. change their straight flight to an irregular circling motion before they land on the sepal or petal; they also approach gauze-bagged flowers that may have blurred visual cues while still being able to release fragrance; they even approach tightly closed flower buds of *S. flava*, which have apparent fragrance; all these observations indicated olfactory cues provided by the flower (Schnell 1983). Burr (1979) recorded a rose-like odor for flowers of *S. purpurea* at anthesis and minty-fruity constituent in the odor of their soft buds. To detect the sources of the floral fragrance, Schnell (1983) dissected and separated different floral structures and found that petals present the strongest fragrance, and the stylar umbrella canopy presents relative strong fragrance, while the stylar umbrella stalk and ovary present zero to low fragrance. In addition, Schnell (1983) reported that the nectar of *S. flava* flower does not have an aromatic component. Thus, secretory stomata (Löfstrand and Schönenberger 2015) and multicellular secretory glands (Shreve 1906) of the stylar umbrella canopy, sepal, and petal are more likely to be involved in providing the olfactory properties of the stylar umbrella canopy, even though the compounds of their non-nectarial secretion are still unclear and deserve future studies. In this study, in addition to their densely cytoplasmic anatomical characteristic, patches of smooth epidermal cells among striated normal epidermal cells are often found near stomata and/or secretory glands, indicating that their cuticular striations are covered by secretions, which further support the secretory nature of the stomata and glands. Patches of smooth epidermal cells that do not occur near stomata
and/or secretory glands may be covered by the nectar drops from the ovary wall on the roof of the pollination chamber.

Micromorphological data on the distribution of secretory glands on the petal and stylar umbrella canopy from this study also showed interesting patterns. Secretory glands are found in higher density around the margins of the stigma lobe on the inner epidermis of the stylar umbrella canopy and are only found on adaxial and abaxial epidermal surfaces of the middle and proximal regions of the R1 segment of the petal, which overlaps with the stigma lobe to form a wall of the pollination chamber. Thus, the wall may exhibit stronger floral odor or fragrance and, therefore, may provide an olfactory cue for the pollinator to find the door of the pollination chamber in combination with the potential visual cues discussed above.

Furthermore, biochemical analysis of volatiles from pitcher leaves of four Sarracenia spp. showed that scent compounds emitted from the leaves are those typically found in floral odors, many of which are attractants of nectar-feeding insects (Jürgens et al. 2009). It will be interesting to compare the volatiles that gives rise to the fragrance from the flowers of Sarracenia spp. with those of the flower-like odor from the nectar glands of their pitcher leaves (Juniper et al. 1989) to investigate potential pollinator-prey conflict through olfactory cues in addition to the temporal separation of anthesis and active prey capturing time of pitcher leaves (Horner 2014).

_Tactile cues and stimuli during pollinator-flower interactions_

Papillate or conical cells with cuticular striations of petals have been shown to provide extra grips and tactile cues or stimuli for insect pollinators (Kevan and Lane 1985; Whitney et al. 2011; Papiorek et al. 2014). The occurrence of papillate cells on the abaxial (outer) epidermis of
the distal regions (R1 segment) and the middle region (R2 segment) of the petals found in this study is consistent with the observations that pollinators first land on the pendulous petals and/or the more or less horizontal sepals of the flower of *S. purpurea* (Jones 1908; Mandossian 1965; Burr 1979). This study found that epidermal cells of the stylar umbrella canopy are convex and have cuticular striations, which may also provide tactile stimuli after the pollinators enter the pollination chamber.

This study found that elongated trichomes on the inner epidermis of the stylar umbrella canopy and the adaxial epidermis of the lid of the pitcher leaf are both highly abundant. This study also showed that the micromorphology of elongated trichomes that are distributed on the inner epidermis of the stylar umbrella canopy, where pollinators collect pollen, is similar to that of the trichomes found on the adaxial epidermis of lid of the pitcher leaf of *S. purpurea* (also shown in Adams and Smith 1977; Juniper et al. 1989; Nolan 2015) in that they are all unicellular, unbranched, and vertically striated. However, the cuticular striations on the trichomes of the lid of the pitcher leaf have almost perfectly parallel cuticular striations (also shown in Nolan 2015) while those of the umbrella canopy are more or less intertwined, which may provide extra grip for pollinators within the pollination chamber. In contrast, the downward-pointing trichomes on the adaxial epidermis of the lid of the pitcher leaf of *S. purpurea* are proposed to guide insects, and their parallel vertical striations make them hard for insects to maintain their footing (Adams and Smith 1977; Juniper et al. 1989). While in this study most trichomes on the inner epidermis of the stylar umbrella canopy of *S. purpurea* (Fig. 6A) stand upright, most trichomes on the inner epidermis below the stigma and of the peripheral region of the ray point approximately 45 degrees away from the stigma in both directions (Fig. 3O), potentially providing the tactile cue to lead insects to exit the pollination chamber through the
sides of the stigma lobe, rather than the top of the stigma lobe where the stigma is located. In fact, Schnell’s (1983) data showed that 76.8% of entrances of pollinators to flowers of *S. flava* are over the stigma lobes, while only 34.1% of exits are over the stigma lobes.

**Conclusions**

This study provided detailed anatomical and micromorphological data on the natural history of pollen grain capture and pollen tube transmission of *S. purpurea*: pollen grains are retained by the unicellular and unbranched papillae of the dry stigma where they germinate and grow between the stigma papillae towards the semi-solid PTTT of the umbrella canopy which consists of both ectotrophic and endotrophic conducting tissues; the pollen tubes then travel through the ectotrophic conducting tissue of the PTTT of the umbrella stalk before they reach the ovary. In addition to illustrating the stylar umbrella’s role as part of the female reproductive structure of the flower of *S. purpurea*, this study showed anatomical and micromorphological data that support the crucial roles that the stylar umbrella may play as the floor and part of the wall of the pollination chamber and by providing visual, olfactory, and tactile cues during pollinator-flower interactions. This study also demonstrated how different floral organs of *S. purpurea* work together to form a specialized pollination chamber—an amazing floral innovation in eudicotyledons.

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References


Table 1. Micromorphological and anatomical characteristics of the stylar umbrella canopy, sepal, petal, and lid of the pitcher leaf of *Sarracenia purpurea*.

<table>
<thead>
<tr>
<th>Secretery Glands</th>
<th>Trichomes</th>
<th>Stomata</th>
<th>Shape of Normal Epidermal Cells</th>
<th>Cuticular Striations</th>
<th>Epidermal Tannins</th>
<th>Subepidermal Tannins</th>
<th>Chloroplasts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outer Epidermis</strong></td>
<td>Present (Fig. 6D)</td>
<td>Present (Fig. 6B)</td>
<td>HA; Absent at the ray region</td>
<td>Between Rays: Convex; Isodiametric</td>
<td>Present; Intense; With patches that are absent</td>
<td>CoT, TDe</td>
<td>TDe</td>
</tr>
<tr>
<td><strong>Stylar Umbrella Canopy Inner Epidermis</strong></td>
<td>Present (Fig. 6C)</td>
<td>Present (Fig. 6A)</td>
<td>A; Absent at the center and ray regions</td>
<td>Between Rays: Convex; Isodiametric</td>
<td>Present; Intense; With patches that are absent</td>
<td>CoT, TDe</td>
<td>TDe</td>
</tr>
<tr>
<td><strong>Abaxial Epidermis</strong></td>
<td>R1: S</td>
<td>R2: S</td>
<td>Absent</td>
<td>R1: HA</td>
<td>R1: Flat-Convex; Irregular</td>
<td>R1: TDe, CoT</td>
<td>R1: TDe</td>
</tr>
<tr>
<td>Sepal</td>
<td>R3: S</td>
<td>R2: R</td>
<td>Absent</td>
<td>R2: HA</td>
<td>R2: Flat-Convex; Irregular</td>
<td>R2: TDe, CoT</td>
<td>R2: TDe</td>
</tr>
<tr>
<td>Adaxial Epidermis</td>
<td>R1: S</td>
<td>R2: A</td>
<td>Absent</td>
<td>R3: Absent</td>
<td>R3: Flat-Convex, Slightly Elongated</td>
<td>R3: TDe, CoT</td>
<td>R3: TDe</td>
</tr>
<tr>
<td><strong>Abaxial Epidermis</strong></td>
<td>R1d: A</td>
<td>R1m: R</td>
<td>Absent</td>
<td>R1d: S</td>
<td>R1m: S</td>
<td>R1d: TDe, CoT</td>
<td>R1d: TDe</td>
</tr>
<tr>
<td>Petal</td>
<td>R1p: R</td>
<td>R2: Absent</td>
<td>Absent</td>
<td>R2: A</td>
<td>R2: Convex-Papillate; Isodiametric</td>
<td>R2: TDe, CoT</td>
<td>R2: TDe</td>
</tr>
<tr>
<td><strong>Abaxial Epidermis</strong></td>
<td>R1d: Absent</td>
<td>R1m: S</td>
<td>Absent</td>
<td>R1d: Absent</td>
<td>R1m: R</td>
<td>R1d: TDe, CoT</td>
<td>R1d: TDe</td>
</tr>
<tr>
<td><strong>Adaxial Epidermis</strong></td>
<td>R1p: Absent</td>
<td>R2: Absent</td>
<td>Absent</td>
<td>R2: Absent</td>
<td>R2: Convex-Papillate; Isodiametric</td>
<td>R2: CoT</td>
<td>R2: TDe</td>
</tr>
<tr>
<td><strong>Lid of Pitcher Leaf Abaxial Epidermis</strong></td>
<td>S</td>
<td>S; Patchy</td>
<td>HA</td>
<td>Flat; Irregular</td>
<td>Absence of intense striations</td>
<td>TDe</td>
<td>TDe</td>
</tr>
<tr>
<td><strong>Abaxial Epidermis</strong></td>
<td>S</td>
<td>HA; Uniform</td>
<td>A</td>
<td>Flat; Irregular</td>
<td>Absence of intense striations</td>
<td>TDe</td>
<td>TDe</td>
</tr>
</tbody>
</table>

**Notes:** No obvious difference is observed for the micromorphology of the secretory gland and stomata between the abaxial and adaxial epidermis of the sepal, petal, and lid of the pitcher leaf, respectively. A = abundant; Convex-Papillate = cell shape that is in-between convex and papillate; CoT = condensed tannins; Cuticular Striations = intensely uneven cuticles that cover the normal epidermal cells; D = distal; Flat-Convex = cell shape that is in-between flat and convex; HA = highly abundant; M = middle; P = proximal; R = rare; Ray = epidermal region of the umbrella canopy that is above or below the PTTT; R1, R1d, R1m, R1p, R2, R3 = anatomical regions as designated in Figure 7A for sepal and Figure 8A for petal; S = sparse; TDe = tannin deposits.
**Fig. 1.** Floral morphology and pollination chamber of *Sarracenia*. A: A floral diagram of *Sarracenia*, with three bracts (brown half ovals), five sepals (dark red crescents), five petals (pink waves), five stigma lobes (dark green “V” shape), approximately 80 stamens (yellow circles), and a syncarpous gynoecium at the ovary level (light-green hexagon). B: A flower of *Sarracenia purpurea*. Five sepals, five petals, and the umbrella canopy of the style are visible. C: Top view of the umbrella-shaped style showing the outer surface of the stylar umbrella canopy with five pollen tube transmitting tracts (PTTTs; rays), which converge at the center. Dotted lines indicate morphological regions of the stylar umbrella canopy cited in the results of this study. D: Side view of the umbrella-shaped style showing the ovary, the cylindrical stylar umbrella stalk, the flattened and broadened stylar umbrella canopy with five stigma lobes, each of which is terminated with a stigma at the inner distal region (solid arrowheads), and the rays on the inner surface of the stylar umbrella canopy. E: Schematic diagram showing the configuration of the pollination chamber. The colors used for each structure corresponds to the color used in the floral diagram. IE = inner epidermis of the umbrella canopy; OE = outer epidermis of the umbrella canopy; Pe = petal; Sp = sepal.

**Fig. 2.** Micromorphology and anatomy of the stigma of *Sarracenia purpurea*. A and B: Distal (A) and proximal (B) outer regions of the stigma facing the top of the pendulous flower, as shown in Fig. 1E. C: Side view of the distal outer region of a naturally pollinated stigma showing unicellular stigma papillae and germinating pollen grains with pollen tubes growing between the papillae; the semi-hollow interior (center of photo) is also shown. D: Longitudinal section of a naturally pollinated stigma showing the semi-hollow interior of its distal region and numerous germinating pollen grains with pollen tubes growing between the stigma papillae and then into...
the conducting cells of the PTTT. E and F: Close up of stigma papillae on the distal (E) and
middle (F) outer regions of the stigma showing their cuticular striations. G–L: Serial transverse
sections of a naturally pollinated stigma from the distal region (G) to proximal region (K), and
the transition region (L) between the stigma and the stigma lobe region of the umbrella canopy,
showing the transition from the semi-hollow interior of the stigma to the PTTT of the stigma
lobe region. Asterisk indicates a vascular bundle. M: Longitudinal section of a naturally
pollinated stigma showing pollen tubes (thin lines; stained dark red) embedded in secretions
(stained light blue) surrounded by conducting cells (stained blue with red stained nucleus;
connected with each other at the ends forming a thread) and transmitting track cells (outermost
layer of the PTTT; stained dark red). Dotted oval outline indicates a segment of a pollen tube.

CC = conducting cell; GP = ground parenchyma; LCM = lateral carpellary margin(s); Pa =
stigma papillae; PG = pollen grain; PT = pollen tube; PTTT = pollen tube transmitting track; Se
= secretions; SG = secretory gland; Sm = stoma; Tr = trichome; TT = transmitting track cell.

Refer to previous figure legends for all other abbreviations. Scale bars: A, C, D, G–K, M = 100
µm; B, L = 200µm; E, F = 10 µm.

**Fig. 3.** Micromorphology and anatomy of the pollen tube transmitting tract (PTTT) of the stylar
umbrella canopy of *Sarracenia purpurea*. A: Median longitudinal section of the stigma showing
that the transmitting tissue of the stigma is connected with the distal region of the PTTT of the
stigma lobe region of the umbrella canopy. B–E: Transverse sections of the PTTT and its
surrounding tissues at the stigma lobe (B), peripheral (C), middle (D), and near center (E)
regions of the umbrella canopy. Solid arrowhead indicates a conducting cell, which is more or
less circular in transverse section and stained lighter compared to the pollen tubes (indicated by
short arrows) within the PTTT. F and G: Anatomy (F) and micromorphology (G) of longitudinal sections of the PTTT of the middle region of the umbrella canopy showing the distribution of pollen tubes (darkly stained filaments within the PTTT), secretions (lightly stained areas within the PTTT), conducting cells (thick and darkly stained cells at the peripheral region of the PTTT), and transmitting track cell (darkly stained outer most layer of the PTTT) and its accompanying major vascular bundle showing vessels with helical secondary thickening of the lateral walls. H: Close up of a longitudinal section of the PTTT showing that pollen tubes are embedded in secretions. I and J: Close up of conducting cells from the interior of the PTTT of the stigma lobe (I) and peripheral (J) regions of the umbrella canopy. Conducting cells have tapered ends and are covered by secretions. K–N: Ray region of the outer epidermis (above the PTTT as shown in panels 3B–3E) at the stigma lobe (K), peripheral (L), middle (M), and near center (N) regions of the umbrella canopy. O–R: Ray region of the inner epidermis (below the PTTT as shown in panels 3B–3E) at the stigma lobe (O), peripheral (P), middle (Q), and near center (R) regions of the umbrella canopy. BC = bundle cap; Ch = chloroplasts; Ph = phloem; sTr = short trichome; tGP = tightly packed ground parenchyma; VB = vascular bundle; Xy = xylem. Refer to previous figure legends for all other abbreviations. Scale bars: A = 200 µm; B–F, K–R = 100 µm; G, H = 50 µm; I = 20 µm; J = 10 µm.

**Fig. 4.** Anatomy of the center of the stylar umbrella of *Sarracenia purpurea*. A–F: Serial longitudinal sections of the stylar umbrella from the near center region (A) to the center region (F) showing the transition from the umbrella canopy to the umbrella stalk, the transition from a horizontal distribution of the PTTT to a vertical distribution, the gradual formation of the funnel-shaped cavity, and the tanniniferous cells lining the interior surface of the funnel-shaped cavity.
G–L: Serial transverse sections of the stylar umbrella from the top of the funnel-shaped cavity (G) to the proximal region of the umbrella stalk (L) where the funnel-shaped cavity is almost closed, showing the anatomical characteristics of the ground parenchyma, the distribution of vascular bundles, and the gradual horizontal expansion of the PTTT. Bracket indicates a PTTT. Solid arrowhead indicates that the PTTT is intact at a particular region where postgenital fusion of the lateral carpellary margins is maintained while PTTTs that are not indicated by an arrowhead are open at a particular region because of pollen tube growth and the breakage of the postgenital fusion of the lateral carpellary margins. Scale bar next to panel L also applies to panels H–K. M: Close up of an intact PTTT from the transverse section in panel G, showing the presence of conducting cells in the PTTT at the transition region between the umbrella canopy and the umbrella stalk. N and O: Close up of an intact PTTT (N) and an open PTTT (O) from the transverse section in panel I of the distal region of the umbrella stalk, showing the absence of conducting cells in the PTTT. In panel O, some transmitting track cells are detached and the tanniniferous cells are originally continuous before pollen tube growth. P: Close up of an intact PTTT from the transverse section in panel L of the proximal region of the umbrella stalk, showing two layers of transmitting track cells, some of which are detached, and the absence of conducting cells in the PTTT. CoT = condensed tannins; cVB = carpellary vascular bundle; FC = funnel-shaped cavity; iVB = intercarpellary vascular bundle; TC = tanniniferous cell. Refer to previous figure legends for all other abbreviations. Refer to previous figure legends for all other abbreviations. Scale bars: A–F, H–L = 200 µm; G = 400 µm; M–P = 100 µm.

**Fig. 5.** Anatomy and micromorphology of the proximal region of the stylar umbrella stalk of *Sarracenia purpurea*. A and B: Transverse sections at approximately two-thirds of its length to
the distal end of the umbrella stalk showing the closure of the central cavity, the convergence of the PTTTs, and their surrounding tissues. Only three of the five PTTTs are shown in panel A. C–E: Close up of PTTTs at three different developmental stages from panel B: the PTTT is initially closed with two layers of transmitting track cells on both sides beginning to produce secretions (C); more secretions fill the cavity of the PTTT and push it wide open (D); pollen tubes growing inside the PTTT absorbing most secretions (E). F and G: Longitudinal sections of the PTTT with pollen tube growth and its surrounding tissues at the proximal region of the umbrella stalk, showing two layers of transmitting track cells and numerous pollen tubes embedded in secretions. H and I: Transverse sections of the proximal end of the umbrella stalk (H), which is sunken into the ovary, showing the opening up of the central cavity where the five PTTTs are connected, and the distal region of the ovary (I) showing that PTTTs connect the central cavity with the locules. J: Transverse section of epidermal and subepidermal layers of umbrella stalk showing condensed tannins in the epidermal tanniniferous cells and their cuticular striations. CuS = cuticle striations; Ep = epidermis; mXy = metaxylem; pXy = protoxylem; sEp = subepidermis. Refer to previous figure legends for all other abbreviations. Scale bars: A = 100 µm; B = 500 µm; C–E, G = 50 µm; F = 25 µm; H, I = 200 µm; J = 20 µm.

**Fig. 6.** Micromorphology and anatomy of the regions of the umbrella canopy between the rays of *Sarracenia purpurea*. A–D: Schematic diagrams of a unit of the umbrella canopy with a ray (PTTT region; dotted line) at the center, showing the distribution and density of trichomes on the inner (A) and outer (B) epidermal surfaces and secretory glands on the inner (C) and outer (D) epidermal surfaces. Absence is indicated by white; higher density is indicated by darker greys: HA = highly abundant; A = abundant; S = sparse; black colored structure indicates stigma, which
is surrounded by the stigma lobe region of the umbrella canopy. E–G: Transverse sections of the peripheral (E), middle (F), and near center (G) regions of the umbrella canopy, which were sectioned perpendicular to a PTTT with the outer epidermis facing the upper side. H: Close up of the cuticular striations of a trichome on the inner epidermis of the stigma lobe region showing its cuticular striations. I: Group of epidermal structures, including trichomes, a stoma, and a multicellular secretory gland on the inner epidermis. J: Inner epidermis showing numerous pollen grains that are clustered together and are retained by the trichomes. K and L: Close up of a secretory gland (K), which is surrounded by subsidiary cells, and a stoma (L) on the outer epidermis. The cuticular layer of the top secretory cells (in panel K) is peeled off from the cell wall. M: Group of epidermal structures, including several stomata and a multicellular secretory gland on the outer epidermis. N and O: Close up of a multicellular secretory gland (N) and a stoma (O) on the outer epidermis and their surrounding tissues in the middle region of the umbrella canopy. P and Q: Normal epidermal cells of the inner (P) and outer (Q) epidermis of the umbrella canopy between the rays, showing their intense cuticular striations. Cu = cuticle; GC = guard cell; maVB = major vascular bundle; miVB = minor vascular bundle; SeC = secretory cell; SuC = subsidiary cell; TDe = tannin deposits. Refer to previous figure legends for all other abbreviations. Scale bars: E–F, I, J, M = 100 µm; G = 200 µm; H = 5 µm; K = 50 µm; L, P, Q = 20 µm; N, O = 25 µm.

**Fig. 7.** Micromorphology and anatomy of the sepal of *Sarracenia purpurea*. A: Diagram of a sepal showing three anatomical regions of the distal (R1), middle (R2), and proximal (R3) segments. B, E, and H: Transverse sections of the R1 (B), R2 (E), and R3 (H) segments of the sepal. The abaxial epidermis of the sepal faces the upper side. C and D: Abaxial (C) and adaxial...
(D) epidermis of the R1 segment. F and G: Abaxial (F) and adaxial (G) epidermis of the R2 segment. I and J: Abaxial (I) and adaxial (J) epidermis of the R3 segment. K and L: Secretory gland on the abaxial (K) and adaxial (L) epidermis of the sepal. M and N: Stoma on the abaxial (M) and adaxial (N) epidermis. AB = abaxial epidermis; AC = apical cell of the secretory gland; AD = adaxial epidermis. Refer to previous figure legends for all other abbreviations. Scale bars: B, E = 100 µm; C, D, F, G, I, J = 50 µm; H = 200; K, M = 10 µm; L, N = 25 µm.

Fig. 8. Micromorphology and anatomy of the petal of Sarracenia purpurea. A: Diagram of a petal showing five anatomical regions of the distal (R1d), middle (R1m), and proximal (R1p) regions of the R1 segment, and the middle R2, and proximal R3 segments. B and E: Transverse sections of the R1d (B) and R1m (E) regions of the R1 segment. The abaxial epidermis of the petal faces the upper side. C and D: Abaxial (C) and adaxial (D) epidermal surfaces of the R1d region showing the papillate cells with intense cuticular striations. F and G: Abaxial (F) and adaxial (G) epidermal surfaces of the R1m region showing that the majority of papillate cells have cuticular striations while those near the stomata or secretory glands (indicated by solid arrowheads) have a more or less smooth surface. H and K: Transverse sections of R2 (H) and R3 (K) segments. The abaxial epidermis of the petal faces the upper side. I and J: Abaxial (I) and adaxial (J) epidermis of the R2 segment. L and M: Abaxial (L) and adaxial (M) epidermis of the R3 segment. N and O: Secretory gland on the adaxial epidermis. P and Q: Stoma on the abaxial epidermis. Refer to previous figure legends for all abbreviations. Scale bars: B, E, H = 100 µm; C, D, I, J, L, M = 20 µm; F, G = 50 µm; K = 200 µm; N, P = 10 µm; O, Q = 25 µm.
**Fig. 9.** Micromorphology and anatomy of the lid of the pitcher leaf of *Sarracenia purpurea*. A–C: Transverse sections from the distal (A), middle (B), and proximal (C) regions of the leaf lid. The adaxial epidermis of the leaf faces the upper side. D: Transverse section of the midrib of the proximal region of the leaf lid. E and F: Adaxial (E) and abaxial (F) epidermis of the leaf lid. G: Close up of the irregular shaped epidermal cells of the adaxial epidermis. H: Abaxial epidermis showing the distribution of trichomes, secretory glands, and stomata. I and J: Trichome on the adaxial epidermis (I) with a close up of its cuticular striations (J), which are straight and parallel. K and L: Trichome on the abaxial epidermis (K) with a close up of its cuticular striations (L), which are wavy. M and N: Secretory gland on the adaxial epidermis. O and P: Stoma on the adaxial epidermis. Refer to previous figure legends for all abbreviations. Scale bars: A–D, H, I = 100 µm; E, F = 50 µm; G = 20 µm; J, M, O = 10 µm; K, N, P = 25 µm; L = 5 µm.