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THE ANATOMY AND CYTOLOGY OF
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INTRODUCTION

The Rafflesiaceae, established by Robert Brown in 1834, is a remarkable family of parasitic plants. Among its eight genera and approximately sixty species are included not only the largest flower of the world, *Rafflesia arnoldii*, but also some of the smallest flowers, those of *Pilostyles* spp. All members of the family are obligate parasites on the stems or roots of their respective host plants. The vegetative portion of members of this family is so reduced that it consists only of filaments or plate-like masses of tissue within the host plant tissues. Flowers occur singly in all genera except *Bdallophyton* and *Cytinus*, which have multiple-flowered inflorescences. Most species in the Rafflesiaceae are dioecious.

Members of the family are widely distributed throughout the tropical, subtropical, and warm-temperate regions of the world. All large-flowered species belong to the tribe Rafflesieae and are restricted to the region of Malaysia. The Mitrastemoneae contains only one genus, *Mitrastemon*, which parasitizes roots of some Fagaceae in southern Mexico, Central America, southern Japan, and Malaysia. *Cytinus* and *Bdallophyton* comprise the tribe Cytineae. The former genus parasitizes roots of species of Cistaceae in the Mediterranean region, Malagasy Republic, and South Africa, whereas the latter is a root parasite on species of Burseraceae in Mexico. The largest tribe in the family, in terms of number of species, is the Apodantheae. Representatives of one of the two genera in this tribe, *Apodanthes*, parasitize stems of the Flacourtiaceae, and they are found in southern Mexico, Central America, and northern South America. The other genus, *Pilostyles*, is a stem parasite on Fabaceae (Leguminosae) and is found primarily in tropical and subtropical regions of the world. Most species of *Pilostyles* are located in North and South America, but there are at least two species in Africa, one in Syria and Iraq, and one in south-west Australia. This remarkable pattern of distribution led Good (1964) to include *Pilostyles* in his 'anomalous genera of the tropical zone.'

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Ever since Guillemin established the genus *Pilostyles* in 1834, there has been confusion as to what constitutes a species within this genus. A survey of the literature indicates that many *Pilostyles* species, particularly those of North and South America, are extremely difficult to distinguish from one another. Some of the confusion regarding species determination has resulted from publication of keys that either are vague or inaccurate. More important, however, is the fact that sufficient collections have not been available for study. Thus, the degree of variation of most species is unknown. Several species have been described on the basis of material collected from only one locality. This is the first in a series of papers designed to study as many facets as possible in the life history of a *Pilostyles* species. I hope results obtained from this research on *P. thurberi* will prove useful as a guideline for the study of other species within the genus, and eventually lead to a revision of the genus itself.

Pilostyles thurberi was first collected by George Thurber in 1850 when he was with the Mexican Boundary survey party commanded by Colonel Emory. His specimens contained only fruiting material, but Asa Gray recognized that the material represented a new species and described it in 1854. The type locality given is, "On a small mountain, near the Gila River, June, 1850." This locality is in the extremely arid Lower Colorado Valley subdivision of the Sonoran Desert (Shreve, 1951). In this region *P. thurberi* is parasitic upon *Dalea emoryi*, the Indigo Bush, whereas in Texas it is found on *Dalea formosa* and *Dalea frutescens*. All collections for this research were made from *P. thurberi* populations located in the Sonoran Desert.

MATERIALS AND METHODS

All anatomical and cytological studies were made from materials collected by the author. Voucher specimens have been deposited in the Rancho Santa Ana Botanic Garden herbarium.

Freshly collected materials for embryological and cytological investigation were preserved in three parts absolute ethyl alcohol and one part concentrated propionic acid. When glacial acetic acid was substituted for propionic acid, considerable difficulty was experienced in staining the chromosomes. Herbarium material utilized for certain anatomical investigations was expanded in 2.5% NaOH for 24 hours at room temperature, rinsed in several changes of water, and then treated as liquid preserved material.

Because of their hardness, it was necessary to pretreat portions of *Dalea emoryi* branches, used in the anatomical study of the endophytic system of *Pilostyles thurberi*, in concentrated hydrofluoric acid for two weeks. After this period, the material was thoroughly washed in several changes of water and then processed in the usual manner.

Fig. 1-4.—Fig. 1-3. Arrangements of female *Pilostyles* flowers and fruit on host stem. Fig. 1-2, $\times 0.75$; Fig. 3, $\times 1.25$.—Fig. 4. Zonation in flower formation. *Pilostyles thurberi* flowers formed at a uniform distance behind apices of host branches. $\times 0.4$.

Johansen's (1940) tertiary-butyl alcohol series was utilized for infiltration and embedding of material in paraffin. Sections for embryological study were cut at 5–10 μ , whereas those for other anatomical slides were cut at 10–15 μ . A safranin O - fast green combination was used to stain all sections (Johansen, 1940). Vessel element size and percentage of stainable pollen protoplasts are based upon 100 observations.

Cytological material was stained in propiono-carmin, and permanent squash amounts were made in Hoyer's solution as described by Beeks (1955). Chromosome numbers are based upon twenty observations.

ANATOMY

THE ENDOPHYTIC SYSTEM

The vegetative tissues of members of the Rafflesiaceae are contained entirely within the host plant tissues. Only the flowers break through these host tissues and become visible (Figs. 1–3).

From the time of the earliest anatomical studies of the vegetative portion of members of this family there has been little uniformity in terminology. Several authors, Solms-Laubach (1874, 1875, and 1898) and Harms (1935) among others, have used the terms 'thallus,' 'mycelium,' and 'hyphae' to describe this stage. Although these authors recognized the lack of homology between the structures defined by these terms and those that they were trying to describe, they could offer no better substitute so they utilized them anyway. These terms have been defined for use in wholly unrelated groups of plants and should be restricted to those groups. Pierce (1893) referred to the vegetative elements in this family as "extremely reduced roots . . . so reduced in structure that they are nothing more than embryonic tissue." At the present time there is no positive evidence that the vegetative portion of the Rafflesiaceae is homologous with either the roots or the stem. On this basis it would seem best to adopt the phrase 'endophytic system' to describe the vegetative portion of members of the Rafflesiaceae. This phrase was introduced by Thoday and Johnson (1930) to describe the vegetative body of *Arceuthobium pusillum* in its host *Picea nigra*. As Kuijt (1960) points out, "The phrase endophytic system is especially useful because it is morphologically noncommittal. . . ."

As described by Kuijt (1960) *Pilostyles thurberi* exhibits a somewhat isophasic pattern of parasitic growth. This he defined as, "A mode of growth in which the growth of the endophytic system keeps pace with that of the apices of the host." Figure 4 shows how flower formation in all branches takes place at a rather uniform distance below the shoot apices. This distance is relatively constant from year to year. Kuijt also believes

Fig. 5–8.—Fig. 5. Transection of *Dalea* stem 5 cm below shoot apex. Cells of endophytic system (arrow) have more densely stained cytoplasm and prominent nucleoli. $\times 310$.—Fig. 6. Axillary bud of *Dalea emoryi*, with cell (arrow) of *Pilostyles thurberi* endophytic system just below apex. $\times 240$.—Fig. 7. Transection of heavily infected *Dalea emoryi* stem. Masses of dark *Pilostyles* cells occupy host phloem and send numerous sinkers along rays into the xylem. $\times 65$.—Fig. 8. Radial section of *Dalea* stem. Sinker follows ray into xylem (to left). $\times 170$.

that the Australian *Pilostyles hamiltonii* on *Daviesia* spp. and the Syrian *P. haussknechtii* on *Astragalus* spp. represent this pattern of growth.

Despite repeated attempts, I have not succeeded in germinating seeds of *Pilostyles thurberi*. To my knowledge no one ever has germinated the seeds of any species of this genus, although Heinricher (1917) was able to germinate seeds of another member of the Rafflesiaceae, *Cytinus hypocistis*. Until seeds are germinated and seedlings produced, anatomical observations on *Pilostyles* must be restricted to floral and vegetative material of mature specimens.

Solms-Laubach (1875) accurately described the basic anatomical features of the endophytic system of *Pilostyles thurberi* from material sent to him by Asa Gray. Rather than repeat all of his observations, I shall attempt to clarify, expand, and illustrate important features.

As noted by other authors (Endriss, 1902; Brown, 1912) the vegetative cells of the parasite are readily distinguished from those of the host by their larger nuclei, usually with two nucleoli, and by more densely staining cytoplasm (Fig. 5).

In agreement with Solms-Laubach, my material shows that the vegetative cells of the parasite do not extend into the actively growing shoot apex of *Dalea emoryi*, as those of *P. haussknechtii* were found to do in *Astragalus* spp. (Solms-Laubach, 1874), but remain some distance behind it. In serial transections and longisections I have been unable to distinguish parasitic cells closer to the shoot apex than 5 cm. At this distance the shoot normally has begun to form its second annual growth ring and the endophytic system is represented in the phloem by filaments and small diameter strands of cells (Fig. 5). As shown in figure 6, the vegetative cells are present in the dormant axillary buds of the host so that the infection will be carried into any new shoot.

In regions of flower-bearing age, i.e., 2-3 year-old host stems, the endophytic system is concentrated in the secondary phloem. In transverse sections it may appear as several cord-like strands or as a plate of tissue (Fig. 7). In tangential sections of the *Dalea* stem this system is repeatedly broken up by the elements of the phloem, mostly parenchyma. Prior to flower-bearing age no vegetative cells of *Pilostyles thurberi* occur in the xylem of the host, nor have they been observed in the pith, as reported for *P. ingae* in its host, *Calliandra* (Endriss, 1902). Its absence from the pith is not surprising, however, because pith cells acquire lignified secondary walls during the first year of shoot growth. This makes it unlikely that the parasite would be able to penetrate this tissue even if it reached it.

Wedge-shaped projections of parasitic tissue, termed sinkers, which extend into the xylem, are produced at the time of flower formation. They develop on the inner side of the tissue mass in the secondary phloem which gives rise to the flower. A sinker normally is formed in conjunction with a ray and follows the ray where it crosses the cambium and extends into the xylem. Figure 7 is a transverse section of a *Dalea emoryi* stem bearing a close cluster of numerous flowers of the parasite. Many sinkers

have formed, but none extend to a great depth into the xylem. In other examples, I observed that sinkers may extend far into the xylem, almost to the pith. The shape of the sinkers approximates that of the tall, multi-seriate rays of the host (Fig. 8).

Tracheary elements may or may not be present in the sinkers. Those sinkers in which they are present normally are situated directly below a flower and usually are somewhat larger than sinkers which lack them. A discussion of the vascular tissue is presented in the section on floral anatomy.

Fig. 9. Endogenous nature of bud primordium. Arrow indicates thin layer of endophytic system beneath which the *Pilostyles* flower primordia arise. $\times 75$.

In general, any given portion of the endophytic system will produce flowers for only one year; however, as the major region of flower production keeps pace with the tip of the host stem, flowers occasionally are produced in the older portions of the system. As a region of the endophytic system ceases to produce flowers, most of its cells become filled with tannin-like deposits and cease to function. A few cells appear to remain viable, but the length of time that they remain in this state is not known.

As in *Pilostyles ingae* (Endriss, 1902), flower primordia of *P. thurberi* originate from a mass of tissue, the 'floral cushion' of Solms-Laubach (1875), in the secondary phloem of the host. Endriss found the primordium to arise exogenously in *P. ingae* whereas that of *P. thurberi* arises endogenously. The endogenous nature of the primordium is detectable even into the advanced bud stage because of the presence of a thin layer of parasitic cells which surrounds the bud in the cortex of the host (Fig. 9). In the later stages of bud formation these cells are destroyed. Harms'

(1935) summary of the origin of the flower primordia for the family indicates that those of all known species, except for *Pilostyles ingae*, are endogenous in origin.

Sections show that parasitic tissue is lacking entirely in the roots of *Dalea emoryi*.

FLORAL ANATOMY

Figures 10–14 show the general morphological features of the male and female flowers and seeds of *P. thurberi*. The reader may refer to these for orientation throughout the discussion of anatomical features.

Few anatomical studies have been made of *Pilostyles* flowers. Endriss (1902) presented the first extensive anatomical study when he described the anatomy of *P. ingae*. Kummerow's (1962) observations of *P. berterii* closely follow those of Endriss. Anatomical features of both male and female flowers are so similar that, unless otherwise noted, descriptions given will apply to the flowers of both sexes.

Perianth Segments.—Although individual segments in the three whorls vary from one another in form, they are identical in their anatomy. Endriss (1902) and Hunziker (Harms, 1935) found the perianth segments of *Pilostyles ingae* and *Rafflesia patma*, respectively, to be composed of an abaxial epidermis, undifferentiated mesophyll, and an adaxial epidermis. This pattern also is present in *Pilostyles thurberi*, as seen in figures 15 and 16.

As seen in paradermal section (Fig. 17) the epidermal cells are somewhat polygonal in shape. In transection, cells of the central portion of the abaxial epidermis appear much elongated compared to those nearer the margin and to those of the adaxial epidermis. A prominent cuticular layer with ridged, elongate pattern of relief covers both surfaces of the segments.

Sunken stomata are present only on the abaxial surface of the segments of all three whorls. They are more frequent in the median and basal portions of the segments than in the upper portion. In transection and paradermal section (Fig. 17, 22) the guard cells appear to be normal; however, they lack chloroplasts and the uneven thickenings generally characteristic of these cells. They usually are associated with a substomatal chamber which is connected to the intercellular space system in the mesophyll. Subsidiary cells occur in an anomocytic pattern. The number of stomata, their association with substomatal chambers, and their gross appearance suggests that they are functional, as Endriss suggests; however, the lack of chloroplasts and wall thickenings makes their ability to function in a normal manner questionable. Porsch (Harms, 1935) found reduced, nonfunctional stomata in *Cytinus hypocistis*. In his work on the stomata of *Rafflesia* and *Brugmansia*, Cammerloher (1920) found that the guard cells often continued to divide until a ring of 5–6 cells is formed. Because there was a stoma between the guard cells, he concluded that they were functional to some extent, possibly in transpiration or the release of the fetid odor associated with these flowers.

The undifferentiated mesophyll is composed of isodiametric parenchyma cells in which there is no sign of chloroplasts. In transection, xylary elements form an interrupted band across the tissues near the adaxial epidermis. Near the margin of a segment xylary elements often are enclosed between the two epidermal layers, with no intervening mesophyll cells.

Fig. 10-14.—Fig. 10. Female flower showing habit. $\times 11$.—Fig. 11. The same longitudinal section; drawn without ovules. $\times 11$.—Fig. 12. Male flower showing habit. $\times 16.5$.—Fig. 13. The same, longitudinal section. $\times 16.5$.—Fig. 14. Seed. $\times 40$.

As shown in figures 19 and 20, the remainder of the flower, receptacle, ovary wall, and column, of both sexes has essentially the same structure as the perianth segments, i.e., undifferentiated parenchyma, with included vascular tissue, enclosed by epidermal cells covered by cuticle having an elongate relief pattern. As reported for *Pilostyles ingae* (Endriss, 1902), storage in *P. thurberi* is in the form of oil and fat droplets. Starch is not present in any tissue of this species.

Stylar Canal.—As a result of nonfusion of ovary primordia in the female flower or column primordia in the male flower, a stylar canal is formed (Fig. 18–20). In the female flowers the canal is continuous with the locule, whereas in the male it usually terminates in the column between the lower level of the anthers and the nectary. Epidermal cells which bear the same reticulate-patterned cuticle as the epidermal cells of the perianth line the canal. Interior to this epidermal layer, in the female, lies a layer of transmission tissue. Its cells are more densely cytoplasmic than those of the neighboring ground tissue. This layer parallels the stylar canal and is continuous with the small, densely cytoplasmic cells that line the locule. The epidermal layer of the male canal is bordered by highly vacuolate parenchyma cells rather than the more densely staining cells found in the female.

Stigma.—Figures 10, 11, 18, and 19 show the structure of the stigma in *Pilostyles thurberi*. The elongate, unicellular stigmatic hairs are derived from the epidermal cells. Xylem elements extend into the stigmatic region as a column that surrounds the stylar canal, but they do not form branches near the stigmatic surface.

Whorls of giant cells occupy the same position in the male flower as does the stigma in the female flower (Fig. 12–13, 20). Like the stigmatic hairs, they stain densely and are derived from epidermal cells. These oversize cells are much larger and have more pointed apices than the stigmatic hairs. Solms-Laubach (Endriss, 1902) believed them to be rudiments of the stigma, whereas Endriss felt that this comparison could not be made because of differences in size and shape. In *Pilostyles thurberi* the giant cells are normally in 1–2 whorls, but they may be in as many as five whorls. *Pilostyles blanchetii* has 4–5 whorls of large cells (Harms, 1935). When several whorls of oversize cells are present, there is often a gradation in size, with larger cells in the basal whorl and somewhat smaller cells in the uppermost whorl. The presence of these intermediate stages tends to substantiate Solms-Laubach's interpretation of the origin of these oversize cells.

Nectary.—The secretory tissue of the ring-like nectary, present in both male and female flowers, is composed of small, thin-walled cells rich in cytoplasm. This tissue is covered by an epidermal layer of small cells. Stomates are present and have much the same appearance as those found in the perianth segments (Fig. 21). No substomatal chambers are associated with these stomata. Endriss observed stomata in *Pilostyles* and suggested that they might be slime pores or possibly small nectaries; how-

Fig. 15-16. Perianth segment anatomy.—Fig. 15. Median longitudinal section of segment from median whorl of female flower.—Fig. 16. Transection half way up segment from lower whorl of male flower. (The scale = 100μ)

ever, he termed the entire nectary ring the 'annulus' and believed that it functioned in the opening of the perianth segments. Kummerow (1962) observed nectar in the axils of the perianth segments of *Pilostyles berterii*, an indication that a similar nectary is present in this species.

Crystals.—Small, plate-like, octagonal calcium oxalate crystals occur in the ground parenchyma of the flower (Fig. 31). Their number increases with the age of the flower, and they are most abundant during fruit development.

Vascular System.—Several authors (Endriss, 1902; Guillemin, 1834; and Solms-Laubach, 1875) have reported the presence of xylem elements in various species of *Pilostyles* and observed their connection with the xylem of the host plant. In all cases the individual xylary elements were classified as tracheids because of their helical-to-reticulate secondary wall thickenings. Indeed, when the xylem elements of *P. thurberi* are studied in sectioned material they appear very much like tracheids, but when studied in macerations it is easy to see that they are in fact vessel elements (Fig. 23–24). They are somewhat angular in cross section and have simple perforation plates. Hunziker (Metcalf and Chalk, 1950) reported that the older xylem elements in *Rafflesia patma* have spirally thickened lateral walls, lack transverse walls, and therefore tend to have the structure of vessels. Kuijt (1960) reported that vessels similar to those in *P. thurberi* occur in another reduced parasitic genus, *Arceuthobium*.

Size is variable; however, all elements are quite small (Table 1).

TABLE 1. Vessel-element dimensions, in μ .

	LENGTH	DIAMETER
Maximum	65.8	26.0
Minimum	18.9	6.3
Average	39.7	12.6

As shown in figures 25 and 26, the vessel elements join directly into highly modified vessel elements of *Dalea emoryi*. This junction of elements may occur just inside the cambium or deeper within the xylem if the sinker penetrates the ray deeply.

Tracheary elements arise from procambial strands produced by the floral meristem. The first elements mature in the floral cushion, or mass of tissue from which the flower originates, before the young bud breaks through the host cortical tissue. Further maturation of xylary elements is acropetal.

Fig. 17–22.—Fig. 17. Paradermal section through abaxial epidermis of perianth segment. $\times 155$.—Fig. 18. Transection of stigmatic region in female flower. Note stylar canal, ring of xylem, and stigmatic hairs. $\times 45$.—Fig. 19. Longitudinal section of mature female flower. Prominent are the stylar canal and nectaries at base of column. $\times 45$.—Fig. 20. Median longitudinal section through male flower. $\times 45$.—Fig. 21. Longitudinal section through nectary. $\times 380$.—Fig. 22. Transection of perianth segment near margin; abaxial surface above. $\times 260$.

Fig. 23-27.—Fig. 23-24. Vessel elements from base of mature flower. Macerated preparation. Note simple perforation plates and helical-reticulate secondary wall thickenings. $\times 575$.—Fig. 25-26. Juncture of host and parasite vessel elements. D = *Dalea* vessel elements; P = *Pilostyles* vessel element. Fig. 25, $\times 365$; Fig. 26, $\times 340$.—Fig. 27. Transection of vascular bundle in female flower. Thick-walled cells are vessel elements. $\times 370$.

Vascular bundles in the mature flower are simple in structure (Fig. 27). They contain from 2–20 vessels associated with elongate, densely staining cells with prominent nuclei. Endriss reported cells similar to these in *Pilostyles ingae*, as did Solms-Laubach for *Cytinus hypocistis* (Endriss, 1902). According to Endriss, Goebel viewed these cells as remnants of the sieve tube elements. In *P. ingae*, as well as in *P. thurberi*, these cells lack sieve plates. However, because conduction of large amounts of solutes over long distances is not required, such cells may function adequately. Each vascular bundle is surrounded by ground parenchyma.

Fig. 28–29. Vascular patterns in *Pilostyles thurberi* flowers.—Fig. 28. Male.—Fig. 29. Female. (UW = upper whorl; MW = median whorl; LW = lower whorl; C = column.)

Vascular patterns in the flowers have not been represented in any of the works cited above. From a taxonomic point of view one would like to know the extent of variability within a given species. Figures 28 and 29 illustrate the vascular patterns in male and female flowers of *Pilostyles thurberi*. Even in such simplified patterns there is considerable variation. In female flowers most variation in pattern occurs in the number of carpellary traces, 4–8, and in the continuity of the column of vascular tissue, which may form a solid or broken ring, in the style. Variation in male

30

31

32

33

Fig. 30-33.—Fig. 30. Anther. Note lack of characteristic wall thickenings in endothelial cells. Stippled cells represent tapetum. $\times 255$.—Fig. 31. Crystal complement found

flowers occurs in the number of traces which extend into the column. In some flowers the two original traces do not divide, whereas in other flowers they do divide to give four traces in the upper column region (Fig. 28). Absence or fusion of perianth segments results in variation in pattern. Traces into the perianth segments broaden upon entering the segment and become much branched in various patterns. Neither anthers nor ovules contain vestiges of traces. In male flowers tracheary elements terminate at the level of the whorl of hairs above the anthers; in the female they terminate at the upper limit of the stigma.

MICROSPOROGENESIS

Microsporogenesis and associated steps in anther formation are like those described for *Pilostyles ingae* by Endriss (1902). Three whorls of densely staining lobed swellings appear immediately below the floral meristem prior to emergence of the flower through the host's cortical tissues. Each of the swellings becomes an anther. Figure 30 represents the stage of development attained shortly after the bud has broken through the host tissues. The thin wall between adjacent pollen sacs is formed by 2-3 layers of elongate parenchyma cells. The outer walls are uniformly three cell layers thick. In addition to the outer layer, the epidermis, there are two layers of parietal cells, the innermost of which is the tapetum. The intermediate layer, which would correspond to the endothecium in other angiosperm anthers, lacks the characteristic secondary wall thickenings. The tapetum remains cellular throughout microspore development.

Pollen mother cells are numerous. Their meiotic divisions are normal and give rise to tetrads of both the tetrahedral and isobilateral types. Although Ernst & Schmid (1913) observed that wall formation is successive in *Rafflesia patma*. It appears to be simultaneous in *Pilostyles thurberi*.

MEGASPOROGENESIS AND EMBRYOLOGY

Initiation of ovule development occurs after the female buds have broken through the cortical tissues of the host and attained a diameter of 0.75-1.0 mm. At this stage of floral development, the stigmatic hairs have not yet developed and the perianth segments have not opened. The locule, which is ellipsoidal to spherical in the mature flower (Fig. 11, 42), is a slit-like cavity at this stage. Placentation is parietal.

Stages in ovule and seed development follow closely those described for *Pilostyles ingae* (Endriss, 1902), *Rafflesia*, and *Brugmansia* (Solms-Laubach, 1898; Ernst & Schmid, 1913).

Ovule development is initiated by an enlarged sub-epidermal cell (Fig. 34). Through periclinal divisions it normally forms an axial row of cells which is surrounded by a single layer covering the initial. An oblique division in early stages of axial row formation may lead to the formation of more than one row of cells, but this condition normally lasts for only a few

in mature *Pilostyles thurberi* flowers and fruits.—Fig. 32. Meiosis; metaphase II; $n = 30$.—Fig. 33. Somatic chromosomes at very late prophase. (Scales = 10μ)

divisions (Fig. 35). When the axial row is 9–12 cells long the column begins to curve slightly (Fig. 36). By the time the ovule has a curvature of 45–90°, the developing inner integuments are quite evident (Fig. 37).

The enlargement of the terminal cell in the axial row which forms the megaspore mother cell occurs simultaneously with, or slightly before, the appearance of the inner integument initials. This enlargement is very rapid and results in a cell conspicuous for its size and its large nucleus (Fig. 37).

The appearance of the inner integuments serves to delimit the bounds of the nucellus and the funiculus. The nucellus is of the tenuinucellate type, and no variation on this type was observed. In the early stages the nucellus stains with the same intensity as surrounding cells. For a short distance below the megaspore mother cell there remains an axial row, but this is obscured from the region of the inner integuments to the base of the funiculus by longitudinal divisions (Fig. 38).

Shortly after the formation of the inner integuments, initials for the outer integuments arise below the inner integuments. These initials, like those of the inner integument, result from oblique divisions in the epidermal cell layer (Fig. 38, 43).

At approximately the time of outer integument formation, the cells of the funiculus derived from the axial row become highly vacuolate and begin to degenerate. This process is complete by the proembryo stage of seed development; the resulting funiculus is a hollow cylinder of cells.

Until curvature of the ovule into the anatropous condition is completed, and until the integuments have almost completed their elongation, there are no new tissues or structures produced. During integument elongation, the inner integuments always elongate more rapidly than the outer until their final length is achieved, at which time both are of about equal length. Throughout their development the inner integuments are composed of two cell layers. Except at the micropylar end, where a few of the terminal cells may undergo periclinal divisions and form two layers of cells, the outer integument consists of only one cell layer. The long, narrow micropyle is formed by the inner integument (Fig. 45).

At the time the integuments have elongated and reached the tip of the nucellus the megaspore mother cell begins to enlarge, and concomitant with this enlargement is an increase in the cytoplasmic density of the nucellar cells (Figs. 44–45). By the time the megaspore mother cell undergoes its first division the integuments have completed their elongation, and the ovule has become completely anatropous.

Megasporogenesis is of the usual type, in which a linear tetrad of megaspores is formed, with the chalazal megaspore functional. Occasionally the micropylar dyad cell does not divide again so that there are only two degenerate cells instead of three (Fig. 39). Whether two or three degenerate cells remain, they become flattened and quite conspicuous between the nucellus and developing embryo sac until the latter has completed its development (Fig. 45). Embryo sac development is of the normal or 'Polygonum' type (Fig. 39–41).

Fig. 34-41.—Fig. 34-38. Early stages in ovule development. In Fig. 37-38 note oblique divisions which initiate integument formation. $\times$ 340.—Fig. 39-41. Embryo sac formation. In Fig. 41 the polar nuclei have been omitted because they were not seen. Degenerate megaspores remain conspicuous during these stages. $\times$ 415.

42 **43** **45** **44** **46**

Unfortunately, sections showing the stages between the completed embryo sac and a young embryo were not available for study. Because the findings of Endriss (1902), Solms-Laubach (1898), and Ernst and Schmid (1913) agree closely with one another, and with my work to this stage, the stages lacking in my material are probably quite similar to those in the material studied by these authors.

After fertilization, the micropyle becomes occluded by small, rectangular cells which appear to be derived from the inner cell layer of the inner integument (Fig. 41, 46-47). Solms-Laubach (1898) observed their derivation from the inner layer of the inner integument in *Rafflesia rochussenii*, but Endriss (1902) reported that both nucellar and inner integument cells contributed to the occlusion. More careful study needs to be made of their origin in *Pilostyles thurberi* because in my material the nucellus and inner layer of the inner integument have already begun to collapse, so that it is difficult to determine from which tissues the occlusion cells arise. These cells remain in the micropyle into the mature seed.

Embryo development is like that described for *Pilostyles ingae* by Endriss (1902). The mature embryo normally is composed of four tiers of two cells each. The suspensor is composed of two cells (Fig. 48). The number of tiers of cells in the embryo is constant but the number of cells in the suspensor may vary from 1-3. In one nearly mature seed two complete embryos were found (Fig. 49).

The few-celled endosperm is of the nuclear type, with cell walls formed after a period of free nuclear division. Starch is not found in the endosperm, but oil drops are present. This condition also was observed by Endriss (1902) in *Pilostyles ingae*.

In agreement with the work of Endriss, at least one of the synergids persists until the embryo approaches maturity. At no time was more than one seen in a single seed. It has a thickened wall and stains very intensely (Fig. 50).

Seed coat formation in *Pilostyles thurberi* is similar to that in other members of the genus. In the outer cell layer of the inner integument there is a gradual increase in wall thickness, starting at the micropylar end and progressing toward the chalazal end of the seed, where it is interrupted by thin-walled cells derived from the chalaza (Fig. 47-48). Sclerification of the cell walls follows and advances toward the chalazal end. This results ultimately in a layer of brachysclereids with very small lumina. The inner layer of the inner integument and the nucellus degenerate during embryo development and are visible in the nearly mature seed only as much flattened cells.

Fig. 42-46.—Fig. 42. Transection of female flower with young ovules. Note four generalized placental regions. $\times 44$.—Fig. 43. Developing ovule with integument initials. $\times 510$.—Fig. 44. Longitudinal section through ovule just before megasporogenesis. Note much enlarged megaspore mother cell. $\times 595$.—Fig. 45. Longitudinal section through ovule with two-nucleate embryo sac. Note micropyle formed by inner integuments, tenuinucellate nucellus, and 'cap' of degenerate megaspores. $\times 540$.—Fig. 46. Young seed micropyle occluded by small rectangular cells. $\times 245$.

The single-layered outer integument remains quite prominent, and its cells become densely cytoplasmic in the nearly mature seed (Fig. 51). The cells are glandular in function and secrete a substance which makes the mature seed sticky to the touch. This is characteristic of the genus *Pilostyles*.

Kummerow (1962) reported a homogenous few-celled embryo for *Pilostyles berteri*; however, his illustration shows an oblique longitudinal section through the seed that apparently missed the embryo. The 'embryo' he has figured corresponds to the cellular endosperm of *P. thurberi* and *P. ingae*.

DISCUSSION

On the basis of this study and the earlier studies of Endriss (1902) and Kummerow (1962) one sees that certain structures have little anatomical variability at the specific level while others may vary greatly.

Enough work has been done on the anatomy of the endophytic system to show that there is more than one type present in the family and within the genus *Pilostyles* itself (Solms-Laubach, 1875; Endriss, 1902). According to Solms-Laubach, *Pilostyles blanchetii* and *P. caulotreti* have less massive endophytic systems than *P. aethiopica*, which in turn has a less massive system than *P. thurberi*. *Pilostyles haussknechtii* (Solms-Laubach, 1874) has the most reduced endophytic system known in the genus. Sinkers are more abundant in *Pilostyles blanchetii* than in *P. aethiopica* and are absent entirely in *P. ingae* (Endriss, 1902). Although the endophytic system is quite reduced in this family, we see from the above information that differences do exist in the degree of reduction. It is felt that these differences will be of considerable value in taxonomic decisions at the species level.

Most aspects of floral anatomy are in close agreement with those found by Endriss in *Pilostyles ingae*. Cuticular relief pattern and crystal complement are constant in *P. thurberi*, and variation in these patterns should be watched for in other species. Vascular patterns are quite variable in *P. thurberi* and probably will not be too useful in the comparison of closely related species. They may prove useful, however, in tracing the fusion of the stamens to the column in *P. holtzii* and *P. blanchetii*. The former species represents the primitive condition in which the stamens remain distinct from the column and possibly still contain a vascular trace. *Pilostyles blanchetii* represents the median condition and possibly contains vestiges of the original vascular traces.

Microsporogenesis, megasporogenesis, and embryology are very similar

Fig. 47–51. Embryology and seed structure.—Fig. 47. Median longitudinal section showing occlusions in micropyle and at chalazal pole of seed. $\times 265$.—Fig. 48. Nearly mature seed with mature embryo and cellular endosperm. Note sclereid layer of seed coat derived from outer layer of inner integument. $\times 230$.—Fig. 49. Longitudinal section through seed with two embryos. $\times 200$.—Fig. 50. Synergid (small, dark cell) persistent at base of developing embryo. $\times 285$.—Fig. 51. Transection through seed at level of upper tier of embryo cells. $\times 205$.

in *Pilostyles thurberi* and *P. ingae*. Thus far not enough variation in these stages has been noted to be of taxonomic value within the genus.

CYTOLOGY

OBSERVATIONS

Few cytological studies have been made in the Rafflesiaceae. Table 2 summarizes chromosome counts for the family.

TABLE 2. *Chromosome numbers in the Rafflesiaceae*

SPECIES	n	2n	AUTHOR
1. <i>Rafflesia arnoldi</i> R. Br.	12		Olah, 1960
2. <i>Rafflesia patma</i> Bl.	12		Ernst & Schmid, 1913
3. <i>Mitrastemon kawa-sasakii</i> Hayata	20		Watanabe, 1935
4. <i>Mitrastemon yamamotoi</i> Mak.	20	40	Watanabe, 1934
5. <i>Pilostyles berterii</i> Guill.		±12	Kummerow, 1962
6. <i>Pilostyles thurberi</i> Gray	30+0-2B	60+0-1B	

Meiosis takes place in the pollen mother cells after the male flower buds have broken through the host cortical tissues and have attained a diameter of approximately 1.5–1.75mm. Due to the large number of very small chromosomes, it is difficult to find cells with countable chromosomes. Somatic counts were usually made at very late prophase (Fig. 33). Somatic chromosomes, measured at metaphase average 1.4 μ in length. There is a marked tendency for the chromosomes to exhibit stickiness (Fig. 53, 55–56), which makes it particularly troublesome to obtain counts at meiotic metaphase I. Pairing is normal at metaphase I and no chromosomal aberrations were found at anaphase I in any of the material observed.

B-chromosomes, not previously reported in this family, are present in *Pilostyles thurberi*. While essentially of the same size and morphology as chromosomes of the normal complement, they may be indentified by their variability in number and separation from the normal complement at metaphase (Fig. 52, 54–55, 57). Variation in the number of B-chromosomes occurs from cell to cell and population to population. Usually only one B-chromosome is present in a pollen mother cell although occasionally there are two (Fig. 54). They seem to occur with greatest frequencies in the population near Yuma, Arizona, with somewhat lower frequencies in the Pinto Wash and Ocotillo populations in Imperial County, California. Because of the difficulty of obtaining good chromosome spreads, no attempt was made to obtain frequency data for the occurrence of B-chromosomes in the various populations.

Normally the B-chromosomes divide at anaphase II. As a result, some of the microspores have $n=30+1B$ chromosomes (Fig. 56, 58) while others have $n=30$ chromosomes (Fig. 32). Occasionally a microspore nucleus will have $n=30+2B$ chromosomes.

DISCUSSION

Cytological work with members of this family has been insufficient for taxonomic use. On the basis of the data in Table 2 the few numbers reported for *Rafflesia* and *Mitrastemon* species are consistent within each

genus. This is not the case in *Pilostyles*. Kummerow (1962) indicates that he had difficulty in obtaining a definite count, and his photographic illustration does not verify his count of $2n=\pm 12$. Further attempts should be made to establish an accurate count for *Pilostyles berterii* Guill.

The two counts reported for *Pilostyles* are problematic with regards to taxonomy. A high number such as $2n=60$ for *Pilostyles thurberi* certainly

Fig. 52-58. Pollen mother cells of *Pilostyles thurberi*.—Fig. 52. Several PMC's, each with a B-chromosome. $\times 770$.—Fig. 53. Polar view of Metaphase I plate. $n = 30 \text{ II} + 1 \text{ B}$. Note stickiness. $\times 1435$.—Fig. 54. Equatorial view of Metaphase I plate. Two B-chromosomes shown. $\times 1460$.—Fig. 55. Polar view of Metaphase I plate. $n = 31 \text{ II} + 1 \text{ B}$. Note stickiness. $\times 1425$.—Fig. 56. Metaphase II, polar view, $n = 30 + 1 \text{ B}$ chromosomes. $\times 1450$.—Fig. 57. Metaphase II, showing single B-chromosome at one pole. $\times 1460$.—Fig. 58. Metaphase II, polar view. $n = 30 + 1 \text{ B}$ chromosomes. $\times 1465$.

indicates that it is of polyploid origin. Whether the base number for the genus is $x=6$ or $x=12$ cannot be determined until further counts are made within the genus. If Kummerow's count is substantiated, it is apparent that chromosome number will prove to be a significant character. On occasion, the morphological features of some chromosomes may be observed at metaphase II. As seen in figure 33, a few of the chromosomes have median or submedian centromeres. Because of the small chromosome size it is doubtful that complete karyotypes will be worked out; however, special marker chromosomes may be discovered.

LITERATURE CITED

- Beeks, R. M. 1955. Improvements in the squash technique for plant chromosomes. *Aliso* 3: 131-134.
- Brown, W. H. 1912. The relation of *Rafflesia manillana* to its host. *Philipp. J. Sci.* 7: 209-226.
- Cammerloher, H. 1920. Der Spaltöffnungsapparat von *Brugmansia Rafflesia*. *Oesterr. Bot. Z.* 7: 153-164.
- Endriss, W. 1902. Monographie von *Pilostyles Ingae* Karst. (*Pilostyles Ulei* Solms-Laub.) *Flora* 91: 209-236.
- Ernst, A., and E. Schmid. 1913. Ueber Blute and Frucht von *Rafflesia*. *Ann. Jard. Bot. Buitenzorg*, 2nd Series. 12: 1-58.
- Good, R. 1964. The Geography of the Flowering Plants. 3rd edition. John Wiley and Sons, Inc., New York.
- Gray, A. 1854. *Plantae Novae Thurberianae*. *Mem. Amer. Acad. Arts* 5: 297-328.
- Guillemin, M. 1834. Memoire sur le *Pilostyles*, nouveau genre de la famille des Rafflesiacees. *Ann. Sci. Nat. (Paris) Sér. 2.* 2: 19-25.
- Harms, H. 1935. Rafflesiaceae. In: A. Engler & K. Prantl, *Die natürlichen Pflanzenfamilien*. 2nd Ed. Band 16b: 243-281.
- Heinricher, E. 1917. Die erste Aufzucht einer Rafflesiaceae, *Cytinus hypocystis* L., aus Samen. *Ber. Deutsch. Bot. Ges.* 35: 505-512.
- Johansen, D. 1940. *Plant Microtechnique*. McGraw-Hill Book Co., Inc., New York.
- Kuijt, J. 1960. Morphological aspects of parasitism in the dwarf mistletoes (*Arceuthobium*). *Univ. Calif. Publ. Bot.* 30: 337-436.
- Kummerow, J. 1962. *Pilostyles berterii* Guill., eine wenig bekannte Rafflesiacee in Mittelchile. *Z. Bot.* 50(4): 321-337.
- Metcalfe, C. R., and L. Chalk. 1950. *Anatomy of the Dicotyledons*. Clarendon Press, Oxford.
- Olah, L. 1960. Cytological and morphological investigations in *Rafflesia arnoldii* R. Br. *Bull. Torrey Bot. Club* 87: 406-416.
- Pierce, G. J. 1893. On the structure of the haustoria of some phanerogamic parasites. *Ann. Bot. (London)* 7: 221-327.
- Shreve, F. 1951. *Vegetation of the Sonoran Desert*. Vol. I. Carnegie Institution of Washington Publ. 59.
- Solms-Laubach, H. 1874. Ueber den Thallus von *Pilostyles Haussknechtii*. *Bot. Zeitung (Berlin)* 32: 49-59, 65-74.
- , 1875. Das Haustorium der Lorantheen und der Thallus der Rafflesiaceen und Balanophoraceen. *Abh. Naturf. Ges. Halle.* 13: 239-276.
- , 1889. Rafflesiaceae. In: A. Engler & K. Prantl, *Die natürlichen Pflanzenfamilien*. III Teil. 1.
- , 1898. Die Entwicklung des Ovulums und des Samens bei *Rafflesia* und *Brugmansia*. *Ann. Jard. Bot. Buitenzorg Suppl.* 2: 11-22.
- Thoday, D. and E. T. Johnson. 1930. On *Arceuthobium pusillum*, Peck. I. The endophytic system. *Ann. Bot. (London)* 44: 393-414.
- Watanabe, K. 1934. Geschlechtliche Fortpflanzung von *Mitrastemon Yamamotoi*. *Proc. Imp. Acad. Japan.* 10: 421-423.
- , 1935. *Mitrastemon Kawa - Sasaki* aus Formosa. *Proc. Imp. Acad. Japan.* 11: 283-285.