REVERSAL IN OVARY POSITION FROM INFERIOR TO SUPERIOR IN THE HAEMODORACEAE: EVIDENCE FROM FLORAL ONTOGENY

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Floral anatomy and development were studied in *Wachendorfia thyrsiflora* and *Lachnanthes caroliniana* of the monocot family Haemodoraceae in order to evaluate the hypothesis that a superior-ovary position arose from an ancestral inferior-ovaried condition. In *Wachendorfia* three conduplicate carpel primordia develop at the floral apex, followed by postgenital fusion forming a superior ovary. The lower receptacular tissue at the central floral axis elongates at about the same extent as the peripheral receptacular tissue. In the extended receptacular region, two lateral commissures develop by differential tissue growth, followed by differentiation of inner epithelial cells to form septal nectaries. The receptacular commissures and nectaries are continuous with carpellary commissures of the superior ovary. Junction of the median outer tepal vein and dorsal carpellary vein occurs well below the septal nectaries at the base of the receptacle. In *Lachnanthes* three small, conduplicate carpel primordia develop at the floral apex. Growth of the lower receptacular tissue at the central floral axis is relatively retarded, such that the peripheral tissues elongate differentially, surrounding the now laterally positioned carpels. Subsequent postgenital fusion results in an inferior-ovary position, relative to perianth insertion. Incomplete postgenital fusion of intercarpellary spaces results in the formation of septal commissures. These commissures later mature into septal nectaries, which extend to the apex of the inferior ovary. Junction of the median outer tepal vein and dorsal carpellary vein occurs just beneath the ovary locules. It is proposed that the floral receptacular region of *Wachendorfia* and of other superior-ovaried Haemodoraceae is homologous with an ancestral inferior ovary with regard to position, development, and vasculature. A superior ovary in the Haemodoraceae arose by the co-option of an ancestral inferior ovary into an elongated receptacle, along with extension of the intercarpellary commissures to the periphery of the receptacular tissue, and modification of those peripheral commissures to nectaries. The adaptive significance of this evolutionary event is unclear but apparently related to a shift in pollination mechanism.

Introduction

The position of the ovary—whether superior, inferior, or intermediate—has long been considered an important, phylogenetically informative character in systematic studies. Within the angiosperms as a whole, an inferior ovary has been assumed by many botanists to be a derived and irreversible feature (Bessey 1915; Sporne 1975; Cronquist 1981). Although an inferior-ovary position has evolved numerous times in various taxonomic groups, its adaptive significance is not certain. It may have arisen in some groups because of selective pressure by floral herbivores or pollinators or in response to an adaptive advantage caused by greater protection of seeds or increased energy allocation to developing ovules (Grant 1950; Stebbins 1974).

Ovary position is uniform within virtually all angiosperm plant families. One exception is the monocotyledon family Haemodoraceae. The Haemodoraceae are a monophyletic group consisting of 13 currently recognized genera and ca. 100 species (Simpson 1990; Anderberg and Eldenäs 1991; see also MacFarlane et al. 1987), with distributions spanning Australia, South Africa, South and Central America, and eastern North America. Of the 13 genera in the family, five (*Barberetta, Schiekia, Pyrrorhiza, Wachendorfia,* and *Xiphidium*) have a superior ovary; the other eight genera have an inferior to half-inferior ovary. Based on a cladistic analysis using data from mor-

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phology, anatomy, palynology, and karyology, Simpson (1990) proposed that the common ancestor of extant Haemodoraceae possessed an *inferior* ovary and that a change in ovary position, from superior to inferior, was a unique evolutionary event shared by all family members. However, this study also concluded that a second change in ovary position, from inferior to superior, occurred for a single clade of the Haemodoraceae, constituting an apomorphy (portrayed in the analysis as an evolutionary reversal) for all five superior-ovaried genera of the family (Simpson 1990). The latter finding was problematic in that, as mentioned above, a reversal in ovary position, from inferior back to superior, generally has been viewed as very unlikely; in addition, no obvious adaptive significance was recognized for such a reversal in the Haemodoraceae (Simpson 1990).

A more recent study of floral anatomy in the Haemodoraceae (Simpson 1993) demonstrated the occurrence of three major types of septal nectaries, based on their number per flower and their position relative to other floral parts. One of these septal nectary types, termed "infralocular," is characterized by having two septal nectaries located almost entirely within the receptacular tissue, well beneath the locules of the ovary but within the vertical plane of the ovary septa (Simpson 1993). Interestingly, the infralocular septal nectary type is found exclusively in four of the five genera with a superior ovary: *Barberetta, Schiekia, Pyrrorhiza,* and *Wachendorfia.* The other superior-ovaried genus, *Xiphidium,* lacks any trace of nectaries in both of its species. (As reviewed in Simpson [1993], Smets and Cresens [1988] proposed that the name "gyno-

pleural" nectary replace that of "septal" nectary in order to indicate possible homology between nectaries that occur on outer carpellary surfaces and those occurring internally. I chose to retain the term "septal" nectary in the 1993 and the present article because of its more widespread use in comparative literature. I recognize the fact that some "septal" nectaries in the Haemodoraceae do not, in fact, occupy the septal regions; however, these nectaries are always vertically aligned along the septal planes of the ovary and are hypothesized to be positionally homologous to septal nectaries that are actually positioned within ovary septa.)

This raises two important questions. First, what is the evolutionary significance of the derived septal nectary anatomy in the above four superior-ovaried genera? And, second, is the evolution of that derived anatomy correlated with the occurrence of a putatively derived superior-ovary position in these taxa? Two of the four genera with infralocular septal nectaries, *Wachendorfia* and *Schiekia*, are unique in having perianth "apertures" (Ornduff and Dulberger 1978; see Simpson 1990, 1993), which consist of two lateral, cup-shaped cavities at the receptacular region of the flower into which nectar is secreted, which is exposed to the outside (fig. 2). The perianth apertures are delimited by decurrent tepal margins that form surrounding flaps of tissue that appear as an opening, hence the term "aperture." A narrow channel leads distally from the apertures to an opening at the front of the flower, allowing nectar to be siphoned by an insect (fig. 1). The two septal nectaries run adjacent to the perianth apertures and extend distally only to the base of the ovary locules, at the level of perianth detachment (see Simpson 1993). From a previous study (Simpson 1993) I concluded that (1) two infralocular nectaries evolved in the common ancestor of all five of the superior-ovaried genera, concomitantly with the evolution of perianth apertures and arising via the evolution of zygomorphy and basal displacement of nectaries into the receptacular tissue; (2) perianth apertures were lost in the genera *Pyrrorhiza* and *Barberetta;* and (3) septal nectaries were probably independently lost in the genus *Xiphidium*, perhaps in response to a shift in pollination mechanism.

The purpose of this article is to report anatomical and developmental findings that help support the hypothesis that ovary position in the Haemodoraceae underwent an evolutionary reversal, from inferior to superior, and to review the possible adaptive significance of that transformation.

Material and Methods

Mature buds or recently opened flowers were preserved in the field in FAA (formalin–acetic acid–alcohol), embedded in Paraplast, and cross-sectioned serially at thicknesses ranging from 8 to 15 μm depending on the developmental stage. Sections were stained with safranin, fast-green, and hematoxylin according to standard procedures (Johansen 1940; Sass 1958; Berlyn and Miksche 1976). Light micro-

scope images were photographed and subsequently scanned. Voucher information is as follows: *Wachendorfia thyrsiflora* L.—R. Ornduff 7691 (UC); *Lachnanthes caroliniana* (Lam.) Dandy [*L. caroliana* (Lam.) Dandy]—M. G. Simpson 14VI80A (DUKE); *Dilatris pilansii* Barker—P. V. D. Meriwe 30X81-2 (STEU); *Haemodorum spicatum* R. Br.—M. G. Simpson 16IX81C (DUKE); *Philydrum lanuginosum* Gaertner—E. F. Constable; U. Hamann 959 (NSW); *Pontederia cordata* L.—M. G. Simpson 28IX81A (DUKE).

In order to better visualize the spatial distribution of major floral components, sequential cross-sections of flowers or flower buds were examined along two radii: one along a midlocular region and the other along an adjacent (in *Wachendorfia*) or opposite (in *Lachnanthes*) midseptal region. At intervals of 1–10 sections, measurements were recorded on a data sheet from the center of the flower to various floral components, including veins and the boundaries of the locule, septal nectary, ovary surface, stamens, and tepals. These were graphically imaged using the computer software Freehand by Macromedia (Macintosh version 5.5). Using this software, rulers were set up on X and Y axes corresponding to micrometer measurements. For each floral component (e.g., dorsal vein) interconnecting points were placed using the curved line function, each point corresponding to the vertical distance from an arbitrary pedicel base and to the horizontal distance from the flower center. Some lines were closed and the areas shaded to represent various floral components. The final graphics represents what is, in effect, a longitudinal section of the flower.

For scanning electron microscopy (SEM) observations, buds were dissected to reveal critical organs, dehydrated to 100% ethanol, and then gradually infiltrated to 100% Freon 113 or methylal, (dimethoxymethane). The material was placed in a metal capsule and critical-point dried with a Tousimis critical-point dryer using pressurized carbon dioxide as the transition fluid. The material was transferred onto a stub covered with double-stick tape, sputter-coated with gold/palladium in a Hummer-4 sputtering apparatus, and photographed on a Hitachi S500 scanning electron microscope (20 kV). Images were captured digitally. All photographic figures were prepared using the Freehand software.

Results

Wachendorfia thyrsiflora Carpel and Septal Nectary Development

The flowers of *Wachendorfia thyrsiflora* are zygomorphic, with three outer and three inner imbricate, glabrous to abaxially hispidulous tepals (fig. 1). The flowers have an extended "receptacular region," defined as the tissue between the ovary base and the short, stalklike pedicel. The outer posterior tepal is fused basally both to the inner posterior tepals and to the upper margins of the outer anterior tepals (fig. 3), forming lateral pouchlike perianth "apertures" (after Ornduff and Dulberger 1978) at their junction in the receptacular region (fig. 2). The perianth apertures function as repositories for secreted nectar. From the front face of the flower, two openings leading to the perianth apertures occur (fig. 1); these apparently serve as conduits for the proboscis of an insect during pollination (Ornduff and Dulberger 1978; Helme and Linder 1992). There are three, free and distinct, incurved stamens positioned opposite the inner tepals (figs. 1,
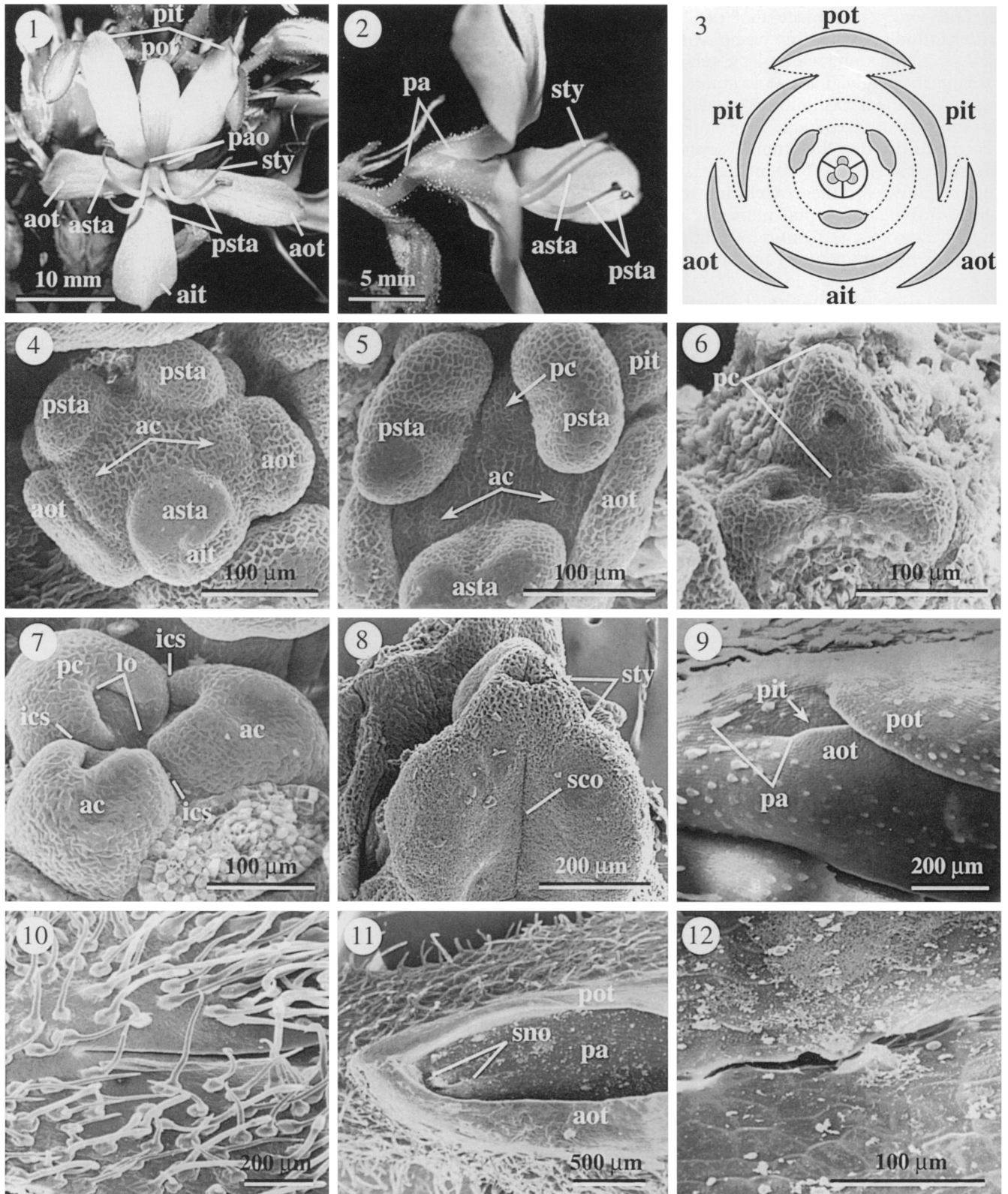
Figs. 1–12 *Wachendorfia thyrsiflora* floral development. Figs. 1–2, Light micrographs of mature flower. Fig. 1, Front view, showing posterior outer tepal (*pot*), two posterior inner tepals (*pit*), anterior outer tepals (*aot*), anterior inner tepal (*ait*), two posterior stamens (*psta*), anterior stamen (*asta*), and oppositely curved style (*sty*). Also note two perianth aperture openings (*pao*). Fig. 2, Side view, showing perianth aperture (*pa*) of receptacular region, style (*sty*), anterior stamen (*asta*), and two posterior stamens (*psta*). Fig. 3, Floral diagram, oriented relative to horizontal flower, posterior above. Abbreviations as above. Figs. 4–12, Scanning electron micrographs of various developmental stages. Fig. 4, Early stage, showing primordia of anterior outer tepals (*aot*), anterior inner tepal (*ait*), anterior stamen (*asta*), posterior

3). The two posterior stamens exhibit little lateral curvature; however, the anterior stamen is strongly bent to one side (fig. 1), either to the left or right, depending on the individual plant. The ovary is superior, ovoid, three-lobed, densely pubescent, with three locules and carpels. There is one ovule per locule, arising from turgid, longitudinally oriented placentae. The style is incurved and strongly bent to the side opposite the anterior stamen, i.e., exhibiting strong enantiostyly (figs. 1, 2).

At an early developmental stage, the four series of floral primordia initiate: outer tepals, inner tepals, stamens, and two anterior carpels (fig. 4). The two anterior carpel primordia appear to develop prior to the posterior carpel primordium (fig. 4). At a later stage, the posterior carpel forms (fig. 5) and becomes comparable in size to the anterior carpels (fig. 6), but differential development of anterior and posterior carpels results in a slight bilateral symmetry of the gynoecium (fig. 7); this bilateral symmetry is evident in that the locule of the posterior carpel is larger and is medially aligned with the center point of the three carpels, whereas the locules of the anterior carpels are smaller and oriented slightly askew to the center point (fig. 7). Carpel primordia differentiate into a typical folded, conduplicate form (figs. 6, 7) with subsequent apparently postgenital fusion of adjacent carpels to form a three-lobed ovary, style, and stigma (fig. 8). This postgenital fusion transforms the intercarpellary spaces (fig. 7) into ovary septal commissures (fig. 8).

Prior to carpellary fusion, accelerated cell divisions occur in the lower, receptacular region of the flower, resulting in vertical ridges or flanks of tissue that are continuous with the margins of the distal, free tepals; these ridges thus appear as "decurrent" extensions of the tepal margins. This accelerated growth also results in an internal cleft of the receptacular tissue (arrow at fig. 13) between each decurrent ridge of the posterior inner tepal and the adjacent anterior outer tepal. With continued differential growth, the upper margin of each decurrent anterior outer tepal overlaps the lower margin of the adjacent decurrent posterior inner tepal at the base of the receptacle (figs. 9, 14); the receptacular cleft between them differentiates into a peripheral septal commissure and an inner "septal" nectary that is vertically aligned with the two anterior septa of the ovary (figs. 14, 15). The immature septal nectary consists of a single layer of densely staining, cuboidal epithelial cells that line the cleft (fig. 15). The septal nectary is contiguous with the septal nectary commissure, which extends to the exterior periphery (fig. 15). The septal nectaries and adjacent commissures in the receptacular region (fig. 14) extend without interruption to the base of the superior portion of the ovary and are confluent with septal commissures of the ovary that developed between the posterior carpel and the two, adjacent anterior carpels (figs. 16; 39B, C). Similarly, protruding flanges of tissue that border the septal commissure in the receptacular region (arrows at fig. 15) are contiguous with the flanges of the two adjacent carpels that flank each commissure and nectary there (arrows at fig. 16).
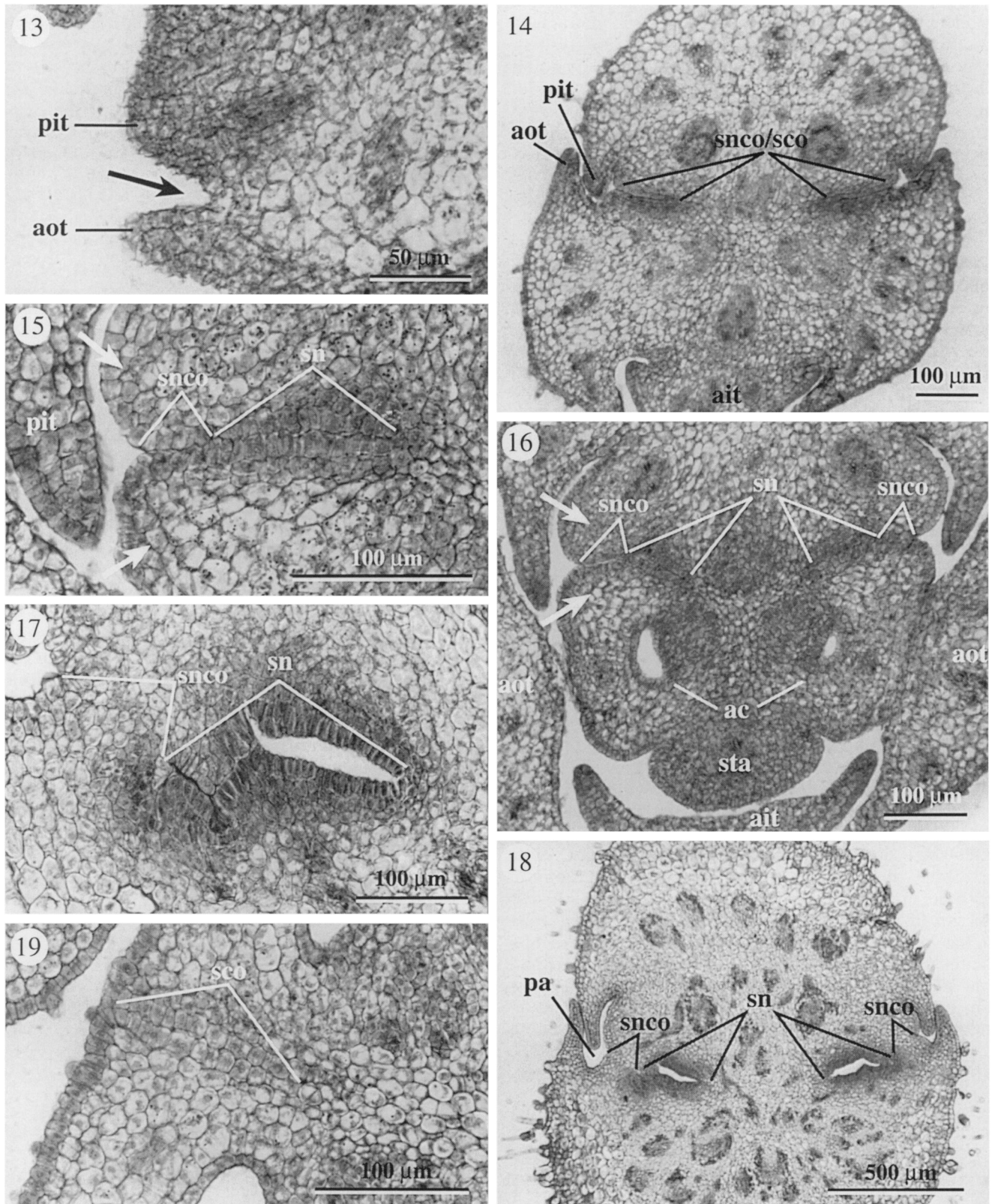
As the flower matures, the margins of the single outer posterior tepal overlap distally those of the adjacent outer anterior tepals (fig. 9). In the receptacular region, these overlapping tepals differentiate to form the "perianth aperture," which becomes bordered by the decurrent outgrowth of outer posterior and outer anterior tepals (figs. 10, 11, 18). The receptacular region undergoes considerable axial growth during development, resulting in significant extension of the septal nectaries (fig. 39A–C). At maturity the septal nectaries become convoluted in outline with an evident gap between the epithelial layers (figs. 17, 18). At this stage, the densely stained epithelial cells are palisade-shaped and are surrounded by two to three layers of slightly less densely stained cells (fig. 17). In the receptacular region, the slitlike commissures extend from the two septal nectary cavities to the exterior (fig. 17). These two septal nectary commissures open fully from the septal nectary to the outside only at the extreme base of the perianth aperture (figs. 11, 12). The septal nectary commissures are confluent with the septal commissures that traverse the entire length of the superior ovary and style (fig. 19). The locules of the ovary remain confluent with a canal that extends the length of the style (fig. 39C).

Wachendorfia thyrsiflora Floral Venation

Early in development, each median (posterior) outer tepal vein and the dorsal carpellary vein are joined together in the basal region of the flower (fig. 39A). At a later stage, the junction between median outer tepal and dorsal carpellary veins becomes positioned at the very base of the receptacle, below the septal nectaries, as the result of intercalary (zonal) expansion of the receptacular axis (fig. 39B, C). Column veins, which extend to the placentae and ovules, develop at

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Figs. 13-19 *Wachendorfia thyrsiflora* floral development, light micrographs of flower cross-sections. Posterior (upper) side of flower at top in all figures. Fig. 13, Very early stage, receptacular region, showing initiation of posterior inner tepal (*pit*) and anterior outer tepal (*aot*). Note cleft between them (arrow), which becomes future septal commissure and nectary. Fig. 14, Intermediate stage, receptacular region, showing overlap of lower margin of posterior inner tepal (*pit*) by the upper margin of anterior outer tepal (*aot*) and development of immature septal nectary commissure/septal commissure (*snco/sco*) between them. Fig. 15, Close-up, showing margin of posterior inner tepal (*pit*), septal nectary commissure (*snco*), and immature septal nectary (*sn*). Note flanges of tissue (arrows) adjacent to septal commissure. Fig. 16,

a later stage and are joined to the dorsal carpellary vein above the outer tepal–dorsal vein junction but still below the septal nectaries (fig. 39B, C). Veins of the inner tepals and stamens are joined below the septal nectaries (fig. 39B, C).

Lachnanthes caroliniana Carpel and Septal Nectary Development

The flowers of Lachnanthes caroliniana are actinomorphic with a biseriate, imbricate, abaxially pubescent perianth consisting of three small, narrow, outer tepals, and three large, relatively wide, inner tepals (figs. 20–22). There are three distinct and essentially free stamens, one opposite each of the inner tepals (figs. 21, 22). The ovary is inferior, ovoid to globose, and externally pubescent to tomentose, with three locules and carpels. There are five to seven ovules per locule, arising from a turgid, peltate placenta. The style is strongly bent to one side opposite one of the outer tepals, but there is no corresponding asymmetric curvature of stamens (fig. 21).

Outer and inner tepal primordia initiate at an early developmental stage (fig. 23). Slightly later in development, stamen primordia develop opposite the inner tepals, with no initial evidence of carpel formation (fig. 24). Earliest carpel development is evident with the subsequent formation of pouch-shaped primordia at the flower apex (fig. 25). Carpel primordia develop by the accelerated growth of cells along three elliptic-shaped rings, which forms the carpel walls, and no detectable growth in the center of each of the three rings, which forms the cavities of the locules. Growth is also minimal between adjacent carpels as well as at the central junction of the three carpels (arrow at figs. 25–27). The region between the walls of adjacent carpels, the intercarpellary space, later becomes the septal region (figs. 26, 32).

At a later stage, accelerated growth at the peripheral region of each of the three elliptical carpel walls occurs, causing the carpels to arch inward (fig. 27) and ultimately come into close contact (fig. 28), assuming a typical conduplicate form. Where the two margins of individual carpels come into close contact, a carpel commissure is evident (figs. 32, 33).

The ovary becomes inferior in position by a combination of differential growth and congenital fusion. Growth is retarded at three general locations: (1) at the central junction of the three carpel primordia (arrows at figs. 25, 26, 32); (2) in the center of each of the three carpel rings, the locular cavities (figs. 25, 26); and (3) between the walls of adjacent carpel walls, the intercarpellary spaces (figs. 26, 32). Growth is accel-

erated in the elliptic-shaped walls of the carpel primordia themselves as well as in the surrounding peripheral tissues of the receptacular region. The surrounding peripheral receptacular tissue grows upward and around the locules and intercarpellary spaces. This, combined with postgenital fusion of the carpel walls at the floral center (see below), results in the ovary being embedded within and surrounded by receptacular tissue (fig. 40A–D).

The locules initially develop as constricted, tangentially oriented slits, associated with the growth of intruded, densely staining placental primordia (fig. 34). In the center of the ovary, the intercarpellary space develops as three, confluent, radiating slits (figs. 34; 40A) that extend distally as septal commissures. The region of this intercarpellary space at the center of the flower becomes filled with tissue by postgenital fusion of adjacent tissues. However, the outer, peripheral portion of the slits persists and differentiates into septal nectaries at about the same time as ovule formation (figs. 35; 40B). Each septal nectary initially consists of two, abutting uniseriate plates of epithelial cells (fig. 36). With further development, the epithelial cells expand and become cytoplasmically dense, and the septal nectary cavity enlarges considerably (figs. 37, 38).
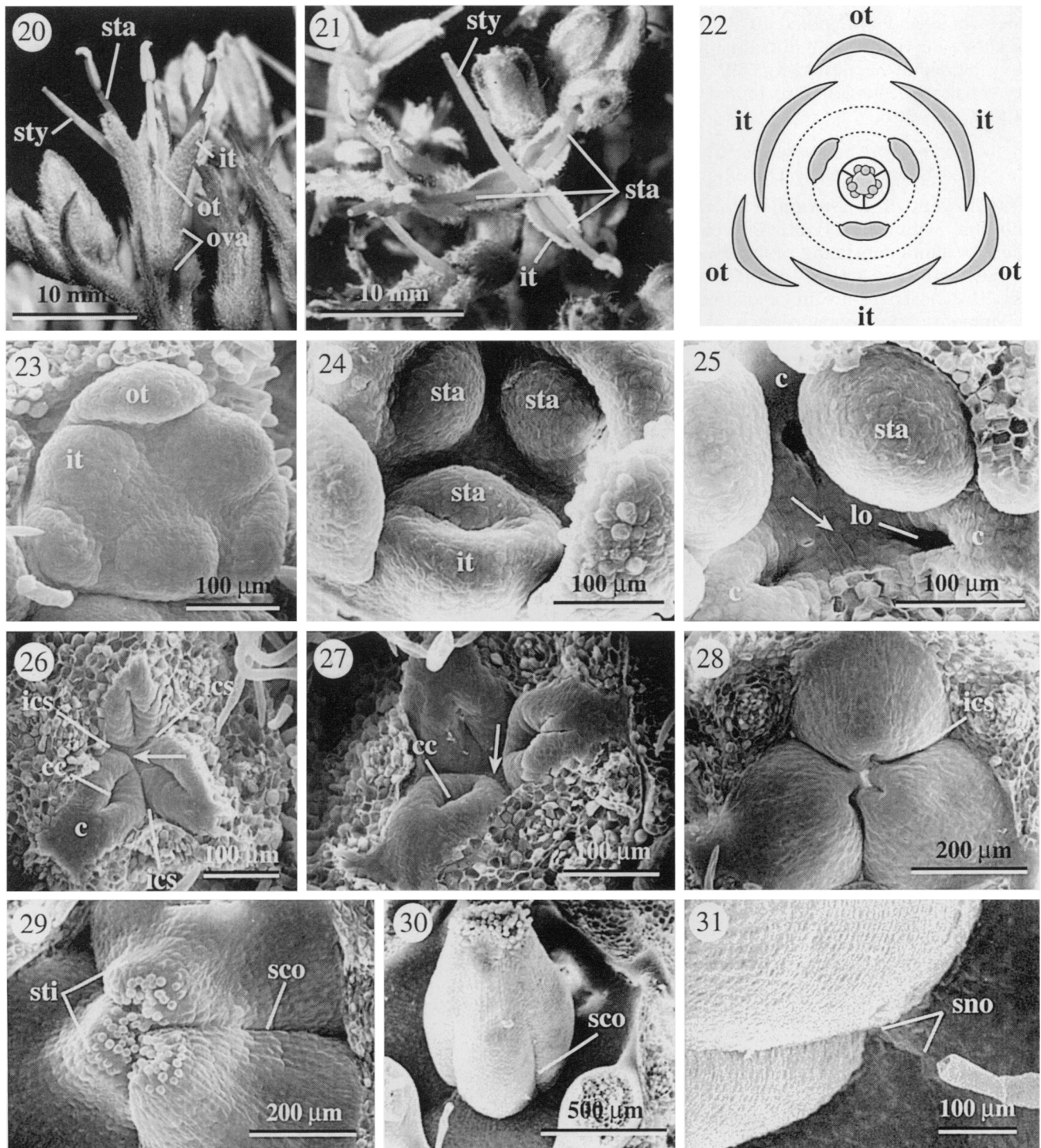
Further accelerated upward growth of the apical region of the three postgenitally fused carpels results in the formation of the style and stigma (figs. 28, 29). As the style and stigma grow (fig. 30), the septal nectaries remain continuous with septal nectary openings at the top of the ovary (fig. 31), the openings being remnants of the original intercarpellary space. These porelike openings function as the site of nectar secretion. The septal nectary cavities are continuous with the original intercarpellary slits (figs. 27, 28) that occur up the length of the style as septal commissures (figs. 29, 30; 40C, D), similar to those seen in the ovary and style of Wachendorfia.

Epidermal cells of the stigmatic region form papillate outgrowths during development (figs. 29, 30). The locules of the inferior carpels remain confluent with a stylar canal that traverses the length of the style (figs. 37; 40C).

Lachnanthes caroliniana Floral Venation

During development, each median outer tepal vein and the dorsal carpellary vein are joined at the apex of the receptacular region, at or below the level of the extreme base of the locule (fig. 40B–D). Column veins, which extend to the placental regions, are joined to the vein that distally extends to the outer tepal and dorsal veins (fig. 40D). In the same region of the flower, a vein leading to the base of the septal nectary

Figs. 20–31 *Lachnanthes caroliniana* floral development. Figs. 20–21, Light micrographs of mature flower. Fig. 20, Side view, showing inferior ovary (*ova*), outer tepals (*ot*), inner tepals (*it*), stamens (*sta*), and curved style (*sty*). Fig. 21, Top view, showing curved style (*sty*) and three stamens (*sta*), the latter opposite the inner tepals (*it*). Fig. 22, Floral diagram. Abbreviations as above. Figs. 23–31, Scanning electron micrographs. Fig. 23, Early stage, showing primordia of outer tepals (*ot*) and inner tepals (*it*). Fig. 24, Slightly later stage, showing formation of stamens (*sta*) opposite inner tepals (*it*). Fig. 25, Later stage, showing more mature stamen (*sta*) and initiation of three, pouch–shaped carpel primordia (*c*) with locules (*lo*). Arrow indicates central junction point of carpels. Fig. 26, Later stage, showing three carpels (*c*), intercarpellary space (*ics*), and carpel commissures (*cc*). Arrow as above. Fig. 27, Later stage, with enlarged carpels, having carpel commissures (*cc*). Arrow as above. Fig. 28, Later stage, showing early, postgenital fusion of carpels and intercarpellary space (*ics*). Fig. 29, Early style development, showing septal commissures (*sco*) of style and papillate cells of incipient stigma (*sti*). Fig. 30, More mature style, showing region of septal commissure (*sco*). Fig. 31, Close-up at base of style, showing slitlike septal nectary opening (*sno*).
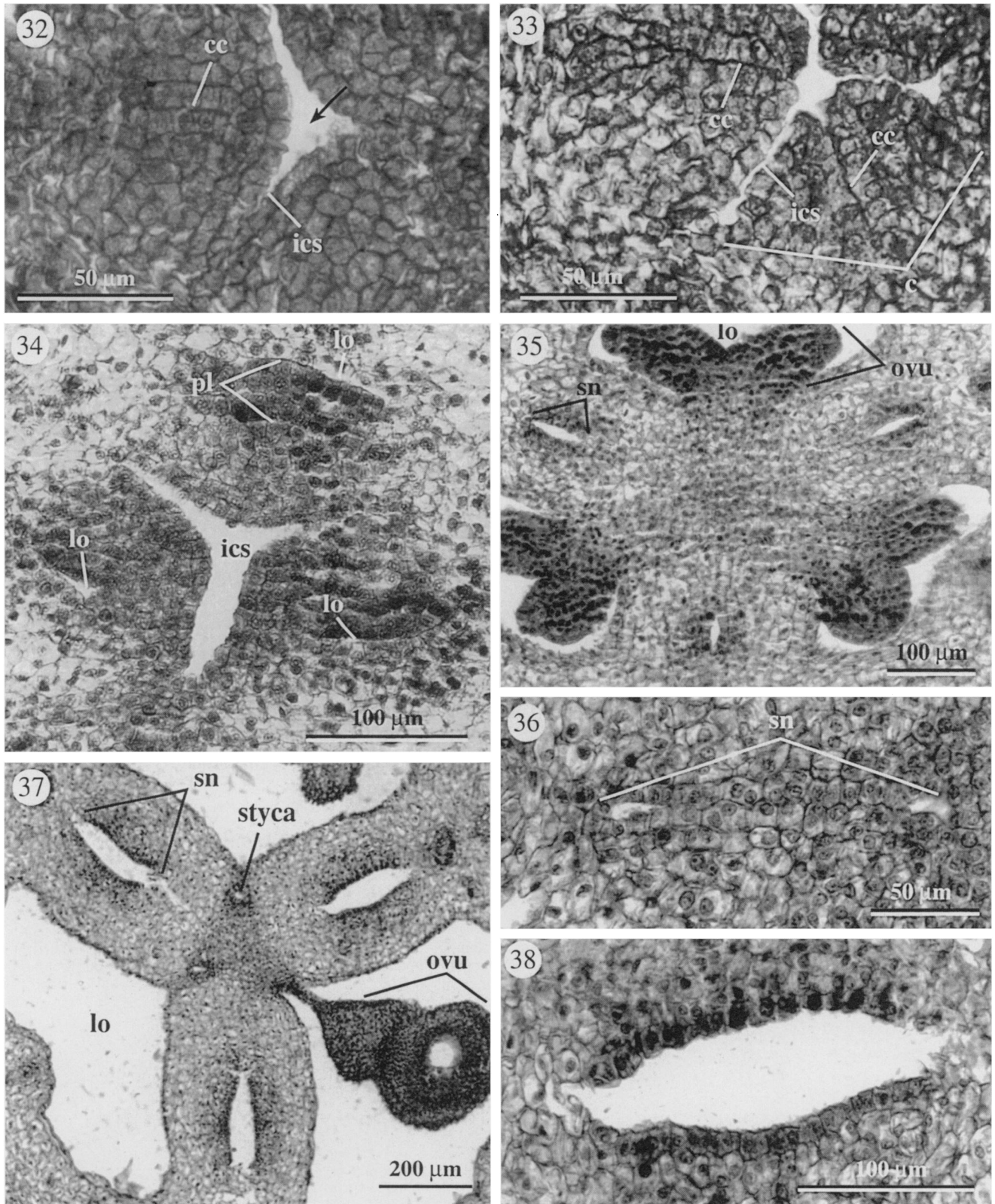
Figs. 32–38 *Lachnanthes caroliniana* floral development, light micrographs of flower cross-sections. Fig. 32, Very early stage, showing young carpels, with incipient intercarpellary spaces (*ics*) and carpel commissures (*cc*). Arrow indicates central junction of carpels. Fig. 33, Later stage, showing carpels (*c*), carpel commissures (*cc*), and intercarpellary spaces (*ics*). Fig. 34, Later stage, inferior ovary cross-section, showing three locules (*lo*), placentae (*pl*), and intercarpellary space (*ics*). Fig. 35, Later stage, inferior ovary cross-section, showing locules (*lo*), ovules (*ovu*), and immature septal nectaries (*sn*). Fig. 36, Close-up of immature septal nectary (*sn*). Fig. 37, Mature stage, inferior ovary cross-section, showing septal nectaries (*sn*), ovule (*ovu*), and stylar canal (*styca*) contiguous with locule (*lo*). Fig. 38, Close-up mature septal nectary.
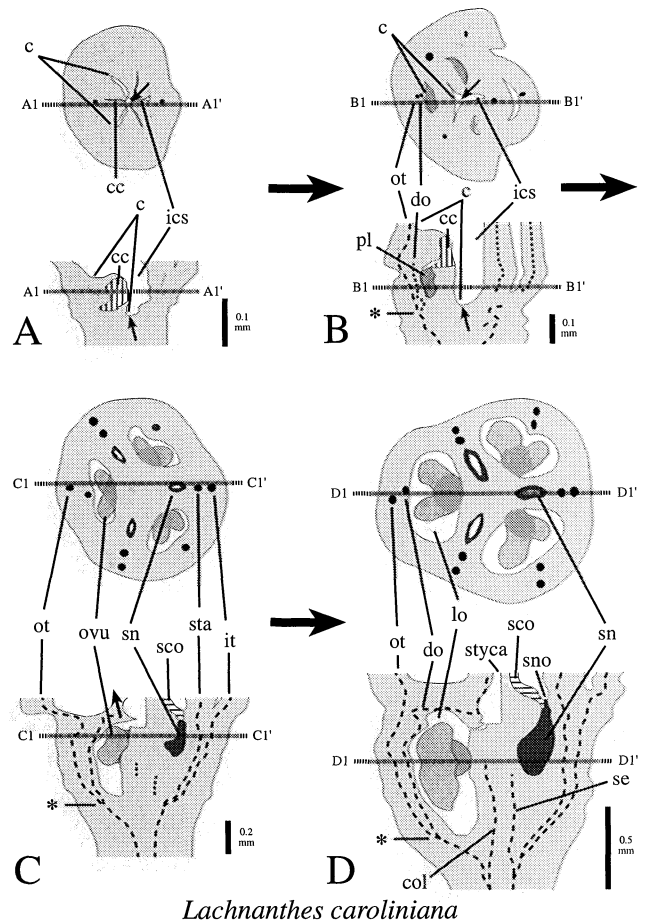
Wachendorfia thyrsiflora
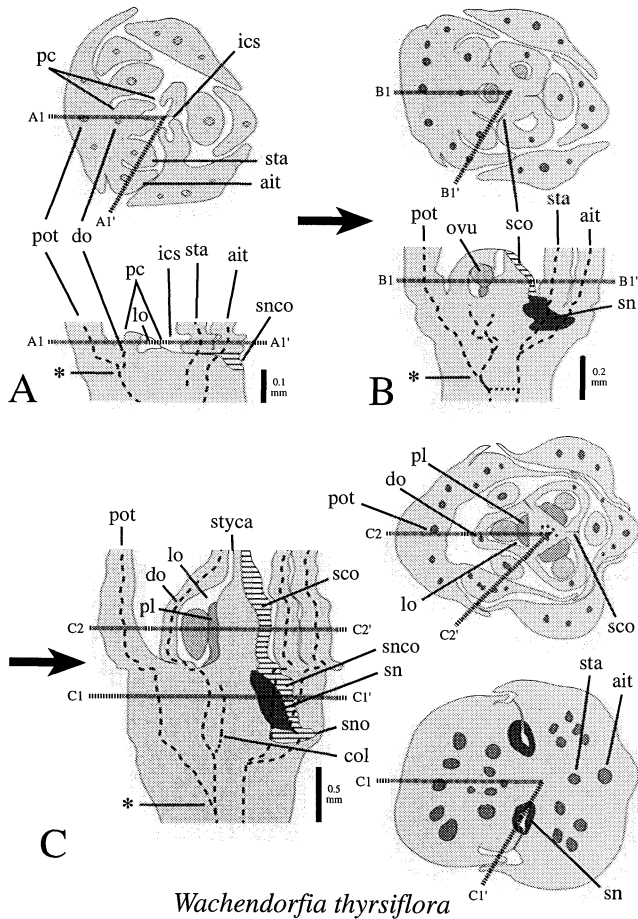
Lachnanthes caroliniana

Fig. 39 *Wachendorfia thyrsiflora.* Diagrams of flower longitudinal sections, below, with corresponding transverse sections, above or to right. In longitudinal views, section through carpel center is at left and through septum or septal region center is at right, the direction corresponding to labeled dashed line in transverse section. Transverse views are at a floral level corresponding to labeled dashed line in longitudinal sectional view. A, Young stage, illustrating unfused posterior carpel (*pc*) and adjacent intercarpellary space (*ics*). B, Middle stage, showing postgenital fusion of carpels. Note contiguous septal nectary (*sn*) and septal commissure (*sco*) of ovary. C, Mature flower, showing septal nectary opening (*sno*) within perianth aperture cavity. Note contiguous locule (*lo*) and stylar canal (*styca*). Also note junction (*) of posterior outer tepal (*pot*) vein and dorsal vein (*do*) at extreme base of receptacular region. Abbreviations and symbols: * = junction of central vein of posterior outer tepal and dorsal carpellary vein; *ait* = anterior inner tepal; *col* = column vein; *do* = dorsal vein; *ics* = intercarpellary space; *lo* = locule; *ovu* = ovule; *pc* = posterior carpel; *pl* = placenta; *pot* = posterior outer tepal; *sco* = septal commissure; *sn* = septal nectary; *snco* = septal nectary commissure; *sno* = septal nectary opening; *sta* = stamen; *styca* = stylar canal.

Fig. 40 *Lachnanthes caroliniana.* Diagrams of flower longitudinal sections. Longitudinal and cross-sectional views as in figure 39. A, Young stage, illustrating unfused carpel (*c*) with carpel commissure (*cc*) and adjacent intercarpellary space (*ics*). Arrow indicates central junction point of carpels. B, Slightly later stage, showing development of placenta (*pl*) and vasculature, including outer tepal (*ot*) and dorsal (*do*) veins. Arrow as above. C, Middle stage, showing inferior ovary position. Note contiguous septal nectary (*sn*) and septal commissure (*sco*) of ovary. D, Mature flower, showing septal nectary opening (*sno*) at top of inferior ovary and septal nectary at same approximate level to that of ovule and upper locule. Locule (*lo*) is contiguous with stylar canal (*styca*). Note junction (*) of outer tepal (*ot*) vein and dorsal vein (*do*) at base of ovary. Abbreviations and symbols: * = junction of central vein of posterior outer tepal and dorsal carpellary vein; *c* = carpel; *cc* = carpel commissure; *col* = column vein(s); *do* = dorsal vein; *ics* = intercarpellary space; *it* = inner tepal; *lo* = locule; *ot* = outer tepal; *ovu* = ovule; *pl* = placenta; *se* = septal veins; *sn* = septal nectary; *sco* = septal commissure; *sno* = septal nectary opening; *sta* = stamen; *styca* = stylar canal.

connects to the vein that leads to the inner tepal and stamen vein (fig. 40*D*). The septal nectaries are positioned at the level of ovule attachment (fig. 40*C, D*). Septal nectaries are continuous with septal nectary commissures that extend into the periphery of the styles (fig. 40*C, D*).

Dilatris pilansii Floral Venation

In mature flowers, each median outer tepal vein and dorsal carpellary vein are joined together slightly distal

to the central region of the locule (fig. 41*A*). Column veins are joined to this common outer tepal-dorsal vein at the base of the inferior ovary and extend to the single ovule and into the apical region of the column tissue (fig. 41*A*). Veins of the inner tepals and stamens are joined near the base of the ovary (fig. 41*A*). Septal veins arise from this common inner tepal-stamen vein slightly proximal to the junction of the latter and extend a short distance into the base of the ovary (fig. 41*A*). The three septal nectaries are vestigial, occurring
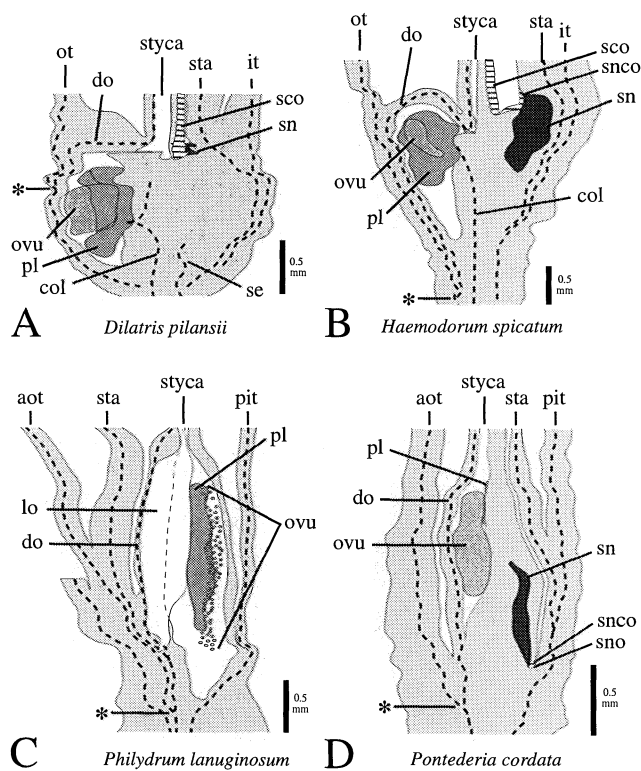
Fig. 41 Diagrams of flower longitudinal sections. Section through carpel center is at left and through septum or septal region center is at right. A, *Dilatris pilansii*. Note reduced septal nectary (*sn*) at apex of inferior ovary. Also note junction (*) of outer tepal (*ot*) vein and dorsal vein (*do*) at middle of ovary. B, *Haemodorum spicatum*. Note septal nectary (*sn*) level with locules of inferior ovary. Note junction (*) of outer tepal (*ot*) vein and dorsal vein (*do*) below base of ovary. C, *Philydrum lanuginosum*. Note absence of septal nectaries and junction (*) of fused anterior outer tepal (*aot*) with stamen (*sta*) veins and dorsal vein (*do*) below base of ovary. D, *Pontederia cordata*. Note septal nectaries at base of ovary. Note junction (*) of anterior outer tepal (*aot*) vein and dorsal vein (*do*) below base of ovary. Abbreviations as in figs. 39, 40.

only at the extreme ovary apex, near the style base (fig. 41*A*).

Haemodorum spicatum Floral Venation

In mature flowers, each median outer tepal vein and dorsal carpellary vein are joined together well below the locule in the apical region of the pedicel (fig. 41*B*). Column veins arise from a lower level (fig. 41*B*). Veins of the inner tepals and stamens are joined near the base of the inferior ovary (fig. 41*B*). No septal veins are evident. The three septal nectaries are large, occurring only in the apical region of the ovary; a commissure opens to allow nectar secretion at the base of the style (fig. 41*B*).

Philydrum lanuginosum (Philydraceae) Floral Venation

Flowers of this species are horizontal and zygomorphic, with a single anterior median stamen and the three posterior tepals fused into one structure. The median outer tepal is anterior in position, opposite that

found in all zygomorphic Haemodoraceae (fig. 41*C*). In mature flowers, the median anterior outer tepal vein and dorsal carpellary vein are joined together just below the locule of the superior ovary (fig. 41*C*). The stamen vein and dorsal carpellary vein are joined slightly above this junction (fig. 41*C*). No septal veins are evident. Septal nectaries are absent, as they are in all members of this family (fig. 41*C*).

Pontederia cordata (Pontederiaceae) Floral Venation

Flowers of this species are horizontal and zygomorphic, with six stamens. The median outer tepal is anterior in position, opposite that found in all zygomorphic Haemodoraceae and similar to that in the Philydraceae (fig. 41*D*). In mature flowers, the median anterior outer tepal vein and dorsal carpellary vein are joined together slightly below the locule of the superior ovary (fig. 41*D*). The median posterior stamen vein and median posterior inner tepal vein are joined slightly above the ovary base (fig. 41*D*). No septal veins are evident. Septal nectaries are present at the base of the locule; a septal nectary commissure extends only a short distance to the extreme ovary base, at the point of the septal nectary opening (fig. 41*D*).

Discussion

The major goal of this study was to evaluate the developmental and anatomical evidence regarding the hypothesis that ovary position in the Haemodoraceae has undergone a shift from inferior to superior in one clade of the family. Previous cladistic analyses of the Haemodoraceae (Simpson 1990, 1993) provide the evidence that a superior ovary, as found in *Wachendorfia* and four other genera, was derived from a preexisting, inferior-ovaried condition (see fig. 43). This hypothesis rests on the totality of morphological, anatomical, embryological, chemical, and karyological characters and the assumptions of character coding and polarity assignment in parsimony analyses. In the cited cladistic analyses, all outgroups (Pontederiaceae and Philydraceae; see Simpson 1990, 1993) have a superior ovary, and the common ancestor (ingroup node) of the Haemodoraceae has an inferior ovary, an apomorphy for the family as a whole. Molecular data (Duvall et al. 1993; Clark et al. 1994) provide evidence that yet another family, the Commelinaceae, is a close relative to the Haemodoraceae. All members of this family also have a superior ovary, supporting the notion that this feature is ancestral relative to the Haemodoraceae, i.e., present at the outgroup node.

The derivation of a superior ovary within the Haemodoraceae would technically be termed a "reversal." This is true only if (as would normally be done) the ovary position of the superior-ovaried taxa is originally coded as homologous to that of the outgroup taxa. However, it should be emphasized that the secondary evolution of a superior ovary in the Haemodoraceae, though termed a reversal, is a unique evolutionary event in itself. This is only the second corroborated example of a reversal in ovary position, the first being
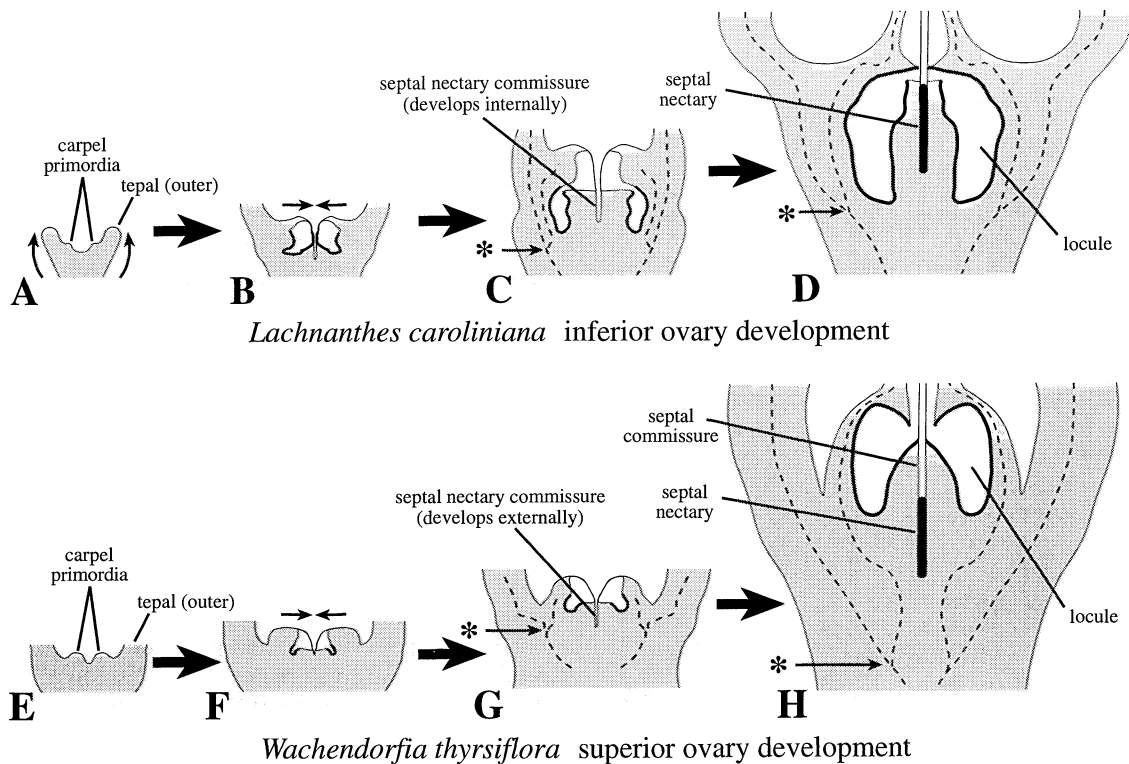
Lachnanthes caroliniana inferior ovary development

Wachendorfia thyrsiflora superior ovary development

Fig. 42 Summary diagram of ovary development, illustrating two adjacent carpels and intervening septal region. A–D, *Lachnanthes caroliniana*. A, Initiation of carpel primordia with retarded growth of central receptacular tissue and accelerated growth of peripheral tissue (arrows). B, Growth of laterally positioned, conduplicate carpels. C, Further growth of carpels and expansion of locules (dark outline) with development of internal septal nectary commissures. D, Relatively mature stage, showing fusion of carpels and differentiation of interlocular septal nectaries. E–H, *Wachendorfia thyrsiflora*. E, Initiation of carpel primordia with relatively accelerated growth of central receptacular tissue. F, Growth of apically positioned, conduplicate carpels. G, Further growth of carpels and locules (dark outline) with external extension of septal nectary commissure. H, Relatively mature stage, showing fusion of carpels and differentiation of infralocular septal nectaries. * = junction of posterior outer tepal vein and dorsal carpellary vein.

that reported by Eyde and Tseng (1969) for *Tetraplasandra* of the Araliaceae, a study not corroborated by a strict cladistic analysis. If we accept that the above hypothesis of ovary position reversal is correct, the observations of floral development and anatomy described here may be considered as to how that transformation took place.

The ovary of *Lachnanthes* becomes inferior in position by a combination of differential growth and congenital fusion. After the three carpel primordia have initiated, growth becomes retarded in three general locations: (1) at the central junction of the three carpel primordia (fig. 32); (2) at the center of each of the three carpel rings, the locular cavities (fig. 25); and (3) between the walls of adjacent carpel walls, the intercarpellary spaces (figs. 26, 32). Growth is relatively accelerated in the elliptic-shaped walls of the carpel primordia (figs. 25–28; 40A, B). However, as illustrated diagrammatically in fig. 42, growth is relatively retarded in the central axis of the receptacle (fig. 42A). This, combined with extension growth of the surrounding peripheral tissue, reorients the carpels to a more lateral position (fig. 42B). This growth is followed by postgenital fusion of the carpel walls at the floral center, with the result that the now inferior ovary

is embedded within and surrounded by peripheral tissue, well beneath the point of insertion of the perianth (figs. 40A–D; 42C, D). Thus, inferior-ovary development in *Lachnanthes* appears to be rather simple differential growth resulting in lateral displacement of typical foliar-like carpels followed by their congenital fusion. No "recurrent" (invaginated) vascular bundles occur, as are present in some inferior-ovaried dicots. (See Douglas 1944 and Sattler 1974 for interpretations on ovary position and carpel development.)

The ovary of *Wachendorfia* also develops by a combination of differential growth and congenital fusion similar to that of *Lachnanthes*. In fact, carpel initiation and development are quite similar in the two taxa (figs. 6–8). The difference is that in *Wachendorfia* the central axis of the receptacle grows and extends upward to about the same degree as the peripheral tissues of the receptacle (figs. 39A; 42E). The carpels become oriented in a more apical position (fig. 42F). Subsequent congenital fusion results in the locules being elevated relative to perianth insertion, forming a superior-ovary position (fig. 42G, H). The ovary and style of *Wachendorfia* develop similarly to the style of *Lachnanthes*.

In interpreting the details of the transformation from

inferior- to superior-ovary position, the concepts of structural and positional homology are used; features that are structurally or positionally similar in two or more taxa may represent an ancestral condition common to those taxa. In addition, as Carlquist (1969) emphasized, it is vital to consider the functional significance of floral anatomy, particularly with regard to pollination mechanism and fruit or seed dispersal. Thus, an overriding question in this study is assessing what possible function the change from inferior- to superior-ovary position had in terms of overall life history and what the possible selective pressure for that change might have been.

One line of evidence for ovary position reversal concerns the vasculature of the flower. In the superior-ovaried *Wachendorfia*, the median outer tepal vein joins with the common dorsal-column vein well below the locule base, at the base of the receptacular region (fig. 42*H*). In the inferior-ovaried *Lachnanthes*, the junction of outer tepal and dorsal vein occurs at or above the locule base (fig. 42*D*). The venation patterns of *Dilatris* and *Haemodorum*, two other inferior-ovaried taxa somewhat closely related to *Lachnanthes*, is somewhat variable. In *Dilatris* the outer tepal–dorsal vein junction occurs in the midregion of the locule (fig. 41*A*). In *Haemodorum*, the outer tepal–dorsal vein junction occurs just beneath the locule (fig. 41*B*). In any case, if floral vasculature in these taxa is positionally homologous, these results indicate that the junction of dorsal and outer tepal veins in the superior-ovaried *Wachendorfia* is positionally homologous with the middle to basal region of an ancestral inferior ovary. The floral venation of two investigated outgroup family species, *Pontederia cordata* (Pontederiaceae) and *Philydrum lanuginosum* (Philydraceae) is similar to that of *Haemodorum spicatum* in that the dorsal carpellary vein and outer tepal vein are joined just beneath the locule, but of a superior (not inferior) ovary. Thus, all outgroup taxa are different in floral vasculature from the superior-ovaried *Wachendorfia* of the Haemodoraceae, indicating a de novo origin of the extended receptacular tissue of the latter.

A second line of evidence that corroborates reversal in ovary position in the Haemodoraceae concerns the positional homology of the septal nectaries. Recall that *Wachendorfia* is one of four superior-ovaried genera unique with two septal nectaries that are infralocular in position, i.e., located beneath the locules, in an elongate receptacular region. (The fifth superior-ovaried genus, *Xiphidium*, lacks nectaries altogether and cannot be evaluated in this regard.) A comparison of comparable developmental stages of *Wachendorfia* (figs. 39; 42*E–H*) and *Lachnanthes* (figs. 40; 42*A–D*) demonstrates that, relative to the insertion of the perianth, the positional relationship of septal nectaries in the receptacular region of *Wachendorfia* is virtually identical with that of septal nectaries within the inferior ovary of *Lachnanthes*. With reference to the insertion point of the perianth, the septal nectaries in all four superior-ovaried genera are similar in position to those of *Lachnanthes* and the genus *Haemodorum*, both of which have "interlocular" septal nectaries, occupying the septal regions between the locules in an inferior ovary (Simpson 1993). Thus, I hypothesize that the receptacular region in at least four of the five superior-ovaried genera of the Haemodoraceae is positionally homologous (homotopous) with the inferior-ovary region in other members of the family. This positional similarity in septal nectary development, therefore, supports the homology between an ancestral inferior ovary and a derived "inferior" receptacular region. With regard to investigated outgroup taxa, all of which have superior ovaries, septal nectaries are absent in *P. lanuginosum* (fig. 41*C*), as they are in all members of the family Philydraceae. Septal nectaries are also absent in all members of the family Commelinaceae, the recently proposed close relative to the Haemodoraceae. The septal nectaries of *P. cordata* are located at the base of a superior ovary, above the point of insertion of the perianth and not beneath in the receptacular region (fig. 41*D*); this is true for all members of the Pontederiaceae that have septal nectaries (M. G. Simpson, unpublished observation; see Burton and Simpson 1996).

A final line of evidence corroborating ovary position in the Haemodoraceae, related to the above, concerns the development of the septal nectaries. Septal nectaries have been interpreted previously as being derived by the incomplete closure of adjacent carpels (Schmid 1985), and this appears to be corroborated for investigated Haemodoraceae. Septal nectaries in all investigated inferior-ovaried taxa of the family develop within intercarpellary spaces, the slitlike sutures between carpels (e.g., figs. 34–38; 42*B–D*). Thus, septal nectary development in these taxa is integrally related to carpel development. In addition, septal nectaries in inferior-ovaried taxa are continuous with the intercarpellary commissures that extend upward to the base of the style (figs. 40*C, D;* 41*A, B;* 42*D*). Septal nectaries in the superior-ovaried *Wachendorfia* differ from those of the inferior-ovaried taxa by developing in continuity with the outside of the receptacular region of the flower (figs. 14, 15; 42*F–H*), rather than on the inside of the inferior ovary. However, nectaries of *Wachendorfia* are similar to those of inferior-ovaried taxa in being completely continuous with intercarpellary commissures that extend into the ovary and style (figs. 16, 19; 39*B, C;* 42*F–H*). The receptacular tissues flanking the septal nectaries in *Wachendorfia* (fig. 15) are continuous with flanking tissues of the carpels at the ovary base (fig. 16). This continuity appears to have arisen via an evolutionary change resulting in relatively accelerated vertical growth of the central receptacular tissue immediately beneath the carpels and extension of the intercarpellary spaces, which form septal commissures, down the length of the outer receptacle, forming the septal nectary commissures and septal nectaries. I hypothesize that this continuity is the result of homology between the externally developing infralocular septal nectaries and the adjacent carpels, further evi-
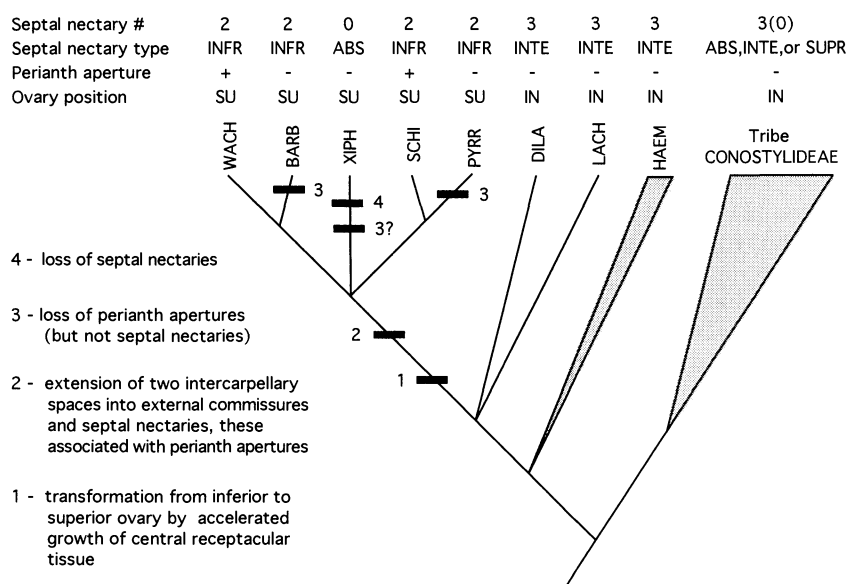
Fig. 43 Cladogram of Haemodoraceae, from Simpson (1990, 1993). Abbreviations as follows: Septal nectary type (from Simpson 1993): *INFR* = infralocular; *ABS* = absent; *SUPR* = supralocular; *INTE* = interlocular. Perianth aperture: "+" = present; "−" = absent. Ovary position: *SU* = superior; *IN* = inferior.

dence that the receptacular region of the superior-ovaried taxa is homologous to the ovary region of the inferior-ovaried taxa. I suggest that the same developmental genes may be involved in the two processes.

In the outgroup taxa with septal nectaries (i.e., members of the Pontederiaceae), septal nectary development has not been studied. However, these outgroup taxa lack any evidence of septal commissures extending into the receptacular tissue (fig. 41D), supporting the hypothesis that septal nectaries evolved independently from those of the superior-ovaried Haemodoraceae.

In conclusion, the scenario hypothesized here for the Haemodoraceae is that an ancestral inferior ovary was evolutionarily transformed into a superior ovary by relatively accelerated, extension growth of the central receptacular tissue, resulting in apically positioned carpels, such that the peripheral receptacular tissue no longer overarched and surrounded the laterally positioned carpel primordia. In addition, the retardation of growth between carpels, in the intercarpellary space, extended to the external tissue of the receptacle. Nectary formation, which ancestrally occurred between adjacent carpel walls, was displaced to the outer receptacle, well beneath the point of insertion of the now superior ovary. A final evolutionary transformation was the loss of the anterior nectary altogether, perhaps correlated with the horizontal orientation of superior-ovaried taxa in the Haemodoraceae (Simpson 1993).

Although the evolution of a superior ovary and of infralocular septal nectaries are portrayed as two separate evolutionary events in figure 43, it is likely that they are correlated and occurred concomitantly. As discussed by Simpson (1993), the selective pressure

for this evolutionary novelty was probably a specialized pollination mechanism associated with the perianth apertures, structures that collect and contain nectar at the outer base of the flower. The perianth apertures may function, in part, to directionally orient an insect (via the perianth aperture openings) for more efficient pollination (Ornduff and Dulberger 1978). The reduction in septal nectary number, from 3 to 2, is correlated with the occurrence of only two perianth apertures, which itself may be related to the evolution of zygomorphy and a horizontal orientation in the superior-ovaried Haemodoraceae (other than *Xiphidium;* Simpson 1993). Two other superior-ovaried members of the Haemodoraceae, *Barberetta* and *Pyrrorhiza,* lack perianth apertures but do have two, infralocular septal nectaries (Simpson 1993). Although not the most parsimonious explanation, it seems most probable that *Barberetta* and *Pyrrorhiza* each independently lost perianth apertures after their ancestral acquisition (Simpson 1993; fig. 43). The evolution of the genus *Xiphidium* within the complex of superior-ovaried taxa of the Haemodoraceae still remains a mystery (fig. 43). One possibility is that *Xiphidium* lost septal nectaries altogether (correlated with a shift in pollination mechanism; see Simpson 1990, 1993) after a transformation to a superior ovary with nectaries in the external receptacular tissue, either before or after reduction in nectary number to two (fig. 43).

Acknowledgments

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