Patterns and Significance of Floral Development in *Whytockia* (Gesneriaceae)

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Abstract: The floral development of *Whytockia* W. W. Smith has been studied in order to explore the developmental basis for the arrangement and differentiation patterns of floral organs, and the evolutionary relationship between *Whytockia* and allies in floral development. The descending imbricate aestivations in both calyx and corolla have remarkably different ontogenetic patterns between calyx and corolla which are derivative with respect to the development of the valvate aestivations in the four-stamened *Rhynchoglossum*. Both corolla lobes and stamens are initiated simultaneously from the same ring meristem. However, the five stamens remarkably precede the initiation of the five corolla lobes. Also, the adaxial stamen is suppressed after initiation to become a staminode, concomitant with retardation of its adjacent organs during development. This situation, together with the non-acropetal order among whorls of floral organs in *Whytockia,* is possibly related to a late expression and a remarkably different expression pattern of *cycloidea*-like genes as compared to *Antirrhinum.* Furthermore, the axile placentation in the bilocular ovary of *Whytockia* is formed by an involute closure of carpels rather than derived from a secondary fusion of two intrusive parietal placentae.

Key words: Floral development, aestivation, ontogenetic pattern, non-acropetal order, axile placentation, *Whytockia*, Gesneriaceae.

Introduction

The genus *Whytockia* W. W. Smith is a small group of species in tribe Epithemateae (Gesneriaceae). It is found in a limestone area from Southwest China to Taiwan Island. Since the 1970s, this genus has attracted considerable interest because it is considered the most primitive representative of Epithemateae (Weber, 1976 a[18], 1982[21]). Based on the morphology of inflorescences and flowers, Weber (1976 a[18], 1982[21]) suggested that *Whytockia* was the phylogenetic progenitor of *Monophyllaea* and *Epithema,* and most other genera in tribe Epithemateae, such as *Rhynchoglossum, Stauranthera* and *Loxonia,* were also derived from a *Whytockia*-like ancestor. Weber's suggestion is supported by some other researchers (Burtt, 1978[2], 1998[3]). Nevertheless, the floral morphology of *Whytockia,* especially the floral developmental morphology, has not been studied intensively in the past, except for observations of the gross external morphology from dried specimens. The homology of some important floral structures between *Whytockia* and its allies has not been well understood. For example, it is curious that both calyx and corolla in *Whytockia* have descending imbricate aestivation (Weber, 1976 a[18]). However, it is unknown how the aestivation is determined during ontogeny and whether it is homologous with that of other Epithemateae. The axile placentation in the Gesneriaceae has traditionally been considered a secondary character derived from parietal placentation (Cronquist, 1981[6]; Wang, Pan, and Li, 1992 a[12], b[13]), but morphological evidence from *Whytockia* and *Rhynchoglossum* seems contrary to this view (Wang et al., 1996[16], 1997[14]; Wang and Pan, 1998[15]). The above issues about the floral morphology and evolutionary trends of some floral structures have come into question with respect to the phylogenetic relationship between *Whytockia* and its allies. However, morphological homology is difficult to interpret and determine without developmental evidence. Developmental data are valuable to decide whether taxonomically important structures of the flower are strictly homologous or convergent, and can offer insights into the origins of particular structures (Crisci and Stuessy, 1980[5]; Kampny and Canne-Hilliker, 1988[8]).

Recently, the gene *cycloidea (cyc)* and its homologues involved in the expression of zygomorphic flowers have been intensively studied in *Antirrhinum majus* (Scrophulariaceae), and have also been isolated in several families, including the Gesneriaceae (Luo et al., 1996[9]; Doebley et al., 1998[7]; Möller et al., 1999[10]; Citerne et al., 2000[4]). Because the Scrophulariaceae and Gesneriaceae are closely related in Scrophulariales, the genetic control of the zygomorphy in the Gesneriaceae should be comparable to the model established in *Antirrhinum.* Besides, the great diversity of floral forms in the Gesneriaceae may be related to different patterns of evolutionary change among the genes homologous with *cycloidea* (Möller et al., 1999[10]; Citerne et al., 2000[4]). Therefore, observations of floral development in phylogenetically key taxa, such as *Whytockia,* may raise more important questions about the evolution of floral symmetry.
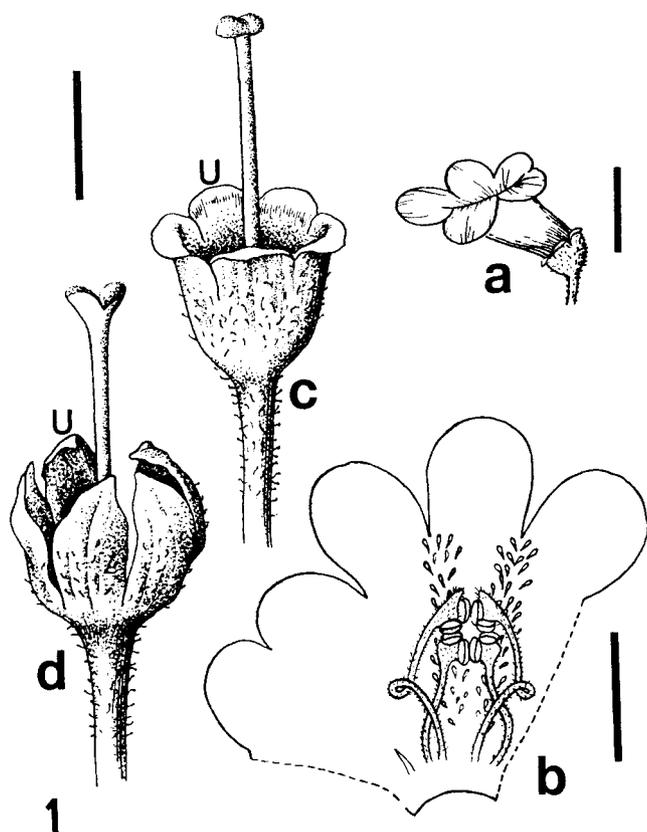
Fig. 1 Diagrammatic illustrations of (**a**) flower, (**b**) corolla and stamens, and (**c**) calyx and pistil of *W. hekouensis,* and (**d**) calyx and pistil of *W. bijieensis.* Note the adaxial (upper) calyx lobe (U) being barely higher than other calyx lobes (**c, d**), and the abaxial (lower) corolla lip with three lobes (the right three lobes in **b**) being longer than the adaxial (upper) corolla lip with two lobes (the left two lobes in **b**), and four didynamous stamens inserted at the lower part of the corolla tube (**b**), scale bar = 11 mm in **a**, 7 mm in **b**, and 3 mm in **c** and **d**. *Figure abbreviations:* As, abaxial (anterior) stamen; C, carpel; Co, column; D, disc; F, floral primordium; L, lateral calyx lobe; Lc, lateral corolla lobe; Lo, ovarian locule; M, carpel margin; N, connective; O, ovary; Ov, ovule; Ps, lateral (posterior) stamen; S, style; Sd, adaxial stamen or staminode; Se, septum; st, stigma; th, thecae; U, adaxial (upper) calyx lobe; Uc, adaxial (upper) corolla lobe; W, abaxial (lower) calyx lobe; Wc, abaxial (lower) corolla lobe; Wo, wall of ovary.

The first goal of our research is to explore the developmental basis for the arrangement and differentiation patterns of floral organs in *Whytockia,* a key genus in the phylogeny of the tribe Epithemateae. Secondly, floral development, especially the early floral development in *Whytockia,* will be analysed in comparison with that found in related genera, which focuses on an understanding of the developmental and evolutionary relationship of some important floral structures between *Whytockia* and allies in Epithemateae. Thirdly, comparing the revealed developmental pattern between corolla and stamens with that in *Antirrhinum,* we attempt to evaluate the possible role of putative genes homologous with *cycloidea* in *Whytockia* so as to shed light on further intensive research required to understand the genetic control and evolutionary path of floral symmetry in the Gesneriaceae.

Materials and Methods

Material of *Whytockia hekouensis* Y. Z. Wang was collected from two natural populations in Hekou County, Yunnan (Wang, 92073; Wang, 93019), and material of *W. bijieensis* Y. Z. Wang et Z. Y. Li (Wang, 95001) was obtained from one natural population in Bijie County, Guizhou. Voucher specimens are deposited in the Herbarium (PE), Institute of Botany, the Chinese Academy of Sciences. Buds and flowers at different developmental stages were fixed in formalin–acetic acid–alcohol (FAA). Samples used for Scanning Electron Microscopy (SEM) were dehydrated in three changes of 100% ethanol, 100% ethanol + amyl acetate (1 : 1) and amyl acetate, then critical point dried with a Hitachi HCP-2 Critical Point Dryer and Au/Pd sputter-coated with a SPI-MODULE Sputter Coater. The prepared samples were examined with a Hitachi S-800 Scanning Electron Microscope and recorded on Shanghai Panchromatic film. Samples used for histological studies were embedded in paraffin, after being dehydrated in an ethanol series. Consecutive microtome sections were cut to a thickness of 6 – 8 μm and stained with safranin (0.5% in 50% ethanol) and fast green (1% in 95% ethanol). Photographs were taken on Shanghai GP3-100, PAN film using a photomicroscope (Leitz Orthoplan).

Results

Organography

At anthesis, flowers in *Whytockia* are highly zygomorphic (Fig. **1 a**). The calyx is five-lobed with the adaxial (upper) lobe barely larger than the others (Figs. **1 c, d**). The calyx tube constitutes almost half the calyx in length in *W. chiritiflora* and *W. hekouensis* (Fig. **1 c**), while a tube is absent in other species, such as *W. purpurascens* and *W. bejieensis* (Fig. **1 d**). The tubular bilabiate, bilaterally symmetrical corolla has an abaxial (lower) trilobed lip that is longer than the adaxial (upper) bilobed one (Figs. **1 a, b**). The aestivation of both calyx and corolla is descending imbricate in buds (Figs. **10, 12, 13, 27**). The androecium consists of four stamens and one staminode inserted at the corolla base. The four stamens are didynamous with the two abaxials (anteriors) longer than the two laterals (posteriors) (Figs. **1 b, 48**). The disc is ring-like. The ovary is bilocular with axile placentation (Fig. **50**), but usually unilocular distally (Fig. **51**). The stigma is deeply bilobed in *W. bijieensis* and slightly bilobed in *W. hekouensis,* with papillate unicellular hairs (Figs. **1 c, d**).

Initiation and development of floral organs

Calyx

Flowers are initiated in pairs on the axis of inflorescences (Figs. **2 – 5**). The floral primordium is initially circular in shape (Fig. **2**), and then elongates to begin the initiation of the calyx (Figs. **3, 4**). As the adaxial side grows faster than the abaxial side, the floral primordium becomes adaxially ascended, and forms a gradually descending slope from the adaxial side to the abaxial side on the floral apex (Fig. **4**). The adaxial lobe arises from the top of the adaxial side (Figs. **4, 5**). Two lateral lobes are initiated simultaneously below and on either side of the adaxial lobe (Figs. **5, 6**). Two abaxial lobes are initiated while the adaxial lobe expands toward the centre of the floral apex (Figs. **6 – 8**).
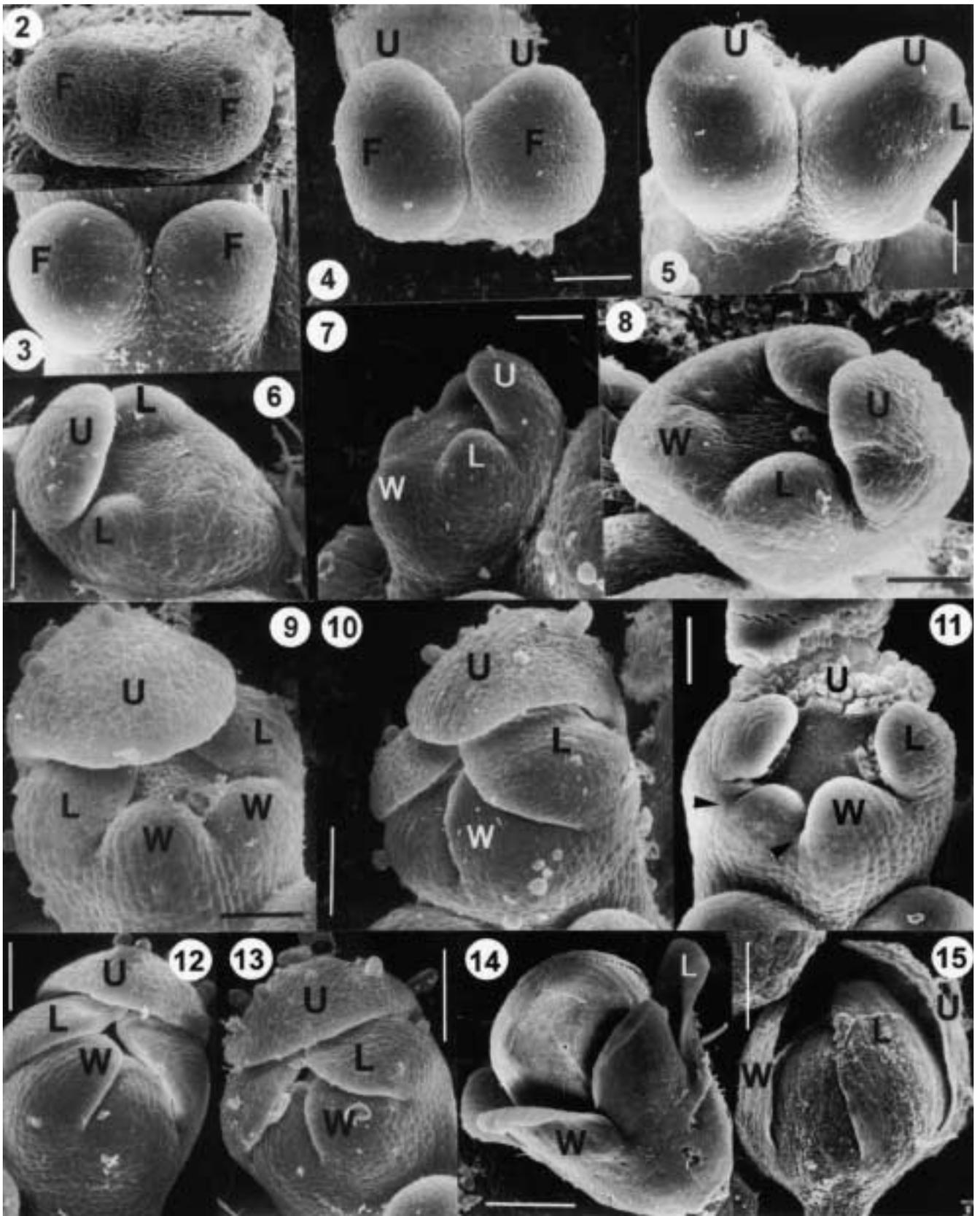
Figs. 2 – 15

◄ **Figs. 2 – 15** Initiation and development of the calyx. *W. hekouensis* in **2 – 6, 8, 11 – 14** and *W. bijieensis* in **7, 9, 10, 15**. (**2 – 4**) Floral primordia and initiation of the adaxial calyx lobe. **2** Polar view: a pair of floral primordia. Scale bar = 60 μm. **3** Oblique view: elongated floral primordia. Scale bar = 65 μm. **4** Oblique view: adaxially ascended floral primordia. Note that the adaxial calyx lobe is becoming visible in the floral primordium. Scale bar = 80 μm. (**5**) Oblique view: appearance of the adaxial calyx lobe followed by two lateral lobes below and on either side of the adaxial one. Scale bar = 78 μm. (**6 – 8**) Initiation of two abaxial calyx lobes. Note the adaxial calyx lobe overarching toward the centre of the floral apex. **6** Oblique view: the two lateral calyx lobes have been initiated and the two abaxial calyx lobes are becoming visible. Scale bar = 70 μm. **7** Lateral view: two abaxial calyx lobes have become visible. Note no obvious lateral growth taking place between adjacent calyx lobes. Scale bar = 66 μm. **8** Oblique view: two abaxial calyx lobes have become visible, and primary lateral growth between adjacent calyx lobes. Scale bar = 55 μm. (**9**) Oblique view: the adaxial calyx lobe overarches strongly toward and almost covers the centre of the floral apex, and overlaps the two lateral sepals. Scale bar = 69 μm. (**10**) Oblique view: unidirectional enclosure of the calyx from adaxial to abaxial side. Note the right overlapping the left in the lateral and abaxial calyx lobes. Scale bar = 101 μm. (**11**) Oblique view: lateral growth (arrowhead) between adjacent calyx lobes. Scale bar = 72 μm. (**12,13**) Oblique view: the left overlaps the right in two lateral and two abaxial calyx lobes in **12** and *vice versa* in **13**. Scale bar = 84 μm in **12** and 112 μm in **13**. (**14**) Lateral view: opened calyx, note the calyx tube. Scale bar = 1262 μm. (**15**) Lateral view: opened calyx, note no obvious calyx tube. Scale bar = 636 μm.

After initiation of the two abaxial lobes, the adaxial lobe over-arches strongly toward and quickly covers the centre of the floral apex (Fig. **9**). Because of the earlier initiation and faster growth, the calyx lobes on the adaxial side are larger than those on the abaxial side (Fig. **9**). The floral apex is enclosed by five calyx lobes, with the adaxial lobe overlapping the two lateral lobes that, in turn, fold down over the two abaxial ones (Fig. **10**). Meanwhile, trichomes form on the dorsal surface of the calyx lobes (Figs. **9,10**). It is chance whether the right or the left overlaps the other in the two lateral and two abaxial lobes. However, it is more frequent that if the right overlaps the left in the two laterals, then the same occurs in the two abaxials, or *vice versa* (Figs. **12,13**). The overlapped calyx opens, accompanying the enlargement of the inner floral organs (Figs. **14,15**). In *W. hekouensis,* the calyx tube is formed by the lateral growth between adjacent calyx lobes (Figs. **8,11,14,16**). However, the calyx tube is not obvious in *W. bijieensis* due to no active lateral growth (Figs. **7,9,15**).

Corolla

While enclosed by calyx lobes, the floral apex increases in height and flattens to become a pentagonal protuberance (Fig. **16**). Then, the ring meristem of the corolla tube is discernible as an elevated flank of the pentagonal floral apex, accompanied by the initiation of stamens (Figs. **17,18**). When the ring meristem is about 35 μm high, the primordia of five corolla lobes arise sequentially from the corners of the ring meristem, which are alternate to five stamen primordia (Figs. **19,20**). The two adaxial lobes appear first, followed by two lateral lobes and one abaxial lobe (Figs. **19 – 21**). During early development, the ring meristem is more active beneath and between the two adaxial lobes (Figs. **22,23**). This growth pattern results in the corolla tube being taller beneath the two adaxial lobes than in

other positions (Figs. **22 – 24**). As a result, the shallowest sinus occurs between the two adaxial lobes, an indication of the earliest onset and greater activity of zonal growth (Figs. **25 – 27**). When the corolla is closed, two adaxial lobes overlap two lateral lobes that, in turn, overlap the abaxial one (Figs. **26, 27**). Thus, the aestivation of the corolla is descending imbricate in buds (Fig. **27**). Afterwards, the lateral and abaxial lobes gradually surpass the two adaxial lobes in length, in which the former will form the abaxial (lower) corolla lip while the latter will develop into the adaxial (upper) corolla lip (Figs. **1 a, b**). At anthesis, the shallowest sinus not only still maintains but also becomes more manifest between the two lobes of the adaxial lip that are smaller than the three lobes of the abaxial lip (Figs. **1 a, b**).
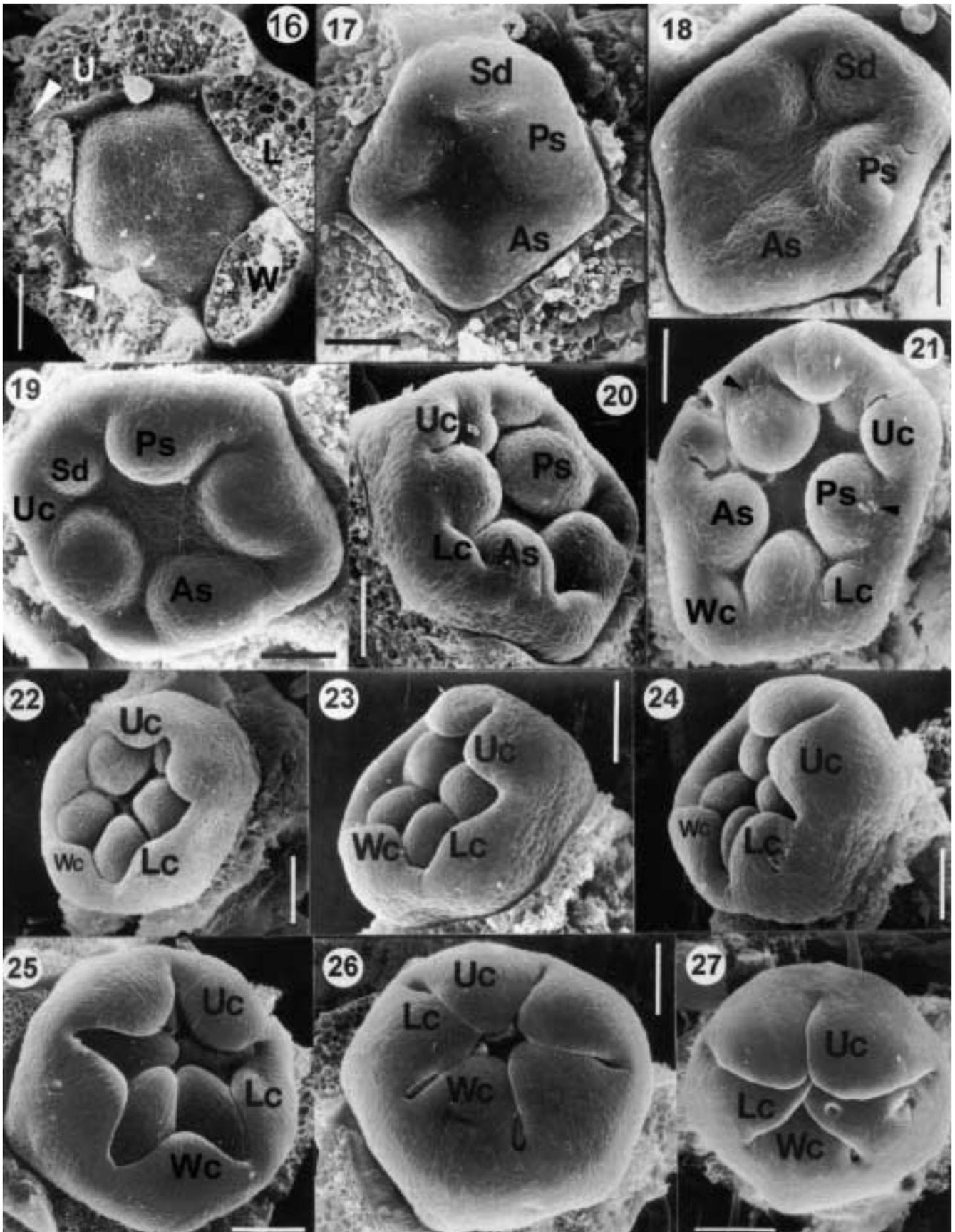
Stamens

Five stamen primordia arise almost simultaneously from the inside of the ring meristem, which remarkably precedes the initiation of five corolla lobes (Fig. **17**). The primary enlargement of stamen primordia is accompanied by the activity of the ring meristem and initiation of five corolla lobes (Figs. **17 – 19**). However, the adaxial stamen primordium is suppressed in growth after initiation, and is destined to become a staminode (Figs. **18 – 21**). Meanwhile, the four other stamen primordia enlarge rapidly, and the two laterals are larger than the two abaxials in size (Figs. **18 – 21**).

While increasing in size, the four young stamens gradually separate from the ring meristem (Figs. **21, 22**). Each stamen enlarges laterally and differentiates into anther and filament, while a median furrow divides an anther into two parts distal with a connective (Figs. **34, 35**). When they are about 380 μm long, the two laterals and two abaxials become nearly equal in size, while the staminode is only 140 μm long (Fig. **35**). A transverse furrow bisects each side of the young anther and defines the thecae (Figs. **35, 40, 41**). As the anthers develop, the filaments elongate, with hairs arising at their upper part, but no differentiation of anther and filament occurs in the staminode (Figs. **40, 41, 46, 47**). In an exceptional case, the adaxial stamen was not suppressed to become a staminode, but developed normally into a fertile stamen that was only slightly smaller than the four other stamens (Fig. **44**). Pollen grains in the anthers of this stamen developed normally, with a polar view of 3-colporate and reticulate surfaces, the same as the pollen grains in other fertile stamens (Fig. **45**). During the consecutive development of the flowers, intercalary growth leads to the further elongation of filaments (Figs. **46, 47**). Near flowering and at anthesis, the filaments are knee-jointed, and the two abaxial filaments (about 7 mm long) are longer than the two lateral ones (about 5 mm long), but the staminode is only 1.5 mm long (Figs. **1 b, 48**).

Carpels

While the corolla is closed, a pentagonal rim is formed around the depressed floral apex (Fig. **28**). Five corners of the rim are alternate to the staminode and four stamens (Fig. **28**). Due to more active growth in the adaxial and abaxial regions, two carpel primordia soon elevate above this rim (Fig. **29**).

Figs. 16–27

◄ **Figs. 16 – 27**   Initiation and early development of corolla and androecium in *W. hekouensis.* (**16 – 18**) Initiation of ring meristem and stamens. **16** A flattened pentagonal protuberance within the closed calyx (removed), note lateral growth between adjacent calyx lobes (arrowhead). Scale bar = 67 μm. **17** Initiation of the ring meristem and five stamens on the floral apex. Scale bar = 59 μm. **18** Early development of stamens accompanied by activity of the ring meristem. Note the adaxial stamen primordium beginning to be suppressed in growth. Scale bar = 39 μm. (**19 – 21**) Initiation of corolla lobes and enlargement of stamens. Note the adaxial stamen is suppressed in growth, and two lateral stamens are larger than two abaxial ones. **19** Oblique–polar view: enlargement of the four lateral and abaxial stamens, and two adaxial corolla lobes being initiated from the ring meristem. Scale bar = 56 μm. **20** Oblique view: two lateral corolla lobes have emerged on the ring meristem and the abaxial corolla lobe is becoming visible. Scale bar = 72 μm. **21** Oblique view: initiation of the abaxial corolla lobe and separation of the stamens from the ring meristem (arrowhead). Note noticeable difference in size between the four stamens and staminode. Scale bar = 59 μm. (**22 – 24**) Oblique–lateral view: early development of the corolla in a sequence from adaxial to abaxial side. Note the corolla tube at the adaxial side is taller than that at the abaxial side, while the two adaxial lobes are larger than the three abaxial lobes. Scale bar = 106 μm in **22**, 112 μm in **23** and 87 μm in **24**. (**25**) Oblique–polar view of the corolla and stamens at a stage similar to **24**, showing two adaxial corolla lobes that are longer than the three abaxial corolla lobes. Scale bar = 115 μm. (**26 – 27**) Enclosure of corolla lobes. Note the shallowest sinus between the two adaxial lobes. **26** Oblique–polar view of corolla: the three abaxial lobes have surpassed the two adaxial lobes in length, when five corolla lobes are to be enclosed. Scale bar = 125 μm. **27** Polar view of corolla: unidirectional enclosure of the corolla lobes from adaxial to abaxial side. Scale bar = 266 μm.

After initiation, the dorsal sides of each carpel grow more quickly than the ventral side. As a result, the dorsal sides overarch toward the floral apex to form a slope from the dorsal distal summit to the ventral base of the carpels (Figs. **29, 30**). Carpel margins at ventral sides progressively invaginate into the depressed floral apex (Figs. **30, 31**). Two carpels infold with contact to the abaxial surfaces (Fig. **31**). As a result of extended marginal growth, the margins of two involute carpels meet and become appressed together in the region of the depressed floral apex (Figs. **31 – 33**). Meanwhile, a fusion takes place along the appressed surfaces of the two involute carpels, upward from the basal part (Figs. **32, 33**). A young ovary is formed as the floral apex is enclosed by the two fused carpels (Figs. **34, 35**). In a young ovary, the lower part is bilocular owing to a fusion of the two involute carpels (Figs. **33, 36**). Further up, the carpel margins separate from the centre of the ovary, which leads to the fused surfaces of the two carpels being situated at the periphery of the ovary (Fig. **37**). The two carpels are still free at the top of the ovary, i.e., two carpellary lobes (Figs. **35, 38**). Meanwhile, the disc is produced by outgrowth around the base of the ovary (Fig. **35**). Then, a stigma differentiates out of the young gynoecium, with papillate cells appearing on top of the two carpellary lobes (Figs. **39, 40**), while a style is produced by the intercalary growth between ovary and stigma (Figs. **39 – 41**). At this stage, the bifid lobes of placentae in each ovarian locule become reflexed, with their swollen tips bearing numerous anatropous and unitegmic ovules (Figs. **42, 43**). Later in development, the gynoecium is surrounded by four fertile stamens (Figs. **46, 47**). During development, two carpellary lobes are gradually fused in the stigmatic region, while two lateral stigmatic lobes are formed

on top of the stigma, bearing papillate unicellular hairs (Figs. **41, 46, 47**). The stigmatic lobes are more obvious in *W. bijieensis* than in *W. hekouensis* (Figs. **46, 47**). At anthesis, the stigma is deeply bilobed in the former while only slightly bilobed in the latter (Figs. **1 c, d**). During maturation, intercalary growth leads to the enlargement of the ovary and further elongation of the style (Figs. **46 – 48**). At anthesis, the style is 2.5 – 3 times longer than the ovary in both species (Figs. **1 c, d, 48**). The placental column in the ovary is swollen from the base to the upper part, with two lobes in each locule (Figs. **49, 50**). However, the septum is discontinuous at the extreme distal end of the ovary in *W. hekouensis,* which forms a compital pore, i.e., paracarpellary region in the transitional area between ovary and style (Fig. **51**).

Discussion

### Ontogenetic interpretation for the aestivation of calyx and corolla

The adaxial ascension of the floral primordium shows an initial floral asymmetry, from adaxial to abaxial side, prior to the initiation of the calyx. This asymmetry becomes more manifest, both in space and time, during the calyx initiation. It is obvious that the descending imbrication of the calyx in the bud originates from the space–time unidirectional sequence of initiation from adaxial to abaxial side, which can be traced back to the initial asymmetry of the floral primordium. The lateral growth between adjacent calyx lobes takes place after the early development in some species, which distinguishes different groups within the genus. The corolla, on the other hand, is initiated primarily as a common ring meristem with stamens that develop into a corolla tube. No adaxial to abaxial asymmetry can be observed in the ring meristem during early development. The five corolla lobes are initiated almost simultaneously or in a quick succession. No sign of descending imbricate aestivation is observed from the corolla initiation. The ontogenetic basis for corolla aestivation in Scrophulariaceae was studied in detail by Armstrong and Douglas (1989[1]). They suggested that the fate of the adaxial or abaxial corolla lobes ending outermost in a bud depended on whether it was upon the adaxial or abaxial side of the floral apex that precocious corolla development took place. In some taxa of legumes, Labiatae and Scrophulariaceae, radial symmetry prevails until failure of a stamen to initiate, or until precocious enlargement of organs on one side of the flower (Tucker, 1987[11]; Armstrong and Douglas, 1989[1]). In *Whytockia,* the precocious growth takes place on the adaxial side of the corolla shortly after initiation, which is indicated by the adaxial portion being higher than the abaxial portion in the corolla tube. The above suggestions (Tucker, 1987[11]; Armstrong and Douglas, 1989[1]) also seem to be true in the corolla development of *Whytockia.* The descending imbricate corolla is gradually formed after initiation through the precocious development on the adaxial side of the corolla. Apparently, the ontogenetic patterns of calyx and corolla are remarkably different from each other, even though the aestivation of both is descending imbricate.

From the available data on floral morphology and development in Epithemateae, the descending imbricate aestivation of both calyx and corolla has been observed only in *Whytockia* and *Monophyllaea* (Weber, 1976 a[18]). The initiation and development of both calyx and corolla also follow a similar pattern
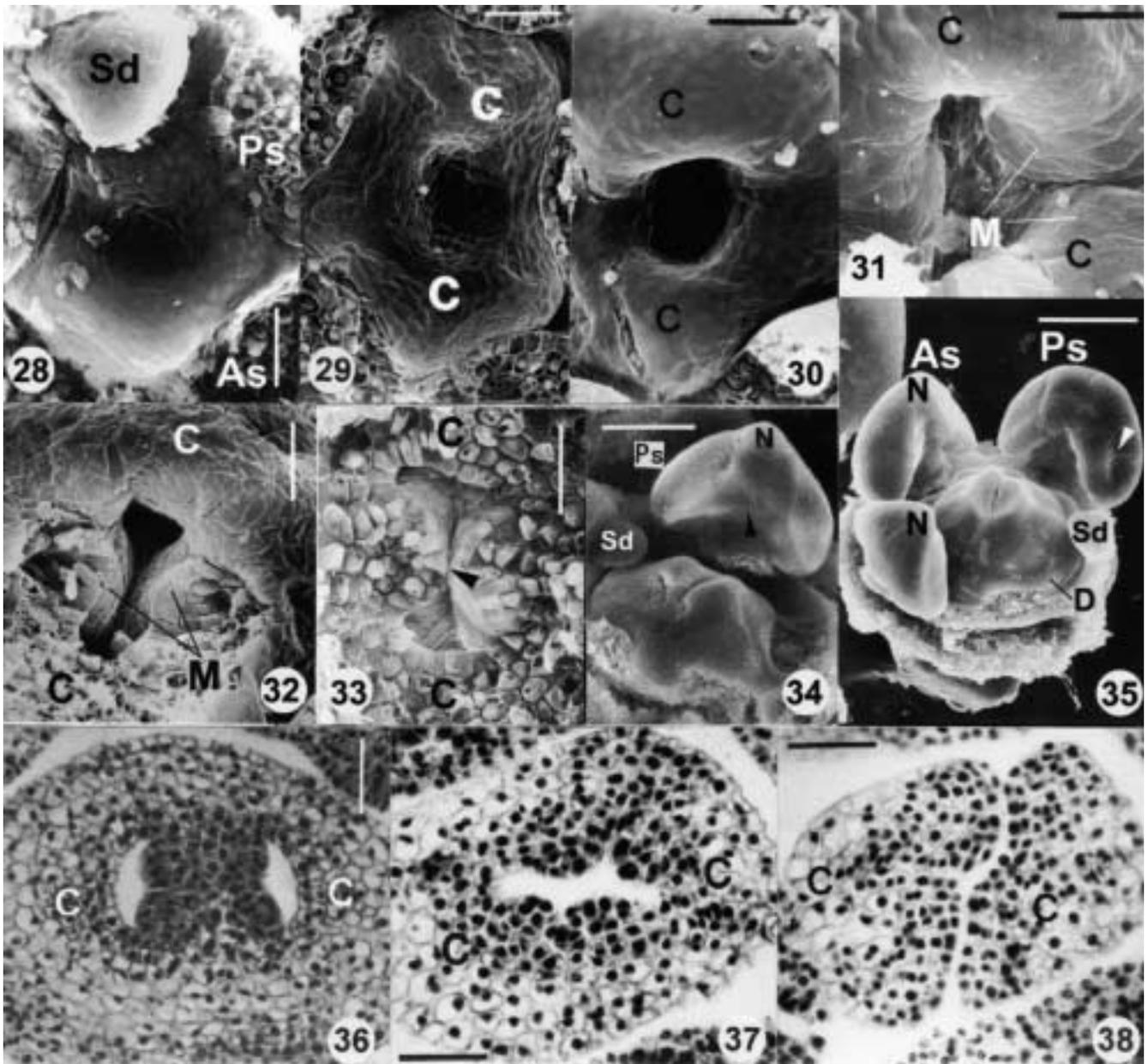
Figs. 28–38 Initiation and early development of the gynoecium, and differentiation of stamens in *W. hekouensis*. (**28**) Polar view: pentagonal rim around the depressed floral apex. Note that five corners of the rim are alternate to the staminode and four stamens (removed). Scale bar = 38 μm. (**29**) Oblique–polar view: a young gynoecium, showing two carpel primordia elevated on the pentagonal rim in the adaxial and abaxial median positions. Scale bar = 36 μm. (**30**) Polar view: a young gynoecium with the dorsal side of each carpel primordium overarching towards the depressed floral apex, and slopes from the dorsal distal summit to the ventral base of the carpels. Scale bar = 33 μm. (**31**) Polar view: ventral margins of one carpel invaginate into the depressed floral apex and infold on its midrib region as an axis. Scale bar = 18 μm. (**32**) Young gynoecium with the top of one carpel removed, showing a fusion between adjacent parts of two involute carpels. Scale bar = 30 μm. (**33**) Young gynoecium with the top of two carpels removed at a slightly late stage compared to **32**, with marginal appression and post-genital fusion of two involute carpels in the region of the depressed floral apex from base upwards. Scale bar = 41 μm. (**34**) Gynoecium with the top of two carpels becoming enclosed, and stamen with form differentiation as anther and filament. Note a median furrow (arrowhead) dividing the anther into two parts distal with a connective. Scale bar = 147 μm. (**35**) Gynoecium with the top of two carpels enclosed and ring-like disc primarily formed around the base of ovary, and anther with a transverse furrow (arrowhead) bisecting each side into two thecae. Note almost equal size between the lateral and abaxial stamens, and a connective becoming visible from the dorsal side of the anther. Scale bar = 285 μm. (**36–38**) Series of transverse sections of the ovary at a stage similar to **35**, showing the carpel margins separate from the centre of the ovary. **36** Transverse section through the lower part of the ovary. Scale bar = 58 μm. **37** Transverse section through the middle part of the ovary. Scale bar = 39 μm. **38** Transverse section through the top of the ovary. Scale bar = 33 μm.
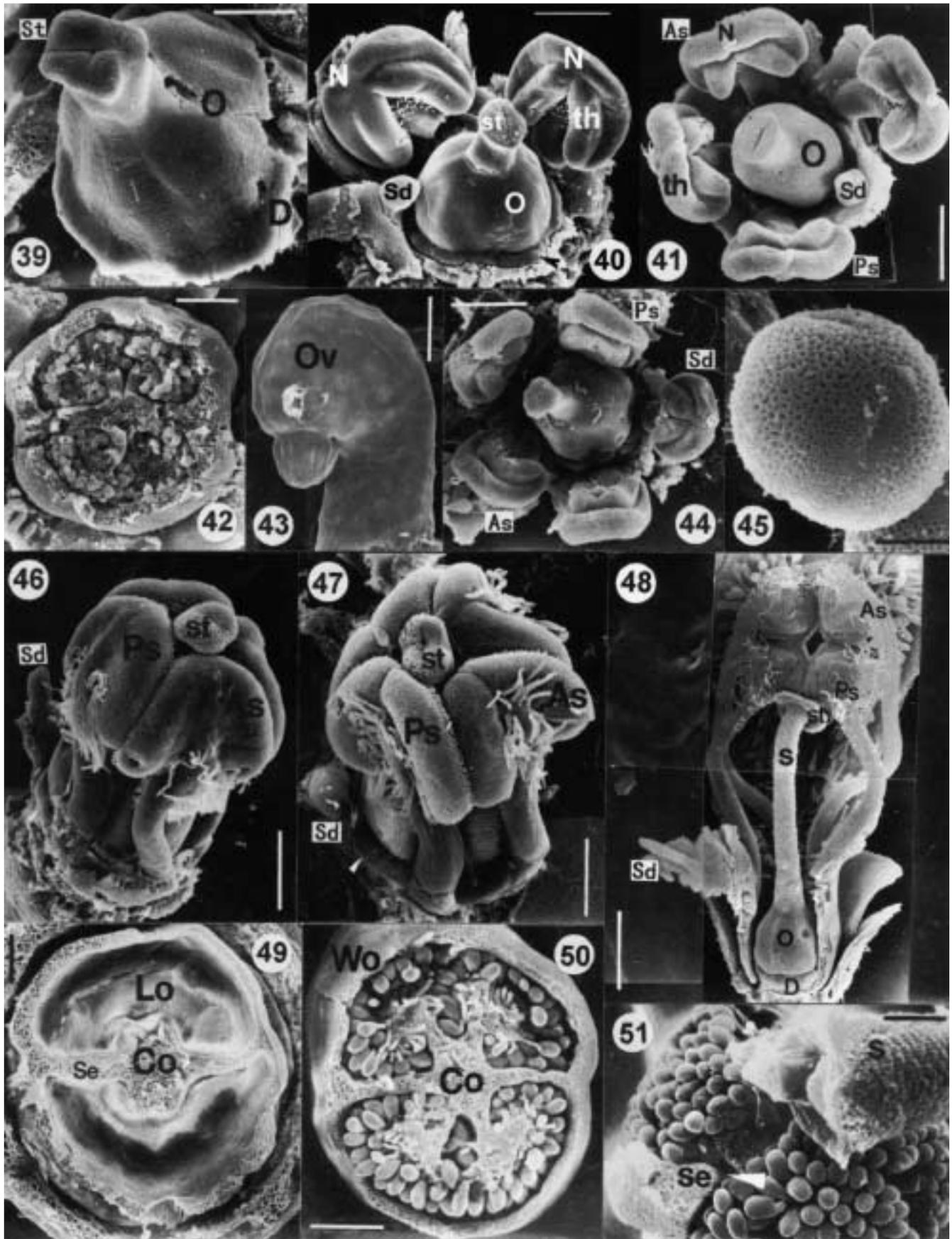
Figs. 39–51

in the two genera (Weber, 1976 b[19]). On the contrary, the aestivation is valvate in both calyx and corolla in the four-stamened species of *Rhynchoglossum* (Wang et al., 1997[14]) and the aestivation is valvate in the calyx and increasing imbricate in the corolla in *Epithema* (Weber, 1976 c[20], 1988[22]). In *Rhynchoglossum omeiense,* five calyx lobes are initiated simultaneously on the floral apex. This initial symmetry is maintained throughout the whole process of floral development (Wang et al., 1997[14]). The initiation of five calyx lobes in *Epithema* is similar to that of *R. omeiense* except for the three adaxial lobes initiating slightly prior to the two abaxial lobes in which the former are also slightly larger than the latter in the valvate bud (Weber, 1988[22]). No elongation and adaxial ascension of the floral primordia takes place in the two genera. The corolla of *R. omeiense* is initiated as five corolla lobe primordia that appear simultaneously, rather than a ring meristem as in *Whytockia.* The corolla tube is formed by the lateral growth between adjacent corolla lobes (Wang et al., 1997[14]). No precocious growth takes place in the corolla development of *R. omeiense.* From the above comparisons, the descending imbricate aestivation of both calyx and corolla in *Whytockia* and *Monophyllaea* is apparently formed by a series of secondary ontogenetic events during development. While, the simultaneous initiation and valvate aestivation of both calyx and corolla in the four-stamened *Rhynchoglossum* demonstrates an original floral symmetry in Epithemateae.

Evolutionary consideration of the gynoecium

The axile placentation within Gesneriaceae is traditionally considered as being derived from parietal placentation. It is suggested that the phylogenetic change from the parietal to the axile has been achieved through the fusion of the two intrusive parietal placentae, meeting in the centre of the ovary (Cronquist, 1981[6]; Wang, Pan, and Li, 1992 a[12], b[13]). In *Whytockia,* the depressed floral apex indicates that the apical meristem is completely used up in carpel formation. During development, two carpel margins invaginate into the depressed floral apex and infold as a result of extended marginal growth. Two ovarian locules are formed through the marginal appression and further post-genital fusion of two involute carpels in the region of the depressed floral apex. Therefore, the septum dividing the ovarian cavity into two locules in *Whytockia* is part of two involute and post-genitally fused carpels rather than the intrusive parietal placentae. In addition, an involute situation of free carpels clearly demonstrated in a bicarpellary and apocarpous gynoecium has been observed in a gynoecial anamorphosis in *Whytockia* (Wang et al., 1996[16]). *Rhynchoglossum omeiense,* a four-stamened species of *Rhynchoglossum,* has an ovary that is bilocular in the lower part and unilocular in the upper part. The gynoecium of *R. omeiense* is initiated as two separate carpellary primordia in the adaxial to abaxial position (Wang et al., 1997[14]). Undergoing an initial lateral growth, the two young carpels infold on each midrib region as an axis. The post-genital fusion takes place between the contact surfaces of the two involute carpels after early development (Wang et al., 1997[14]). The unilocular condition results from carpel margins receding from the centre to periphery, upwards in the ovary (Wang et al., 1997[14]). The independent initiation of two carpels is more obvious in *R. omeiense* than in *Whytockia.* The gynoecial development, both in *Whytockia* and *Rhynchoglossum,* suggests that the axile placentation in the two genera is formed through an involute closure of two carpels rather than derived from a secondary fusion of two intrusive parietal placentae.

From the available data in Epithemateae, ovaries are typically bilocular in *Whytockia, Monophyllaea* (Weber, 1971[17]; Wang and Pan, 1998[15]) and *Gyrogyne* (Yinzheng Wang, unpublished). Ovaries in *Stauranthera* and four-stamened *Rhynchoglossum* (such as that in *R. omeiense*) show a bilocular condition in the lower part (Weber, 1971[17]; Wang et al., 1997[14]). The typical unilocular ovary, correlated with the presence of two fertile stamens plus three staminodes in a flower, is only observed in *Epithema* and two-stamened *Rhynchoglossum* (Weber, 1971[17]; Yinzheng Wang, unpublished). However, four fertile stamens plus one staminode are widely distributed in the species, with typically bilocular or transitional ovaries, with the bilocular condition at the lower part and unilocular at the upper part. The bilocular ovary with axile placentation is a common feature in Epithemateae, which phylogenetically may proceed to the unilocular ovary with parietal placentation.

Developmental relationship in stamens and corolla

The initiation of floral organs in angiosperms is mostly acropetal: calyx, corolla, stamen and carpel. However, a common ring meristem forms first in *Whytockia* upon which corolla lobes and stamens arise. Five stamens remarkably precede the initiation of five corolla lobes. The timing of stamen initiation is perhaps an exception in the Gesneriaceae. A similar situation has been observed in the legumes, in which carpels precede the initiation of the second whorl of stamens in some genera (Tucker, 1987[11]). In addition, the adaxial stamen in *Whytockia*

◄ **Figs. 39 – 51**   Development of stamens and gynoecium. *W. hekouensis* in **39 – 46, 48 – 51**; *W. bijieensis* in **47**. (**39**) Oblique view: a paracarpous stigma becomes visible, with papillate cells primarily appearing on the top of it. Scale bar = 203 µm. (**40**) Oblique view: primary elongation of the style and further form differentiation of anthers which give rise to a connective and four thecae. Scale bar = 430 µm. (**41**) Oblique view: four stamens equal in size. Scale bar = 525 µm. (**42**) Transverse section of ovary at stage similar to **41**: two lobes of the axile placenta in each ovarian locule becoming reflexed with their swollen tips bearing numerous anatropous and unitegmic young ovules. Scale bar = 243 µm. (**43**) Lateral view: a young ovule in the ovary in **42**. Scale bar = 18 µm. (**44**) Oblique view of an occasionally found flower at stage similar to **41** with calyx and corolla removed, showing five fertile stamens almost equal in size. Note the adaxial stamen is only slightly smaller than the other four stamens, rather than suppressed in growth to become a staminode in a normally developing flower as in **41**. Scale bar = 692 µm. (**45**) A normally developed pollen grain in the anther of the adaxial stamen in **44** with polar view of 3-colporate and reticulate surface. Scale bar = 3.4 µm. (**46, 47**) Gynoecium surrounded by four fertile stamens, with hairs at upper part of filaments, ring-like disc around the base of ovary (arrowhead). Note stigmatic lobes more obvious in **47** than in **46**. Scale bar = 737 µm in **46** and 553 µm in **47**. (**48**) Lateral view: a flower at anthesis with calyx and corolla removed. Scale bar = 1900 µm. (**49 – 51**) Transverse sections of ovary at stage similar to **48**. **49** Transverse section in the basal part of ovary: the central swollen column of the axile placenta. Scale bar = 380 µm. **50** Transverse section in the middle part of ovary: the bilocular ovary with axile placentation. Scale bar = 560 µm. **51** Oblique–polar view of the distal part of ovary when the style is removed, showing the compital pore (arrowhead). Scale bar = 190 µm.

is suppressed after initiation and forms a staminode during development. Concomitantly, its adjacent organs, i.e., the two adaxial corolla lobes and two lateral stamens, also become retarded late in development and form a bilabiate corolla and didynamous stamens in *Whytockia.* The spatial and temporal variations are the two important factors determining organic differential pathways. The two adaxial corolla lobes and two lateral stamens are the floral organs most closely adjacent to the staminode, and their retardation takes place after the suppression of the staminode. Since these ontogenetic changes co-occur, the suppression of the staminode and developmental retardation of its adjacent organs are probably related to only one selection event.

In *Antirrhinum majus,* the gene *cycloidea* is expressed only in the adaxial side of the flower, especially in the staminode and two adaxial corolla lobes (Luo et al., 1996[9]; Doebley et al., 1998[7]). This asymmetrical expression results in the suppression of the adaxial stamen to a staminode, together with a bilabiate corolla and didynamous stamens in *Antirrhinum majus.* Actinomorphic flowers, i.e., peloric mutants in *Antirrhinum,* are caused by the loss of function at the *cycloidea* locus (Luo et al., 1996[9]). Putative homologues of the gene *cycloidea* referred to as Gesneriaceae *cycloidea* (G*cyc*), have been found in various groups in the Gesneriaceae (Möller et al., 1999[10]; Citerne et al., 2000[4]). Homology assessment from phylogenetic analysis suggests that G*cyc* belongs to a small gene family which has undergone several duplication and putative gene loss events during the evolution of the Gesneriaceae (Citerne et al., 2000[4]).

In the zygomorphic flowers of *Whytockia,* even though the adaxial stamen is suppressed after initiation and becomes a staminode during development, the five stamen primordia are initiated simultaneously. There is no essential difference between the staminode and four stamens at initiation. In addition, a common ring meristem forms before initiation of stamens and corolla lobes, and no adaxial to abaxial (dorsoventral) asymmetry can be observed in the ring meristem during early development. The five corolla lobes are initiated in quick succession, in which the two adaxial lobes are slightly earlier than the three abaxial lobes. However, the adaxial to abaxial (dorsoventral) asymmetry of stamens and corolla is manifested during primordial initiation in *Antirrhinum,* (Luo et al., 1996[9]). Besides the situation within whorls, the non-acropetal order among whorls of floral organs in *Whytockia* is also remarkably different from that in *Antirrhinum,* in which corolla and stamens follow an acropetal order in initiation (Luo et al., 1996[9]). If the initiation and development of corolla and stamens is related to the expression of *cycloidea*-like genes, the expression pattern of *cycloidea*-like genes in *Whytockia* should be remarkably different from that of *Antirrhinum.* The *cycloidea*-like genes in *Whytockia* may express late compared with *Antirrhinum.* Also, these genes may be activated at the adaxial stamen as an active centre, and then extend to lateral stamens and adaxial corolla lobes, both adaxially and abaxially. Meanwhile, the five fertile stamens occasionally found in androecial development could be caused by an atypical down regulation of *cycloidea*-like genes. Further investigation on the expression of *cycloidea*-like genes in *Whytockia* will provide insights into the different patterns of genetic control of floral symmetry in the Gesneriaceae.

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