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Corolla Tube Formation in the Tubiflorae and Gentianales

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Corolla tube formation was investigated anatomically for 22 species of the Polemoniaceae, Convolvulaceae, Boraginaceae, Verbenaceae, Buddlejaceae, Scrophulariaceae, Gentianaceae, Menyanthaceae and Asclepiadaceae.

The corolla tube formation is basically similar among species, except for the cases of *Nymphoides*, *Dichondra* and *Cuscuta*. The petal bases extend laterally to the interprimordial regions, the upward growth occurring at those regions just beside the petal bases, and connect mutually at the back of stamen primordia. The upward growth at the connected regions co-operates with the growth of the expanding petal margins, resulting in the formation of the upper portion of the corolla tube. However, developmental patterns are not always similar. In *Swertia*, *Nymphoides* and *Menyanthes*, the upward growth at the connected regions is meager. In *Dichondra* and *Cuscuta*, the mutual connection of petal bases is not seen.

The lower portion of the corolla tube is formed by the elongation of the common base of petal and stamen primordia, resulting in the formation of the epipetalous condition of stamens, except for the case of *Nymphoides*. In *Nymphoides*, the lower portion of the corolla tube results from the cup-like structure formed on the floral meristem before the initiation of petal primordia.

Key words: Corolla tube formation — Gentianales — Morphology — Tubiflorae.

It has been reported in many studies on the development of the sympetalous flowers that the corolla tube is formed by the zonal growth in many species (cf. Daniel and Sattler, 1978; Nishino, 1978) and by the combination of the zonal growth and the postgenital fusion in *Vinca rosea* (Boke, 1948). However, in the recent studies on the corolla tube formation in the species of the Solanaceae (Daniel and Sattler, 1978; Nishino, 1978), it was shown that the bases of the petal primordia extended laterally and fused or connected mutually, resulting in the formation of the corolla tube which developed into the upper portion of the corolla tube, and the lower portion of the corolla tube was formed by the elongation of the common base of petal and stamen primordia. Although almost all the upper portion of the corolla tube in the six species of the Apocynaceae is formed by the postgenital fusion, the early processes of the corolla tube formation are similar to those in the case of the Solanaceae (Nishino, 1982). However, there is a great deficiency of detailed observations in other groups among the Tubiflorae and Gentianales.

There were few studies on the corolla tube formation in the species of the

Tubiflorae except for the Solanaceae. Miller and Wetmore (1946) reported that in *Phlox Drummondii* the corolla tube was formed by the zonal growth. Singh and Jain (1975) reported that in *Justicia* the upper portion of the corolla tube was formed by the extension and "ontogenetic union" of the bases of the petals, and the lower portion was formed by the zonal growth in the common base of petal and stamen primordia. Nishino (1976) reported that the corolla tube in *Pharbitis* was formed by the co-operation of the growth at the interprimordial regions and the marginal growth of the petal primordia. Organogenetic studies reported that the petal bases extended laterally to the interprimordial regions, resulting in the formation of the corolla tube in *Calystegia* and *Lanthana* (Sattler, 1973), *Antirrhinum* (Singh and Jain, 1979) and *Digitalis* (Singh, 1979).

There were also few studies on the corolla tube formation in the species of the Gentianales, except for the Apocynaceae. McCoy (1940) reported that in *Frasera* the common petal-stamen zone developed *en masse*, forming a short tube. McCoy also described for the calyx that the cell divisions at the base of the sepal was extended, and a short calyx tube was formed. Robyns (1955) reported that in *Centaurium* the corolla tube arose from the beginning as a circular base bearing the primordia of the lobes, and stamens and the corolla tube arose also as a common structure. Sattler (1973) described in an organogenetic study that in *Asclepias* the interprimordial growth between the petal primordia led to the formation of a corolla tube.

This report presents a comparative study on the corolla tube formation in some species of the Polemoniaceae, Convolvulaceae, Boraginaceae, Verbenaceae, Buddlejaceae, Scrophulariaceae, Gentianaceae, Menyanthaceae and Asclepiadaceae. Particular emphasis is placed on the early stage of the tube formation at the interprimordial regions of petal primordia.

Materials and Methods

In this study, the following species were used. The species of the Tubiflorae are *Phlox paniculata* L. of the Polemoniaceae; *Dichondra repens* Forst. and *Cuscuta australis* R. Brown of the Convolvulaceae; *Symphytum officinale* L., *Trigonotis peduncularis* Benth. and *Ehretia ovalifolia* Hasskarl of the Boraginaceae; *Verbena tenera* Spreng. and *Clerodendron trichotomum* Thunb. of the Verbenaceae; *Buddleja Lindleyana* Fortune and *B. Davidii* Franch. of the Buddlejaceae; *Verbascum thapsus* and *Veronicastrum sibiricum* Pennell var. *japonicum* Hara of the Scrophulariaceae.

The species of the Gentianales are *Swertia bimaculata* Hook. fil. et Thoms., *S. japonica* Makino, *Gentiana scabra* Bunge var. *Buergeri* Maxim. and *Tripterospermum japonicum* Maxim. of the Gentianaceae; *Nymphoides indica* O. Kuntze and *Menyanthes trifoliata* L. of the Menyanthaceae; *Cynanchum caudatum* Maxim., *C. purpurascens* Morren et Decaisne, *C. atratum* Bunge and *Stephanotis floribunda* Brongn. of the Asclepiadaceae.

Materials of *Phlox* were collected from cultivated plants in Chiba Pref.; those of *Symphytum, Verbena* and *Clerodendron* from cultivated plants on the campus of the

University of Tokyo, Komaba, Meguro-ku, Tokyo; those of *Trigonotis* from wild plants on the same campus; those of *Ehretia,* two species of *Buddleja, Verbascum, Veronicastrum, Cynanchum purpurascens* and *C. atratum* at the Botanical Gardens, the University of Tokyo, Bunkyo-ku, Tokyo; those of *Swertia bimaculata, Gentiana* and *Menyanthes* were collected at the Nikko Branch, the Botanical Gardens, the University of Tokyo; those of *Cynanchum caudatum* at Togakushi, Nagano Pref.; those of *Tripterospermum* at Mt. Daisetsu, Hokkaido; those of *Nymphoides* at the Swamp Inba-numa, Chiba Pref.; and those of *Stephanotis* from the cultivated plants at Chiba University.

The materials of *Dichondra* were kindly collected at Shuri, Okinawa, fixed and sent to me by Dr. K. Shimabuku, University of the Ryukyus. The fixed materials of *Cuscuta,* collected at Hibiya, Chiyoda-ku, Tokyo, and those of *Swertia japonica,* collected at Karuizawa, Nagano Pref., were offered to me by Dr. Y. Fukuda, Chiba University.

Materials fixed in formalin/acetic acid/alcohol (FAA) were dehydrated by the tertiary-butyl alcohol method and embedded in paraffin. Serial transections and longisections were cut at 8 or 10 μm and stained with hematoxylin, safranin and fast green. Measurement of the height of a given primordium was made by multiplying the number of serial transections by their thickness.

Results

Part I. Tubiflorae

Gross morphology of the corolla

The corolla of each species is sympetalous and composed of a tube and five or four lobes, having five, four or two epipetalous stamens on the tube in positions alternating with the corolla lobes. Most of the corollas are actinomorphic. One of the petals of a corolla is shown diagrammatically in Fig. 1 for six species. The corolla tube is divided into two parts, the upper (UCT) and lower (LCT) defined by the boundary at the level of the stamen insertion.

Phlox paniculata (Fig. 1A). The corolla is salverform, and has five lobes and a long and slender tube. The aestivation of the corolla lobes is contorted, different from that in other tubiflorae-species studied. Five epipetalous stamens are inserted at the upper part of the corolla tube.

Dichondra repens (Fig. 1B). The corolla is rotate, and has five lobes and a short tube. Five epipetalous stamens are inserted at the sinuses of the corolla lobes; that is the corolla lobes and stamens stand alternately on the corolla tube. So, in this species the corolla tube is considered as only the common structure of petals and stamens.

Cuscuta australis. The corolla is urceolate, and has a relatively long tube. The structure of the corolla is basically similar to that of *Dichondra.*

Symphytum officinale (Fig. 1C). The corolla is urceolate, and has five lobes and a relatively long tube. Five epipetalous stamens are inserted at the middle part of the corolla tube. There are five projections at the middle part of the corolla tube in

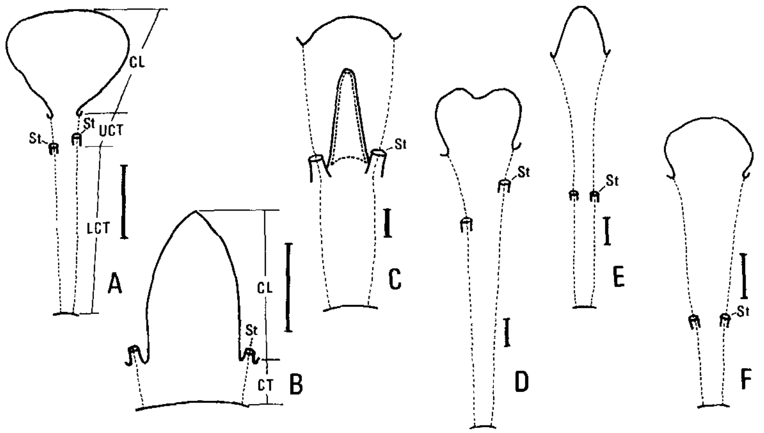

Fig. 1. Diagrams showing one of the petals of a mature corolla. A: *Phlox paniculata.* B: *Dichondra repens.* C: *Symphytum officinale.* D: *Verbena tenera.* E: *Buddleja Lindleyana.* F: *Veronicastrum sibiricum* var. *japonicum.* CL, corolla lobe; CT, corolla tube; UCT, upper portion of the corolla tube; LCT, lower portion of the corolla tube; St, stamen base. UCT and LCT are demarcated by the level of stamen insertion. Lines are 1 cm in A, 0.5 mm in B, 1 mm in C–F.

positions opposite to the corolla lobes. These projections are the folds of this portion of the corolla tube and are curved inside, as reported by Lawrence (1937) for some species of the Boraginaceae including *Symphytum.* The structure of the corolla of *Trigonotis peduncularis* resembles that of *Symphytum.*

Ehretia ovalifolia. The corolla is rotate, and has five lobes and a relatively short tube. Five epipetalous stamens are inserted at the basal part of the corolla tube.

Verbena tenera (Fig. 1D). The corolla is nearly actinomorphic and salverform, and has five lobes and a long and slender tube. The upper portion of the corolla tube is slightly curved. Four epipetalous stamens are inserted at the upper part of the corolla tube. One stamen in an adaxial side of the flower is lacking from the pentamerous plan. The structure of the corolla of *Clerodendron trichotomum* is basically similar to that of *Verbena.*

Buddleja Lindleyana (Fig. 1E). The corolla is nearly actinomorphic and salverform, and has four lobes and a long, slender tube. Four epipetalous stamens are inserted at the relatively lower part of the corolla tube. The structure of the corolla of *Buddleja Davidii* closely resembles that of *B. Lindleyana.*

Verbascum thapsus. The corolla is rotate, and has five lobes and a relatively short tube. Five epipetalous stamens are inserted at the lower part of the corolla tube.

Veronicastrum sibiricum var. *japonicum* (Fig. 1F). The corolla is tubular, and has four lobes and a long tube. Two epipetalous stamens are postero-lateral in positions and are inserted at the lower part of the corolla tube.

Development of the corolla tube

Phlox paniculata. The initiation of petal primordia is later than that of stamen

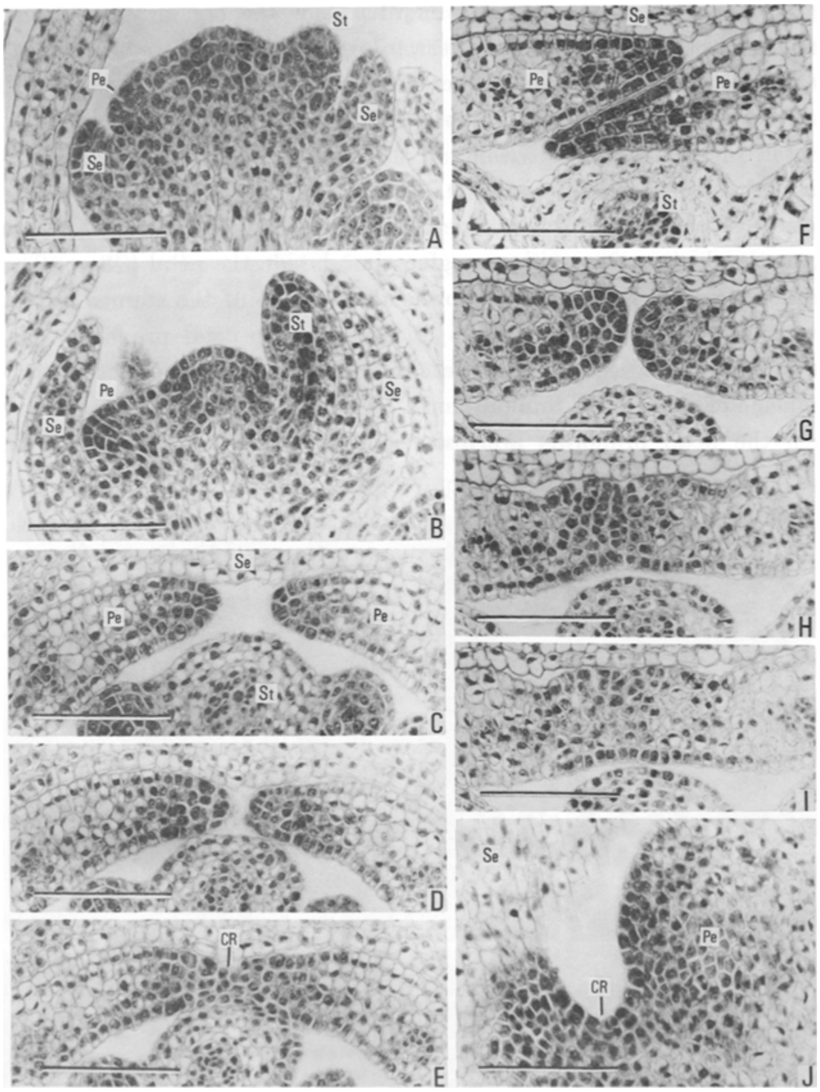

Fig. 2. Development of the corolla in *Phlox paniculata*. A, B: Median longisections of petal and stamen primordia at the stages of petal initiation (A) and of the early development of petal primordia (B) respectively. C–E: Transections of a corolla of about 260 μm in height, showing the lobe margins (C, D) and the connected region (E); C–E, successively lower levels. F–I: Transections of a corolla of about 1 mm in height, showing the lobe margins (F, G), the connected region (H) and the portion just under the connected region (I); F–I, successively lower levels. J: Tangential section through the connected region of petal primordia of about 370 μm in height. CR, connected region; Pe, petal; Se, sepal; St, stamen. Lines are 100 μm.

primordia (Fig. 2A). When the stamen primordia are about 20 μm in height, petal primordia are initiated simultaneously. The initiation of the petal primordia begins with the periclinal divisions of the subsurface cells at the peripheral regions of the floral meristem in positions alternating with the stamen primordia. The initiation and the early apical growth of the petal primordia show the organization of surface and subsurface initials (Fig. 2A, B), but afterwards periclinal divisions of surface cells are occasionally seen in the petal apical meristem. The marginal meristems of the petal primordia also show the organization of surface and subsurface initials in the early stage. Later, many periclinal divisions of the surface cells are seen, especially, at the lower parts of the margins (Fig. 2C, D, F, G).

The bases of petal primordia enlarge laterally to the interprimoridal regions by the upward growth which occurs at those regions just beside the petal bases. The bases of petal primordia extend further to the lower abaxial side of the stamen primordia and connect with them at first. The bases of the adjacent petal primordia continue to extend laterally until they connect with each other at the median of the lower portion of the abaxial side of the stamen primordium. This connection occurs at a relatively later stage when the petal primordia are about 80 μm in height. The region where the

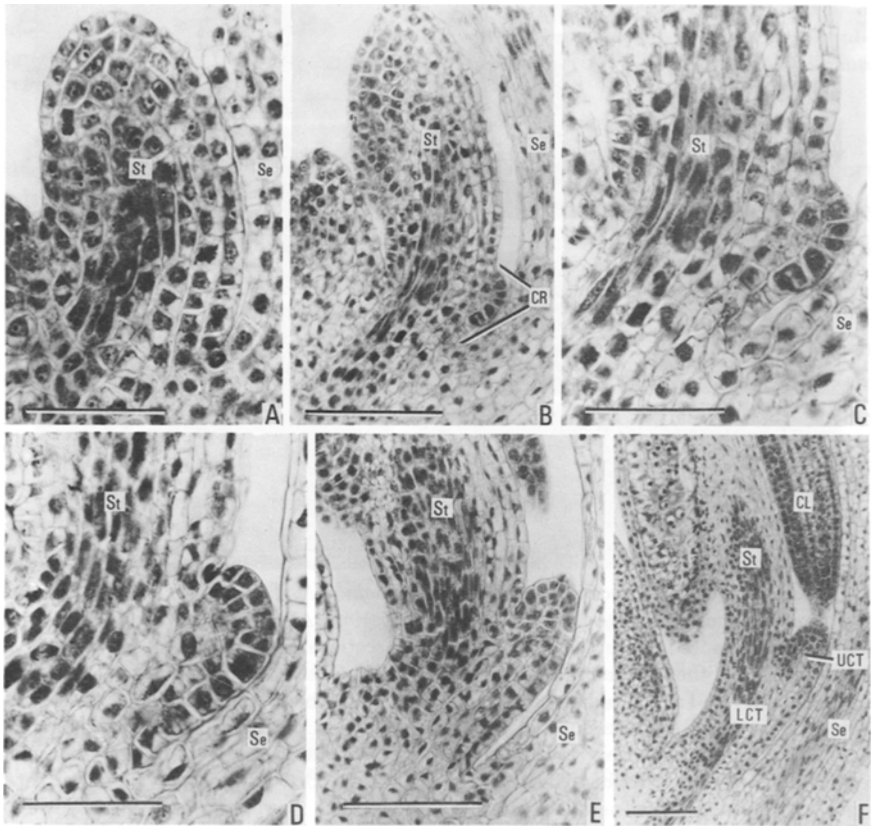

Fig. 3. Development of the corolla in *Phlox paniculata*. A–F: Median longisections of the stamen primordia or radial sections of the connected regions of petal primordia. They are at the stages of petal initiation (A) and of petal primordia of about 100 μm, (B, C), 180 μm (D), 370 μm (E) and 1 mm (F) in height respectively. CL, corolla lobe; UCT, upper portion of the corolla tube; LCT, lower portion of the corolla tube; CR, connected region; Se, sepal; St, stamen. Lines are 50 μm in A, C, D, 100 μm in B, E, F.

mutual connection of petal bases occurs is called the "connected region" as in the cases described in previous studies by Nishino (1978, 1982).

After the connection of the petal bases, the upward growth occurs also at the connected regions (Fig. 3B). This upward growth is easily ascertained in the radial sections through the stamen primordium, as the development of a protuberance at the lower portion of the abaxial side of the stamen primordium (Fig. 3B–F). The connection of the petal bases occurs below the level of the adaxial bases of the stamen primordia (Fig. 3B). So, the extension and connection of petal bases appear in transections as an abaxial thickening of the lower portions of the stamen primordia (Fig. 2C–E). The upward growth at the connected regions three-dimensionally results in the formation of a short corolla tube, and the height of the protuberances indicates that of the corolla tube (Figs. 2E, H, I and 3B–F). Such a corolla tube develops into the upper portion of the corolla tube.

Before the connection of adjacent petal primordia, the cells near the surface of the abaxial side of the stamen primordia are vacuolate (Fig. 3A). However, with the beginning of the upward growth, the cells at the connected region become more meristematic (Fig. 3B, D). A tangential view through a connected region and adjacent lower margins of the corolla lobes reveals that the marginal meristems are continuous through the meristem of the connected region (Fig. 2J). In the early stages of the development of the upper portion of the corolla tube, the connected region grows upwardly by means of the organization of surface and subsurface initials (Fig. 3B, C). But, in the later stages many periclinal divisions occur in the surface cells at this region (Fig. 3D, E).

The connected regions are situated in a lower level than the adaxial bases of the stamen primordia in the early stages (Fig. 3B). The upper ends of the connected regions are raised to a higher level than the adaxial bases of the stamen primordia during the elongation of the common base of petal and stamen primordia (Fig. 3E). This common base elongates extremely in the later stages, resulting in the formation of the long corolla tube (Figs. 1A and 3F).

Dichondra repens. Five petal primordia are initiated simultaneously, and almost at the same time five stamen primordia are initiated. However, the early development of the petal primordia is very slow as compared with that of the stamen primordia. The petal primordia extend their bases laterally to the abaxial side of the bases of the stamen primordia (Fig. 4A, B). By this extension, petal primordia connect with the stamen primordia resulting in the formation of a ring-like common base, on which the petal and stamen primordia stand alternately (Fig. 4C–E). Serial longisections through the stamen primordium indicate that a small interprimordial region between adjacent petal primordia is left during the development of the corolla, and no upward growth occurs at the median of the lower abaxial side of the stamen primordium (Fig. 4F–H). On this point, it is regarded that no mutual connection of the petal primordia occurs. The common base of petal and stamen primordia elongates and develops into the whole of the corolla tube (Figs. 1B and 4B, C–E).

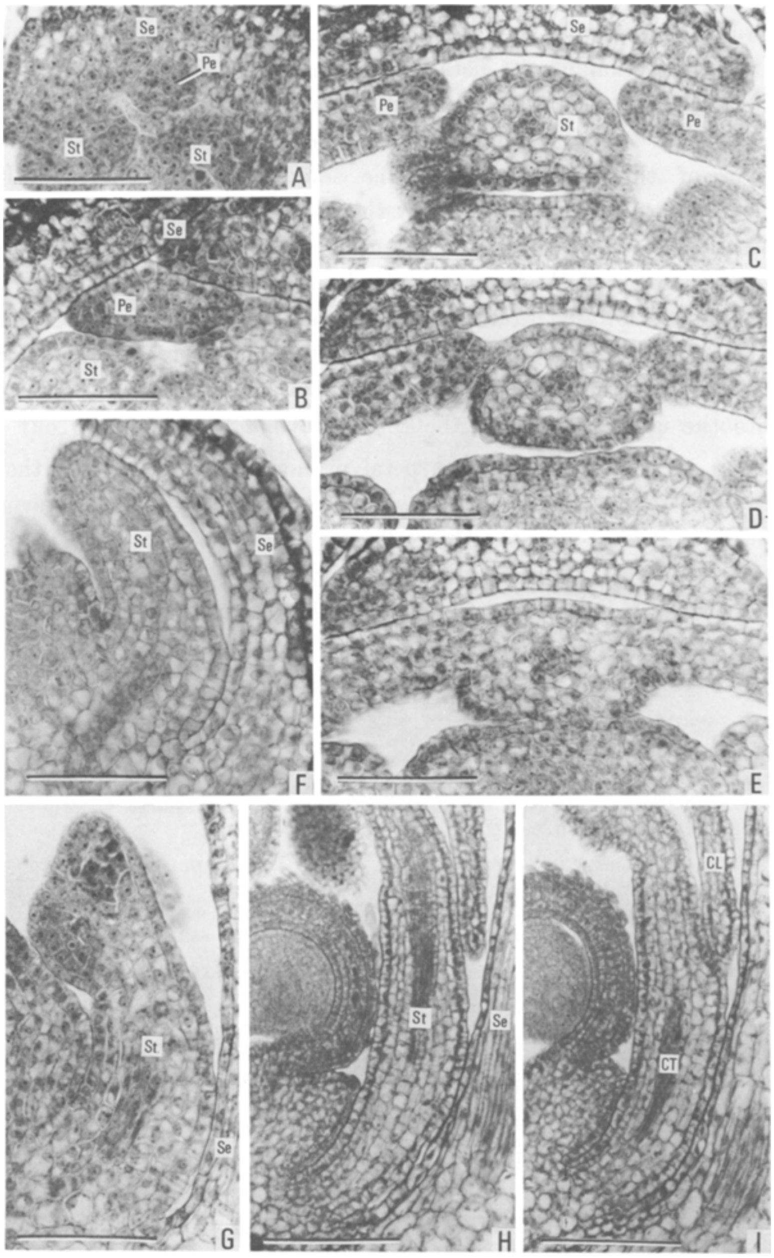

Fig. 4. Development of the corolla in *Dichondra repens*. A, B: Transections of the basal portions of petal primordia of about 16 μm (A), 50 μm (B) in height respetively. C–E: Serial transections of a corolla of about 70 μm in height; C–E, successively lower levels. F, G: Median longisections of the stamen primordia at the stages of the petal primordia of about 50 μm (F) and 110 μm (G) in height respectively. H, I: Longisections of a stamen primordium at the stage of the petal primordium of about 1 mm in height. CL, corolla lobe; CT, corolla tube; Pe, petal; Se, sepal; St, stamen. Lines are 50 μm in A–G, 100 μm in H, I.

Cuscuta australis. The development of the petal and the formation of the corolla tube resemble those of *Dichondra*.

Petal and stamen primordia are initiated at almost the same time. The development of the stamen primordia is faster than that of petal primordia. The petal primordia show no mutual connection at the back of the stamen primordia. The corolla tube is formed only by the elongation of the common base of petal and stamen primordia, resulting in the formation of the epipetalous condition, that the stamens are inserted at the sinuses of the corolla lobes.

Symphytum officinale. Petal and stamen primordia are initiated at almost the same time. The bases of petal primordia extend laterally and connect with each other in a relatively early stage of the corolla development. By this connection, the upper portion of the corolla tube is initiated. After the connection of the bases of petal primordia, the upward growth begins at the connected regions by means of the organization of surface and subsurface initials. However, this organization soon changes to that of only the surface initials without subsurface initials (Fig. 5A), and the same change occurs in the lower marginal meristems of the corolla lobes. The adaxial base of the connected region, which is initially situated on a lower level than the adaxial base of the stamen primordium, is raised to a higher level than that during the elongation of the lower portion of the corolla tube.

The development of five inner projections in a corolla tube begins with the adaxial

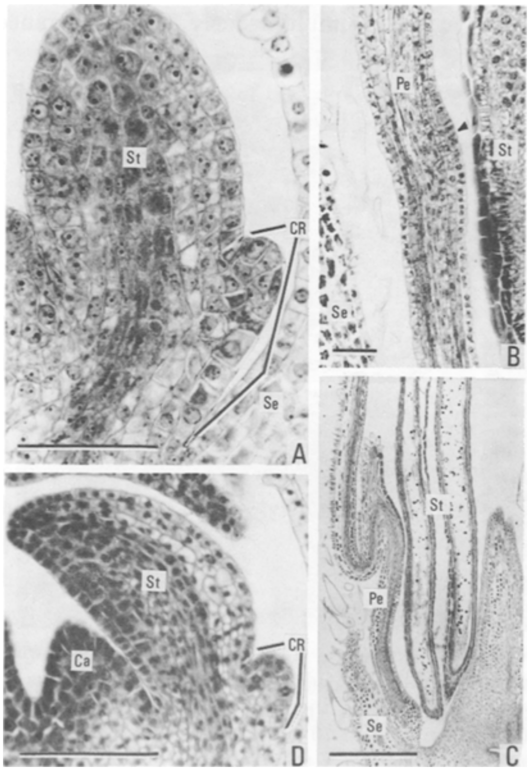

Fig. 5. Development of the corolla in *Symphytum officinale* (A–C) and *Ehretia ovalifolia* (D). A: Radial section of the connected region of petal bases at the stage of the petal primordia of about 65 μm in height. B, C: Longisections of the corollas at the stages of about 1.5 mm (B) and 3.5 mm (C) in height respectively, showing the adaxial growth (arrowhead) and the inner projection. D: Radial section of the connected region of petal bases at the stage of the petal primordia of about 300 μm in height. Ca, carpel; CR, connected region; Pe, petal; Se, sepal; St, stamen. Lines are 50 μm in A, 100 μm in B, D, 0.5 mm in C.

growth of the tube at a very late stage of the corolla development (Fig. 5B). With the protrusion by the adaxial growth, this part of the tube wall itself falls into the inside by the differential growth through the inner and outer sides of the tube, resulting in the formation of the sac-like projection (Fig. 5C). This projection is passed through by the extremely-curved median vein of the petal.

Trigonotis peduncularis. The development of the corolla tube resembles that of *Symphytum.* The petal bases connect mutually at a relatively early stage of the corolla development. In the marginal growth of the corolla lobes and the upward growth at the connected regions, periclinial divisions of the surface cells are seen occasionally. The connected regions are at first situated almost at the same level with that of the adaxial bases of the stamen primordia, and are raised to a higher level than that in the later stage. The development of five inner folds at the throat of the corolla begins with the adaxial growth of the tube at a very late stage of the corolla development.

Ehretia ovalifolia. The early development of the corolla tube resembles that of *Symphytum.* The mutual connection of petal bases is followed by the upward growth at the connected regions. This upward growth shows the organization of surface and subsurface initials in the early stage. Soon, many periclinal divisions occur in the surface cells (Fig. 5D) as well as such divisions at the lower margins of the corolla lobes. The elongation of the common base of petal and stamen primordia is very slow, resulting in the formation of a short lower portion of the corolla tube.

Verbena tenera. Five petal primordia are initiated simultaneously as protuberances,

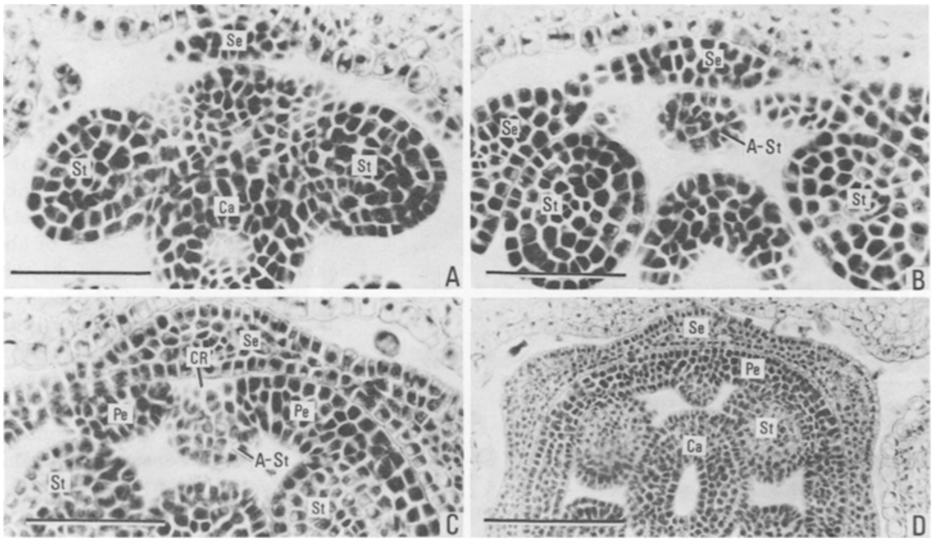

Fig. 6. Development of the corolla in *Verbena tenera.* A–D: Transections of young flowers showing the development of adaxial stamens (A-St). The adaxial side of the flowers as to the inflorescence axis are oriented upward. They are at the stages of petal initiation (A) and of petal primordia of about 24 μm (B), 40 μm (C) and 70 μm (D) in height respectively. Refer to Fig. 5 for abbreviations. Lines are 50 μm in A–C, 100 μm in D.

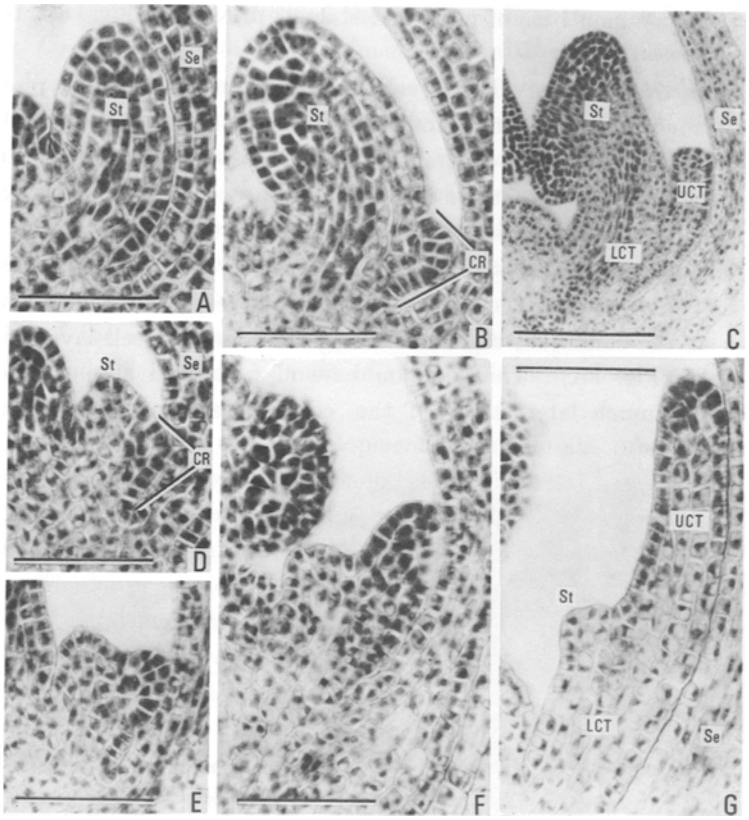

Fig. 7. Development of the corolla in *Verbena tenera*. A–G: Median longisections of stamen primordia showing the development of the connected regions of petal primordia. They are at the stages of petal initiation (A) and of petal primordia of about 40 μm (B), 180 μm (C), 40 μm (D), 70 μm (E), 130 μm (F) and 260 μm (G) in height respectively. The stamens in D–G are the adaxial ones. Refer to Fig. 3 for abbreviations. Lines are 50 μm in A, B, D–G, 100 μm in C.

later than the initiation of five stamen primordia, in which an adaxial stamen is initiated slightly later than the others (Figs. 6A, B and 7A). The development of the adaxial stamen is arrested in very early stages, and the cells of the primordium soon become quite vacuolate (Figs. 6B–D and 7D, E). The bases of petal primordia extend laterally and connect mutually at a relatively early stage of the corolla development. By this connection, the upper portion of the corolla tube is initiated. After the connection, the upward growth begins with the periclinal divisions of the subsurface cells of the lower abaxial side of the stamen primordia. Soon after, active periclinal divisions of the surface cells occur in the upward growth (Fig. 7B, D, E) as well as those in the lower margins of the corolla lobes. The upward growth at the connected region is very active, also at the abaxial side of the abortive adaxial stamen (Fig. 7C, F). During the elongation of the upper portion of the corolla tube, the rudiment of the adaxial stamen comes to be a small projection on the corolla tube (Fig. 7D–G) and finally is indistinguishable. The lower portion of the corolla tube is formed by the elongation of the common base of petal and stamen primordia (Figs. 6C, D and 7B, C, G).

Clerodendron trichotomum. The development of the corolla tube resembles that of *Verbena*. Four stamen primordia are distinguished as being initiated as projections, preceding the appearance of five petal primordia. After the extension and mutual connection of petal bases, the active upward growth occurs at the connected regions (Fig. 8A). This upward growth shows the organization of surface initials as well as that in the marginal growth of the lower margins of the corolla lobes in the corresponding stages. In the early stage of the upward growth, active periclinal divisions of surface cells are seen at the connected regions, making three or four cell layers derived from the surface cells (Fig. 8A). The common base of petal and stamen primordia also elongates at very much later stages of the corolla development, resulting in the

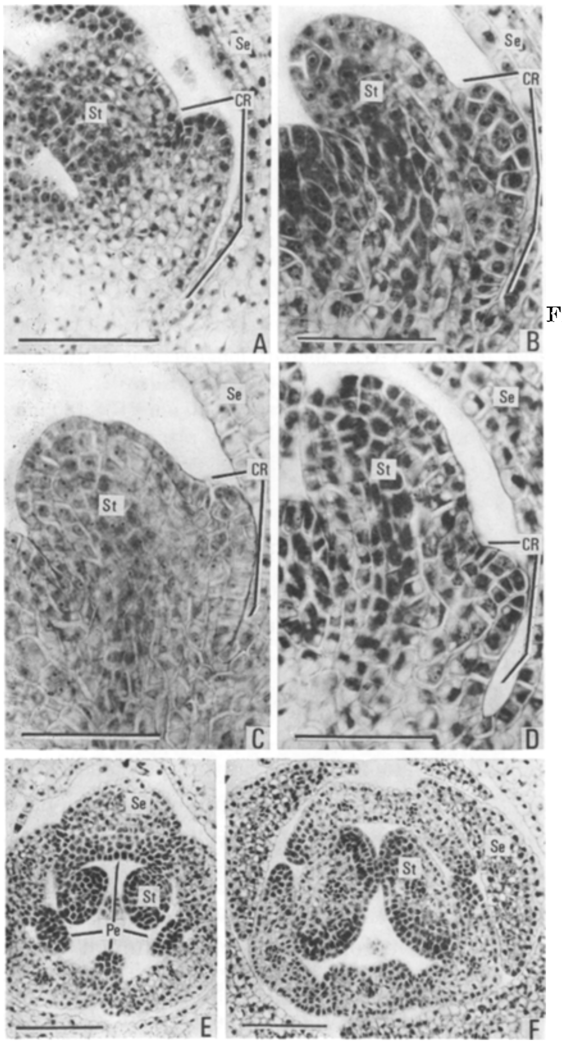

Fig. 8. Development of the corolla in *Clerodendron trichotomum* (A), *Buddleja Lindleyana* (B), *Verbascum thapsus* (C) and *Veronicastrum sibiricum* var. *japonicum* (D–F). A–D: Radial sections of the connected regions of petal primordia at the stages of about 200 μm (A), 75 μm (B), 50 μm (C) and 90 μm (D) in height respectively. E, F: Transections of young flowers, in which the corlolas are at the stages of about 50 μm (E) and 260 μm (F) in height respectively. Transections are oriented with the adaxial side for the inflorescence axis upward. Refer to Fig. 5 for abbreviations. Lines are 50 μm in A–D, 100 μm in E, F.

formation of the long lower portion of the corolla tube.

Buddleja Lindleyana. Petal and stamen primordia are initiated at almost the same time. Periclinal divisions of the surface cells occur occasionally in the apical growth of the petal primordium. Following the lateral extension of the bases of the petal primordia, bases connect mutually at a very early stage of the corolla development, resulting in the initiation of the upper portion of the corolla tube. The subsequent upward growth at the connected regions shows the organization of surface initials, periclinal divisions of which occur very actively there (Fig. 8B), as well as in the marginal growth of the lobes. The lower portion of the corolla tube is formed by the elongation of the common base of petal and stamen primordia.

Buddleja Davidii. The development of the corolla tube closely resembles that of the species mentioned just above.

Verbascum thapsus. The early development of the corolla tube resembles that of *Clerodendron* of the Verbenaceae. The connection of the petal bases occurs at an early stage of the corolla development. The upward growth at the connected regions shows the organization of surface initials (Fig. 8C) as well as the marginal growth of the corolla lobes. The lower portion of the corolla tube is formed by the elongation of the common base of petal and stamen primordia.

Veronicastrum sibiricum var. *japonicum.* The development of the corolla tube resembles that of *Buddleja Lindleyana.* Petal and stamen primordia are initiated at almost the same time. Petal primordia are initiated as four projections, in which a primordium of the adaxial side has a wide base from the beginning (Fig. 8E). Also, the primordium of the abaxial side is small. Two stamen primordia are initiated at the sites alternate with the adaxial petal primordium in position. The bases of petal primordia extend laterally and connect mutually at a relatively early stage. The upward growth at the connected regions shows the organization of surface and subsurface initials in the early stages, and that of surface initials in later stages (Fig. 8D). Thus, the upper portion of the corolla tube is initiated by the connection of the petal bases. In the petal primordium of the adaxial side, two procambia differentiate, while in each of other three primordia one procambium differentiates (Fig. 8F). These procambia develop into the two major veins of the adaxial petal and midveins of other petals of the corolla that is supplied by five traces. The lower portion of the corolla tube is formed by the elongation of the common base of petal and stamen primordia.

Part II. Gentianales

Gross morphology of the corolla

The corolla of each species is actinomorphic and is composed of five lobes and a tube, having five epipetalous stamens on the tube in positions alternating with the corolla lobes. One of the petals of a corolla is shown diagrammatically in Fig. 9 for 5 species. The corolla tube is divided into two parts, the upper (UCT) and lower (LCT) defined by the boundary at the level of the stamen insertion.

Swertia bimaculata (Fig. 9A). The corolla is rotate, and has a short tube. The

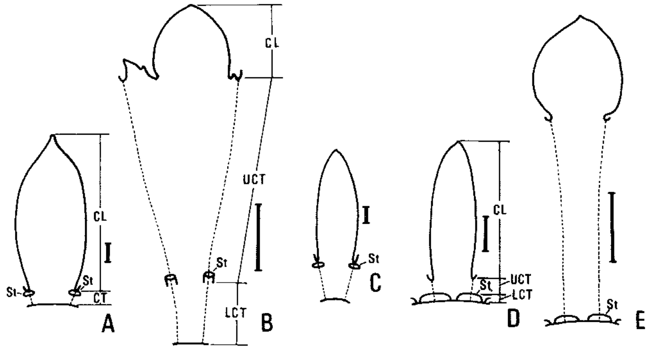

Fig. 9. Diagrams showing one of the petals of a mature corolla. A: *Swertia bimaculata*. B: *Gentiana scabra* var. *Buergeri*. C: *Nymphoides indica*. D: *Cynanchum caudatum*. E: *Stephanotis floribunda*. UCT and LCT are demarcated by the level of stamen insertion. Refer to Fig. 1 for abbreviations. Lines are 1 mm in A, C, D, 1 cm in B, E.

epipetalous stamens are inserted at the sinuses of the corolla lobes; that is the corolla lobes and the stamens stand alternately on the corolla tube. So, in this species the corolla tube is considered as only the common structure of petals and stamens. The structure of the corolla of *Swertia japonica* closely resembles that of *Swertia bimaculata*.

Gentiana scabra var. *Buergeri* (Fig. 9B). The corolla is funnelform, and has a long tube. There are small lobe-like projections on either sides of the prominent corolla lobe. These projections are folded inside in the plicated aestivation of the flower bud. Epipetalous stamens are inserted at the lower part of the corolla tube. The structure of the corolla of *Tripterospermum japonicum* closely resembles that of *Gentiana*.

Nymphoides indica (Fig. 9C). The corolla is rotate, and has a short tube. Epipetalous stamens are inserted almost at the top of the corolla tube. The structure of the corolla of *Menyanthes trifoliata* resembles that of *Nymphoides*.

Cynanchum caudatum (Fig. 9D). The corolla is rotate, and has a short tube. Epipetalous stamens are inserted at the basal part of the corolla tube. The structure of the corolla in *Cynanchum purpurascens* and *C. atratum* closely resembles that of *C. caudatum*.

Stephanotis floribunda (Fig. 9E). The corolla is salverform, and has a long tube. Epipetalous stamens are inserted at the basal part of the corolla tube.

Development of the corolla tube

Swertia bimaculata. Five petal primordia are initiated simultaneously, and slightly later or almost at the same time five stamen primordia are initiated. The entirely meristematic petal primordia broaden their bases laterally and connect mutually at the lower abaxial side of the stamen primordia. This connection occurs at a very early stage, and the following upward growth at the connected regions begins with the periclinal divisions of subsurface cells when the petal primordia are about 20 μm in height (Fig. 10A). The connected regions are situated on a lower level than the adaxial bases of the stamen primordia in the early stage of the corolla development

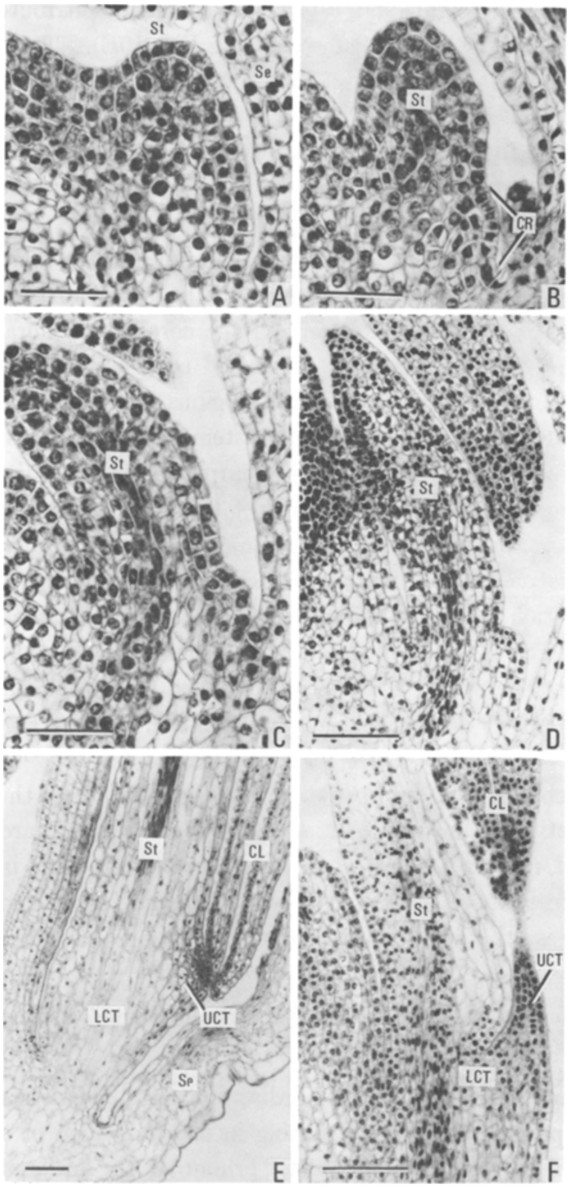

Fig. 10. Development of corolla in *Swertia bimaculata* (A–E) and *Gentiana scabra* var. *Buergeri*(F). A–F: Median longisections of stamen primordia showing the development of connected regions of petal primordia. They are at the stages of petal primordia of about 20 μm (A), 100 μm (B), 200 μm (C), 800 μm (D), 4 mm (E) and 1.6 mm (F) in height respectively. Refer to Fig. 3 for abbreviations. Lines are 50 μm in A–C, 100 μm in D–F.

(Fig. 10B). The upper ends of the connected regions are raised to a higher level than the adaxial bases of the stamen primordia during the elongation of the common base of petal and stamen primordia (Fig. 10C–E). The meristems of the connected regions and the marginal meristems of the corolla lobes are continuous and show the organization of surface and subsurface initials. The upward growth is so slow that the protuberances at the connected regions in radial sections are no more than small swells even when the corolla is about 200 μm in height (Fig. 10C). Afterwards the meristems of the connected regions rapidly become vacuolate (Fig. 10D). The protuberances at the connected regions are meager in height, and afterwards scarcely contribute to the development of the upper portion of the corolla tube (UCT in Fig. 10E). Thus, almost all of the corolla tube is formed by the elongation of the common base of the petal and stamen primordia (LCT in Fig. 10E).

Swertia japonica. The corolla tube formation is similar to that of the species mentioned just above. However, in *Swertia japonica,* the upward growth at the connected regions is active to some extent.

Gentiana scabra var. *Buergeri.* Petal primordia are initiated simultaneously and, following the lateral extension of their bases, connect mutually at the lower abaxial side of the stamen primordia. During the early development of the corolla, the floral meristem increases its volume, and the stamen primordia are raised to a higher level than the petal primordia. The upward growth at the connected regions is slow during the early stages of the corolla development. The tips of the meristems for the upward growth at the connected regions gradually become extremely sharp-pointed in radial sections (Fig. 10F), compared with those of the lower margins of the corolla lobes in transections. Both the upward growth at the connected region and the marginal growth of the corolla lobes are very active, and show the organization of surface and subsurface initials. During the elongation of the common base of petal and stamen primordia, the upper ends of the connected regions are raised to a higher level than the adaxial bases of the stamen primordia. However, this elongation is so slow that the lower portion of the corolla tube is short even when the corolla is 1.6mm in height (Fig. 10F).

The lower margins of the corolla lobes, which are extending their width to form the contorted aestivation, change the direction of growth gradually from lateral to inward and finally clockwise. The connected regions also grow in a clockwise direction in association with the change in the direction of growth of lobe margins. The folded portions in the margins grow actively, resulting in the formation of projections on either side of each prominent lobe.

Tripterospermum japonicum. The development of the corolla tube closely resembles that of *Gentiana* described above.

Nymphoides indica. After the initiation of sepal primordia, the floral meristem increases its height, especially, at the peripheral region, resulting in the formation of a cup-like structure surrounded by the calyx (Fig. 11A, B). Petal primordia are initiated on this cup-like structure (Fig. 11C). Slightly later, stamen primordia are initiated somewhat inside of the cup-like structure, which consequently becomes the common base of petal and stamen primordia (Fig. 11C). The early growth of the petal primordia is not distinct, because the petal growth appears to be the prolongation of the cup formation of the floral meristem (Fig. 11C, D). The bases of petal primordia extend laterally on the cup-like structure and connect mutually at a relatively early stage of the corolla development (Fig. 11C). The marginal meristems of petal primordia are recognizable at the stage of this connection. By the upward growth at the connected regions of petal primordia (Fig. 11D, E), a short tube develops in addition to the cup-

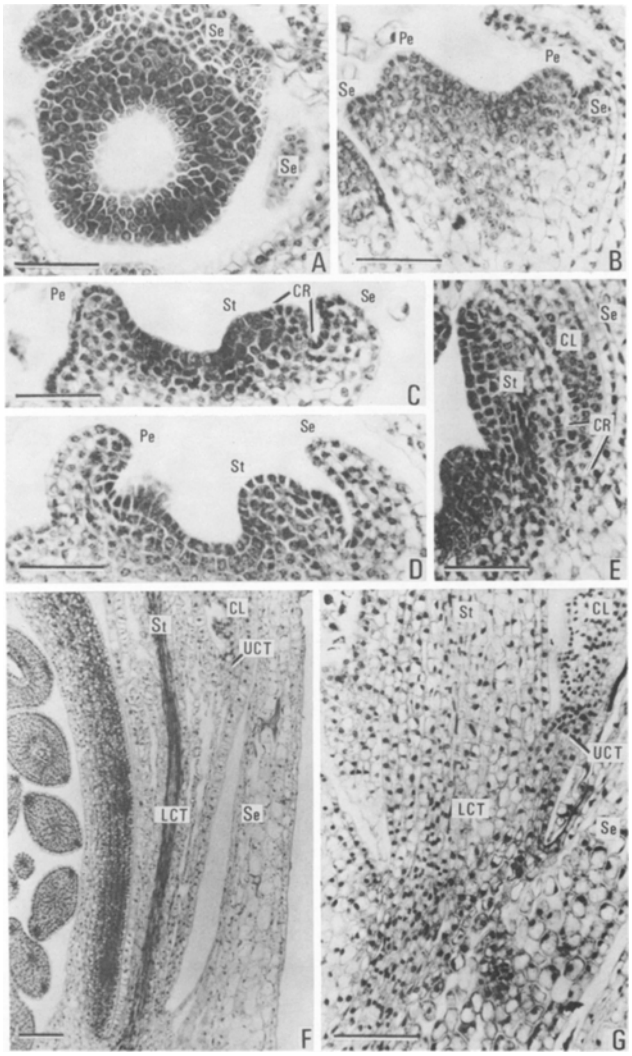

Fig. 11. Development of the corolla in *Nymphoides indica* (A–F) and *Menyanthes trifoliata* (G). A: Transection of a young flower at the stage of petal initiation. B–G: Median longisections of petal and stamen primordia showing the development of petals and connected regions of petal bases. They are at the stages of petal initiation (B), and of petal primordia of about 30 μm (C), 50 μm (D), 110 μm (E), 3 mm (F) and 1.3 mm (G) in height respectively. Pe, petal. Refer to Fig. 3 for other abbreviations. Lines are 50 μm in A–E, 100 μm in F, G.

like structure. However, this upward growth is very slow and afterwards scarcely contributes to the development of the upper portion of the corolla tube (UCT in Fig. 11F). Thus, almost all the corolla tube is formed by the elongation of the cup-like structure which is formed before the initiation of the petal primordia (LCT in Fig. 11F).

Menyanthes trifoliata. Rough observation indicates a development of the corolla tube similar to that of *Nymphoides*. The upward growth at the connected region also scarcely contributes to the corolla tube development (Fig. 11G).

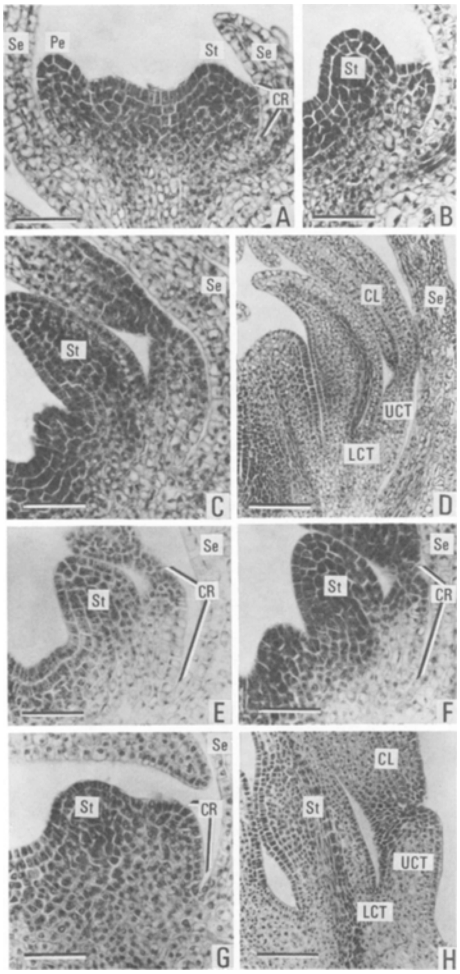

Fig. 12. Development of the corolla in *Cynanchum caudatum* (A–D), *C. purpurascence* (E), *C. atratum* (F) and *Stephanotis floribunda* (G, H). A–H: Radial sections of the connected regions of petal primordia at the stages of about 20 μm (A), 60 μm (B), 150 μm (C), 400 μm (D), 130 μm (E), 150 μm (F), 70 μm (G) and 600 μm (H) in height respectively. Pe, petal. Refer to Fig. 3 for other abbreviations. Lines are 50 μm in A–C, E–G, 100 μm in D,H.

Cynanchum caudatum. Petal and stamen primordia are initiated simultaneously. The mutual connection of the extended petal bases occurs at a very early stage of the corolla development. When the petal primordia are about 10 μm in height, periclinal divisions of the subsurface cells for the beginning of the upward growth are seen at the connected regions of the petal primordia. The upward growth at the connected regions is active (Fig. 12A–D) and shows the organization of surface and subsurface initials. The corolla tube formed by the connection of the petal bases develops into the upper portion of the corolla tube (Fig. 12D). The common base of petal and stamen primordia does not elongate actively (Fig. 12D), resulting in the formation of the short lower portion of the corolla tube at the mature stage.

Cynanchum purpurascens and *C. atratum.* The development of the corolla tubes in these species closely resembles that of the species mentioned just above. The upward growth at the connected regions of the petal bases is also active (Fig. 12E, F), resulting in the formation of the upper portion of the corolla tube.

Stephanotis floribunda. The early development of the corolla tube closely resembles that of *Cynanchum.* The upward growth at the connected regions of petal bases is also active (Fig. 12G, H), resulting in the formation of the upper portion of the corolla tube. This portion of the tube elongates extremely in late stages of the corolla development.

Discussion

The developmental patterns of the initiation and the early growth of petal primordia coincide with those observed in other studies on the development of the corolla in the species of the Tubiflorae by Satina and Blakeslee (1941), Miller and Wetmore (1946), Hicks (1973), Singh and Jain (1975) and Nishino (1976, 1978) and Gentianales by McCoy (1940), Boke (1948) and Nishino (1982). The petal primordia are initiated later than the stamen primordia in *Phlox paniculata*. Such development well coincides with that reported by Miller and Wetmore (1946) in the study on *Phlox Drummondii*, in which petal primordia are described as appearing after the initiation of carpel primordia.

The corolla tube formation in the species studied in this report is basically similar to the cases observed in the Solanaceae by Daniel and Sattler (1978) and Nishino (1978), except for the cases of *Nymphoides*, *Dichondra* and *Cuscuta*. The upper portion of the corolla tube is formed by the extension and the mutual connection of the petal bases. The upward growth at the connected regions co-operates with the growth of the expanding petal margins. However, their developmental patterns are not always similar to each other. The postgenital fusion which is observed in the species of the Apocynaceae (Boke, 1948; Nishino, 1982) does not occur in other species of the Gentianales. Although the Asclepiadaceae is generally regarded to be closely related to the Apocynaceae (Lawrence, 1951), it is indicated from the present study that the Apocynaceae is an uncommon family with respect to the corolla tube formation.

In the mature corolla of *Veronicastrum*, the adaxial petal is supplied by two bundles. Such a structure was reported for the corolla of *Veronica*, and considered to be brought by the congenital fusion of the two petals (Saunders, 1934). In *Veronicastrum*, the adaxial petal primordium is initiated as a wide one, and the ontogeny of the adaxial petal primordium shows no process of fusion in the present study. Such a case may be an example of the true congenital fusion, and further studies are needed regarding this problem.

In *Swertia*, *Nymphoides* and *Menyanthes*, the upward growth at the connected regions of petal bases is meager, and afterwards scarcely contributes to the corolla tube development. Such development results in the condition in which the epipetalous stamens are inserted at the sinuses of the corolla lobes. The reduction of the upward growth at the connected regions is very similar to that observed in the case of the Apocynaceae by Nishino (1982). However, in the Apocynaceae, the postgenital fusion occurs near the connected regions, resulting in the formation of almost all of the upper portion of the corolla tube.

The lower portion of the corolla tube is formed by the elongation of the common base of petal and stamen primordia as reported in the cases of the Solanaceae and Apocynaceae by Nishino (1978, 1982), resulting in the formation of the epipetalous condition of stamens, except for the case of *Nymphoides*. This elongation of the common base may correspond to the "zonal growth", the term which has been used to describe the corolla tube formation in many studies.

As to the corolla tube formation of *Justicia* which has also epipetalous stamens on the corolla tube, Singh and Jain (1975) reported that the upper portion of the corolla tube was formed by the "ontogenetic union" of the petal bases, and that the lower portion was formed by the zonal growth in the common bases of petal and stamen primordia after the "ontogenetic union" of the bases of the petals and stamens. However, they did not show any figures for the tube development. They considered that the "ontogenetic union" in the development of the calyx and corolla of *Justicia* was the same process as that shown clearly in the formation of the upper portion of the corolla tube and a part of the gynoecium in *Vinca* (Boke, 1948, 1949), but they did not describe the process of the fusion. It is probable that the "ontogenetic union" in *Justicia* may be a process equivalent to the "connection" in the present study.

In the development of the corolla tubes of *Dichondra* and *Cuscuta*, the mutual connection of petal bases is not seen at the back of the stamen primordia. So, the entire corolla tube in these species is formed only by the elongation of the common base of the stamen and petal primordia, and may be comparable with the lower portion of the corolla tube of other species studied in the Tubiflorae. However, in *Dichondra* and *Cuscuta* petal and stamen primordia stand alternately on the common base, while in other species of the Tubiflorae the petal bases connect mutually, and the connected region is a part of the common base of petal and stamen primordia. In this respect, it is regarded that there is no real sympetality in *Dichondra* and *Cuscuta*.

In *Nymphoides*, the lower portion of the corolla tube is formed by the elongation of the cup-like structure surrounded by the calyx before the initiation of the petal primordia. Such tube formation has been reported in *Chrysanthemum* by Popham (1963) and in *Echinops* by Leins and Gemmeke (1979), and in *Syringa* by Sattler (1973). The cup-like structure is formed by the upward growth at the peripheral region of the floral meristem also in these species. And the petal and stamen primordia are initiated on this cup-like structure, resulting in the formation of the epipetalous condition of the stamens. Such corolla tubes at the mature state cannot be distinguished from the corolla tube formed by the elongation of the common base, which is initiated after the connection of the petal bases at the back of the stamens.

The present study and the studies by Daniel and Sattler (1978) and Nishino (1978, 1982) show some manners of the corolla tube formation in the species of the Tubiflorae and Gentianales. However, different manners were reported for the cases of the species of the Primulales (cf. Sattler, 1962) and Ericales (Leins, 1964). So further comparative studies are needed on the corolla tube formation in these groups.

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