POLLEN PRESENTER ONTOGENIES OF SELECTED LOBELIACEAE AND CAMPANULACEAE SPECIES

SYSTEMATICS HONOURS PROJECT
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Ontogeny and Phylogeny

In the beginning is the end;
But ends unfold, becoming strange.
Lives- and generations- suffer change.
The tested metabolic paths will tend
To last and shape the range
Of future evolution from the past.
J.M.Burns.
(cited by Gould, 1977)

ABSTRACT

Pollen presenter ontogenies of five selected Camapanulaceae and Lobeliaceae species were described with the aid of SEM. The role of early ontogenetic distinctions, in the formation of morphologically divergent pollen presenters, was investigated. The Campanulaceae and Lobeliaceae follow a similar developmental sequence prior to stylar hair initiation, when morphological divergence occurs. Anther tube formation was found to be a derived trait in the Lobeliaceae, which supports previous suggestions. Within the Lobeliaceae, the developmental sequence of anther tube and anther tuft formation has been reversed in *Grammatotheca bergiana*. The stylar ring hairs of *Monopsis flava* display "congenital displacement" (heterotopy). Within the Campanulaceae, both *Lightfootia longifolia* and *Siphocodon debilis* follow a similar developmental sequence. *S.debilis* has a terminal stigma which is covered by a thin membrane, contrary to previous ideas that it was naked. This membrane may prevent self-pollination. It is additionally postulated that the stigma of *Siphocodon debilis* may aid in pollen presentation and form an intermediate between the deposition-mechanism of pollen presentation in the Campanulaceae and the pump-mechanism of the Lobeliaceae.

1.INTRODUCTION

1.a.Pollen Presenters

Although direct pollen transfer from anther to stigma, is customary among flowering plants, families of the Campanulales-Asterales-complex are characterised by secondary pollen presentation. This mechanism involves the initial deposition of pollen on the style and its associated structures, prior to removal by potential pollinators. Secondary pollen presentation mechanisms and the structures involved have fascinated botanists since at least the time of Linneaus, and many studies have subsequently been conducted in this field (recent works include: Carolin, 1960; Erbar and Leins, 1989; Leins and Erbar,1990; Lammers, 1992; Ladd and Donaldson, 1993; Nyman, 1993 a, 1993 b). "Thus do we, for lack of knowing what has been done, grind over and over the same grist" (Barnes, 1886 cited by Shetler, 1979). In order to avoid repetition of previous work, this study focused on the early developmental stages of secondary pollen presenters, with a view to understanding the evolution of morphological differences between mature Lobeliaceae and Campanulaceae species.

In South Africa pollen presenters are ubiquitous in the Campanulaceae, Lobeliaceae, Asteraceae, Goodeniaceae and Proteaceae (Leins and Erbar, 1990; Ladd and Donaldson, 1993). All flowers are protandrous and display introrse anther dehiscence, which occurs in the bud stage (Erbar and Leins, 1989; Ladd and Donaldson, 1993). Pollen is thus released onto the style and its auxiliary structures: sweeping hairs, retractile pollen collecting hairs (PCH), indusia and anther tubes, which all aid in the collection and presentation of pollen (Carolin, 1960; Erbar and Leins, 1989; Leins and Erbar, 1990). Striking differences exist between secondary pollen presentation mechanisms of the Lobeliaceae and Campanulaceae. In the Lobeliaceae, pollen is actively pushed out of the anther tube by the growing style and subsequently made available for insect pollinators ("pump-mechanism" Leins and Erbar, 1990). While in the Campanulaceae, pollen is passively collected by specialised stylar hairs (PCH's) and presented to insects only once the anthers have shrivelled away, to expose this pollen laden structure ("deposition-mechanism", Leins and Erbar, 1990). Although structural differences are central to this study, reference will also be made to function, as these two concepts are inextricably linked.

The close phylogenetic relationship between Lobeliaceae and Campanulaceae has been recognised by many authors (Dyer, 1975; Cronquist, 1988; Lammers, 1992); although there is no appropriate phylogeny available for the Campanulales. Lammers (1992) claims that the unique invaginating stylar hairs, are the only evident synapomorphy (shared, derived character) for the Campanulaceae. The Lobeliaceae, which do not possess invaginating stylar hairs, have irregular resupinate flowers and connate anthers as the proposed synapomorphies (Heywood, 1985; Lammers, 1992). Among the numerous characters shared by both families, the presence of specialised protandrous pollen presentation is found to be the only morphological synapomorphy, supporting this close relationship (Cronquist, 1988; Lammers, 1992). An investigation into the development of these divergent forms of secondary pollen presentation may thus provide useful information concerning the evolutionary relationship of these two families.

1.b.Why study ontogeny?

Ontogeny and phylogeny are inextricably linked, since evolution proceeds by the modification of successive ontogenies (Hufford, 1988). As a consequence, taxa in any clade are evolutionarily linked by the succession of ancestral ontogenies that they have in common. Darwin (1859) stated this as "community in embryonic structure reveals community by descent". The study of morphological distinctions among taxa and the developmental modes through which they have diverged, should subsequently elucidate the process of morphological evolution.

Ontogenetic studies comparing pollen presenters of these two, closely related, families are rare. Erbar and Leins (1989) have investigated the late developmental stages of pollen presenters in *Jasione, Lobelia* and *Campanula.* The early stages of pollen presenter development have not been explored. Although much work has been done on the pollen presenters of Campanulaceae, most of these studies have focused on *Campanula* (refer to Shetler, 1979 for historical reveiw) and no detailed studies have been carried out on any South African species. Ladd and Donaldson (1993) recently investigated the occurrence of pollen presenters in the South African flora; their study provides a useful foundation for subsequent research, but is lacking in the detail which is imperative in this discipline.

1.c. Objectives

Pollen presenter development was investigated for five species selected from Lobeliaceae and Campanulaceae. The purpose of this study was two-fold:

1) to provide a detailed description of the early development of the pollen presenters, using SEM techniques.
2) to investigate the role of early ontogenetic distinctions in the formation of, functionally and morphologically, divergent pollen presenters.

At what stage in the developmental sequence does morphological divergence commence? The study focused on comparisons of the sequence of events prior to the onset of the male phase (anther dehiscence). This part of the developmental sequence was selected as it is vital for understanding the evolution of morphological change and pollen presenters were considered mature at anther dehiscence. In addition, Erbar and Leins (1989) have already covered the later stages of development in European Campanulaceae and Lobeliaceae species.

2.METHODS

Flowering material was collected at Fernkloof Nature Reserve, in early February of 1994, and fixed in FAA in the field (Fig 1). Flower buds were then transferred to 70% ethanol, where they were stored until later dissection. A preliminary morphological study, using light microscopy, was made of the mature pollen presenters for all collected species. This gave an indication of which forms of pollen presentation were common and which unusual within each family. A subsample of five species was then selected for detailed investigation. The general "pump-mechanism" of the Lobeliaceae is represented by *Grammatotheca bergiana* (Cham.) Presl. and *Lobelia coronopifolia* L. The latter species was only partially investigated to illustrate slight morphological differences in the general pattern. *Monopsis flava* (Eckl. & Zeyh.) E. Wimm. deviates slightly from the common structure and was consequently also selected from the family. In the Campanulaceae, *Lightfootia longifolia* A.DC. possesses the common "deposition-mechanism", while *Siphocodon debilis* (Schltr.) differs from all the other Campanulaceae specimens, and detailed studies of its intriguing structure have been strongly recommended by Ladd and Donaldson (1993).

Morphological differences between mature pollen presenters were investigated and these structures were illustrated for comparative purposes. Alcohol-preserved bud material of the

selected specimens were dissected; the sepals and petals were at least partially removed to expose the stamens and style, and then transferred into 100% formaldehydedimethylacetal (=FDA) for chemical dehydration (after Gerstberger and Leins, 1978). Subsequently the material was critical-point dried directly from FDA using CO_2 as a transitional fluid. Apart from giving excellent results, this method is also less time consuming than conventional methods, and is thus suitable for comparative studies of a large number of specimens (Kurzweil, 1991). The technique was developed in Germany more than 10 years ago and is widely used by botanists in Europe, but is surprisingly little known in South Africa (Kurzweil, 1991). To avoid shrinking, the dried and heated flower buds were left in the pressure chamber until they had slowly cooled down to room temperature. The dried buds were mounted with a mixture of colloidal graphite and glue, on aluminium stubs and sputter-coated with gold in an argon-atmosphere (4 min at 20 mA). The specimens were then examined on a Cambridge (S200) SEM at 5KV. The developmental sequence involved in pollen presenter formation was described for each species and comparisons were subsequently made within and between the two families.

The timing of events, in an ontogenetic sequence, is postulated to play an important role in evolutionary modification of morphology (Gould, 1977; Alberch et al., 1979; Hufford, 1988). In order to understand the role of heterochrony (change in developmental timing), quantitative data are required. Style and stylar hair lengths were recorded for each species from their initiation to maturation. The data were displayed as a series of simple graphs. Pollen presenter formation, for the two families, was illustrated as a hypothetical sequence of developmental events, using bud diameter as a quantitative measure of size (see Fig.2 for measurement positions). Sequence diagrams were constructed to summarise the developmental events, and illuminate differences and similarities within and between the two families.

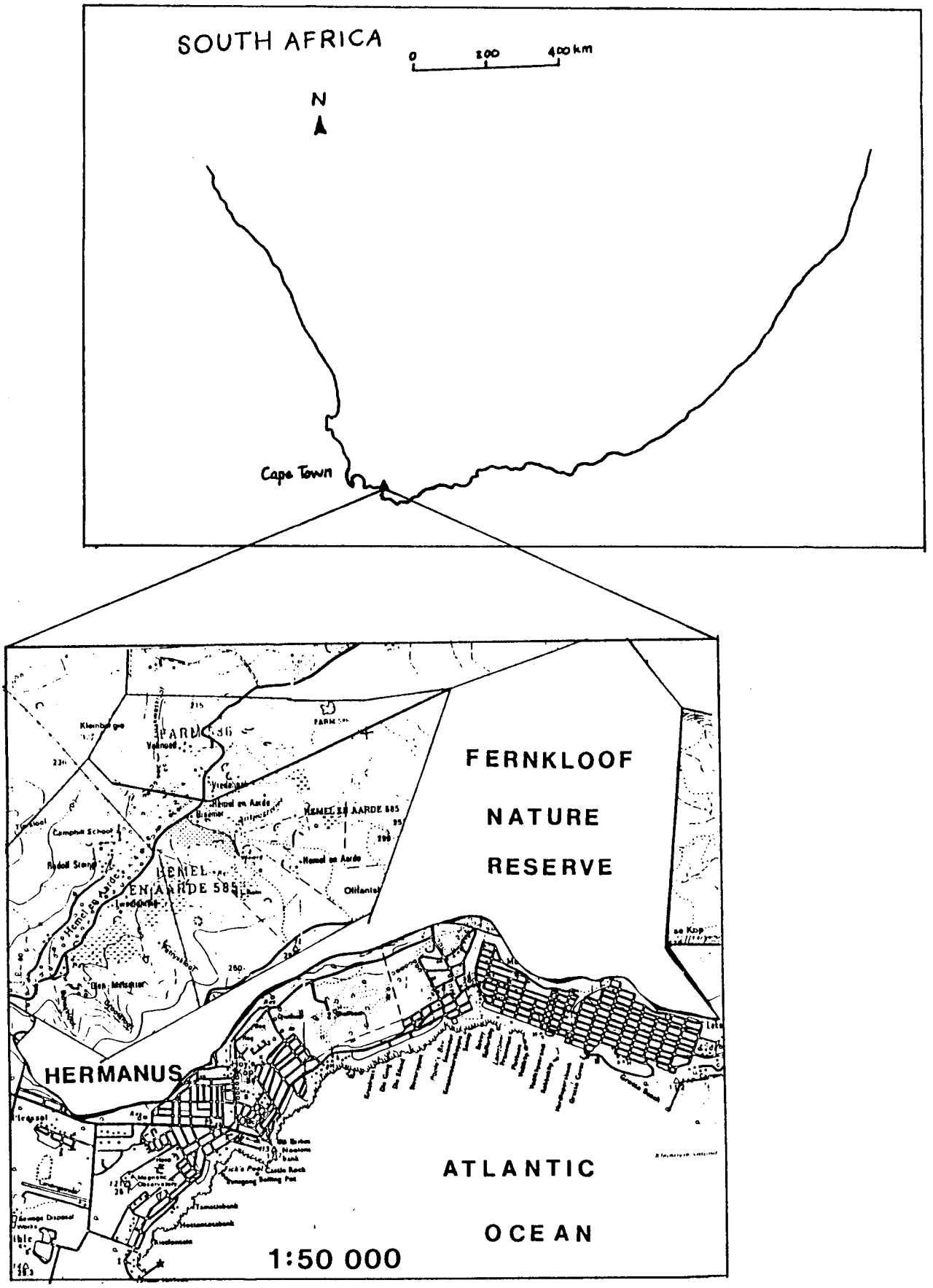


Fig. 1. Map of Fernkloof Nature Reserve, close to Hermanus in the Western Cape, where Lobeliaceae and Campanulaceae specimens were collected.

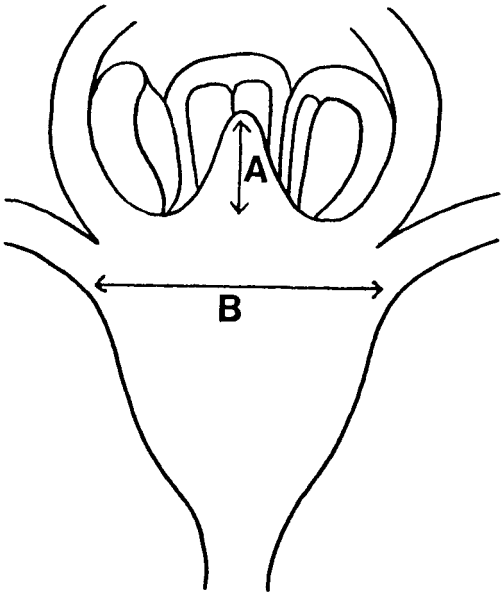


Fig. 2. Measurement positions of young bud and style of Lobeliaceae and Campanulaceae species. A = style length. B = bud diametre. The longest apical stylar hairs were measured for Lobeliaceae species; while an average of three measurements was obtained for ring hairs in Lobeliaceae and stylar hairs in Campanulaceae.

3.RESULTS

3.1.Mature Pollen Presenters

The Lobeliaceae have consistently less stylar hairs than the Campanulaceae (Fig 3). Two types of stylar hairs are present in the Lobeliaceae: the first comprises a cluster of long, tapering hairs which project from the stylar apex; these display intraspecific variation in length. The second type consist of bulbous tipped hairs which are aggregated in a ring, just below the style apex in *G.bergiana* and *L.coronopifolia* and lower down the style in *M.flava*. In comparison, both *L.longifolia* and *S.debilis* have only one type of retractile stylar hair which cover the upper two-thirds of the style. These hairs are equal in length and are randomly orientated in *L.longifolia* while in *S.debilis* they all point downwards.

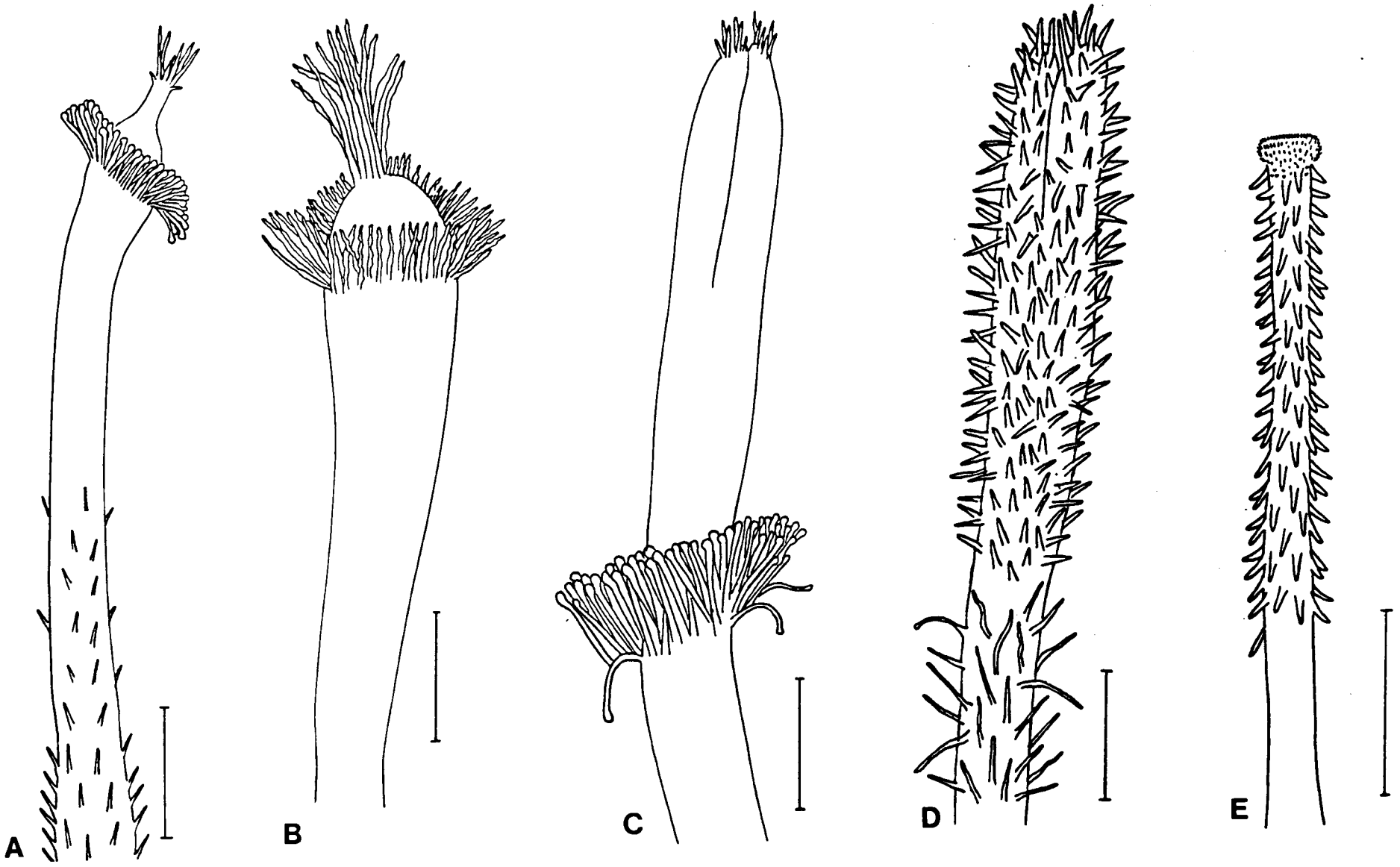


Fig. 3. Mature pollen presenters of five selected Campanulaceae and Lobeliaceae species. A = *Grammatotheca bergiana* (Lobeliaceae). B = *Lobelia coronopifolia* (Lobeliaceae). C = *Monopsis flava* (Lobeliaceae). D = *Lightfootia longifolia* (Campanulaceae). E = *Siphocodon debilis* (Campanulaceae). Scale bars = 500 μ

3.2.Early ontogenetic modifications of the pollen presenters and associated structures

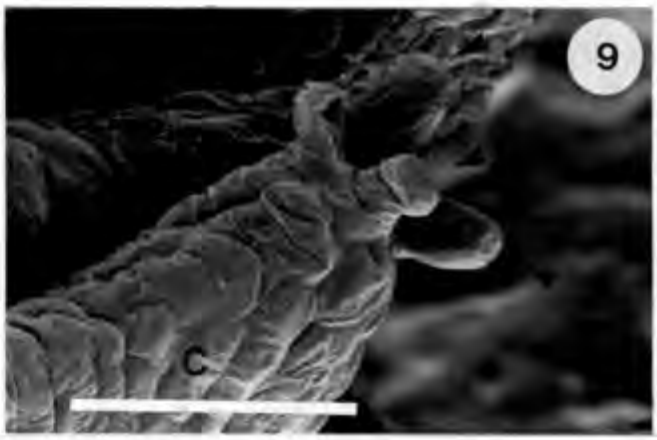
3.2.1.LOBELIACEAE

a.*Grammatotheca Bergiana*

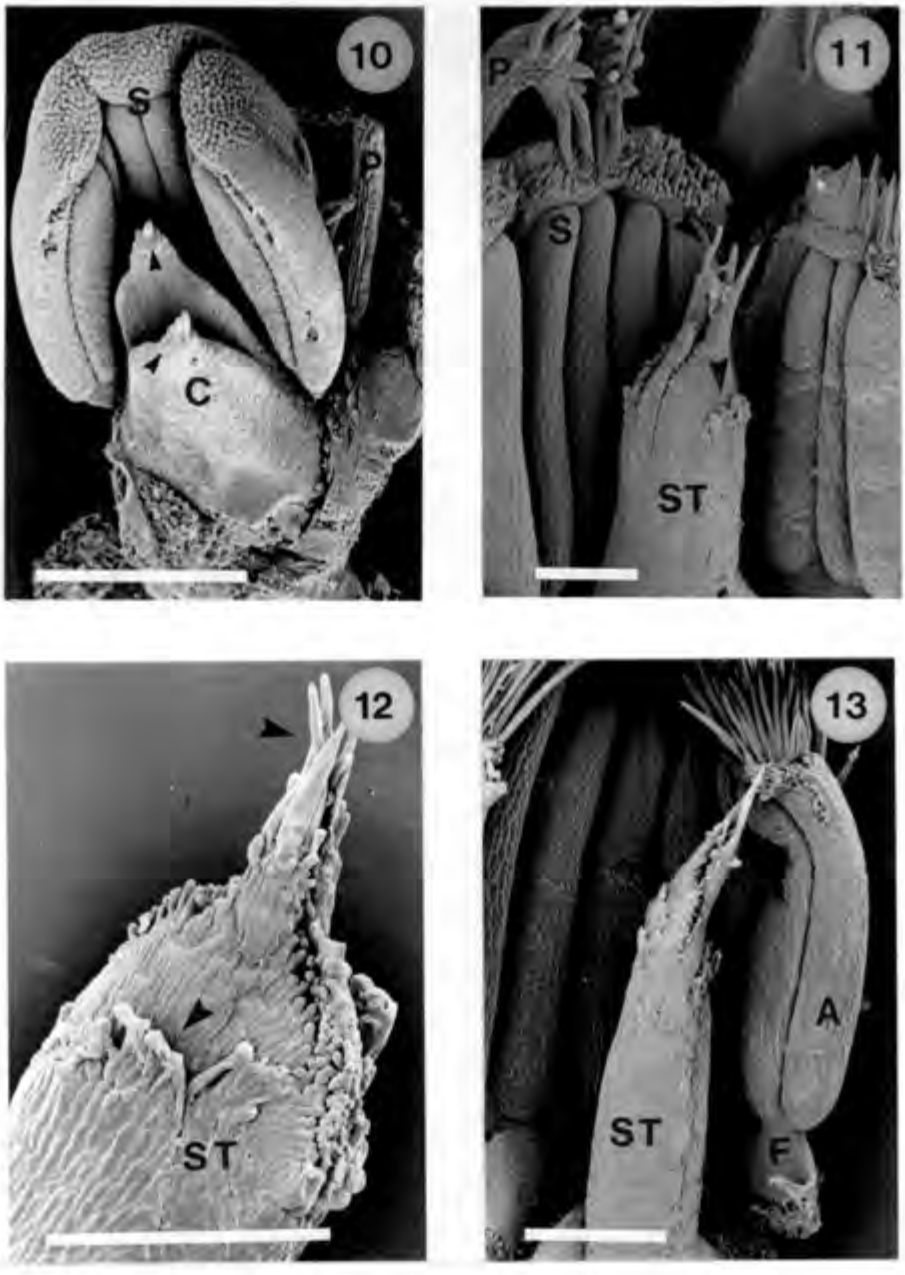
Flower buds develop in the axils of subtending bracts (Fig 4), and the pentamerous corolla is enveloped by 5 separate sepals. Once these peripheral structures are removed, five antisepalous (alternate with petals) stamen primordia are visible (Fig 5). They are glabrous, rounded in shape and of similar size. Since development of the floral organs is centripetal, the androecium is always at a later stage of development, hence protandry. The gynoecium is initiated as two central carpel primordia (Fig 5, 6), which develop into distinct stigmas in the mature flower. The stamens are initially only constituted by anthers: filaments develop at a much later stage in floral ontogeny. The apical epidermal cells of the anthers become swollen early in the developmental sequence (Fig 7); these eventually form apical tufts. Lateral expansion of anthers and the fusion of adjacent cuticles initiates the formation of the anther tube (Fig 8, 10, 11). Differential growth of the anthers is apparent in the young bud: the two adaxial anthers being shorter than the rest (Fig 8, 11). The carpels, which are laterally compressed along their length, each develop two apical hair primordia by elongation of epidermal cells (Fig 8, 9, 10). This is followed by the initiation of apical anther tufts; the shortest anthers have the longest tufts (Fig 11). In addition to the apical stylar hairs, which have elongated extensively, a row of hairs begins to form at the base of each stigma (Fig 11, 12). These lower stylar hairs develop sequentially, from the centre of each stigma outwards so that the longest basal hairs are aligned with the longest apical hairs (Fig 12). This lower 'hairline' eventually encircles the style. Pollen presenter formation is completed once these hairs have matured. Later development of these structures involves extension of the filaments and further elongation of the style (Fig 13), so that the base of the anther tube becomes aligned with the ring of stylar hairs at anther dehiscence.

b.*Lobelia coronopifolia*

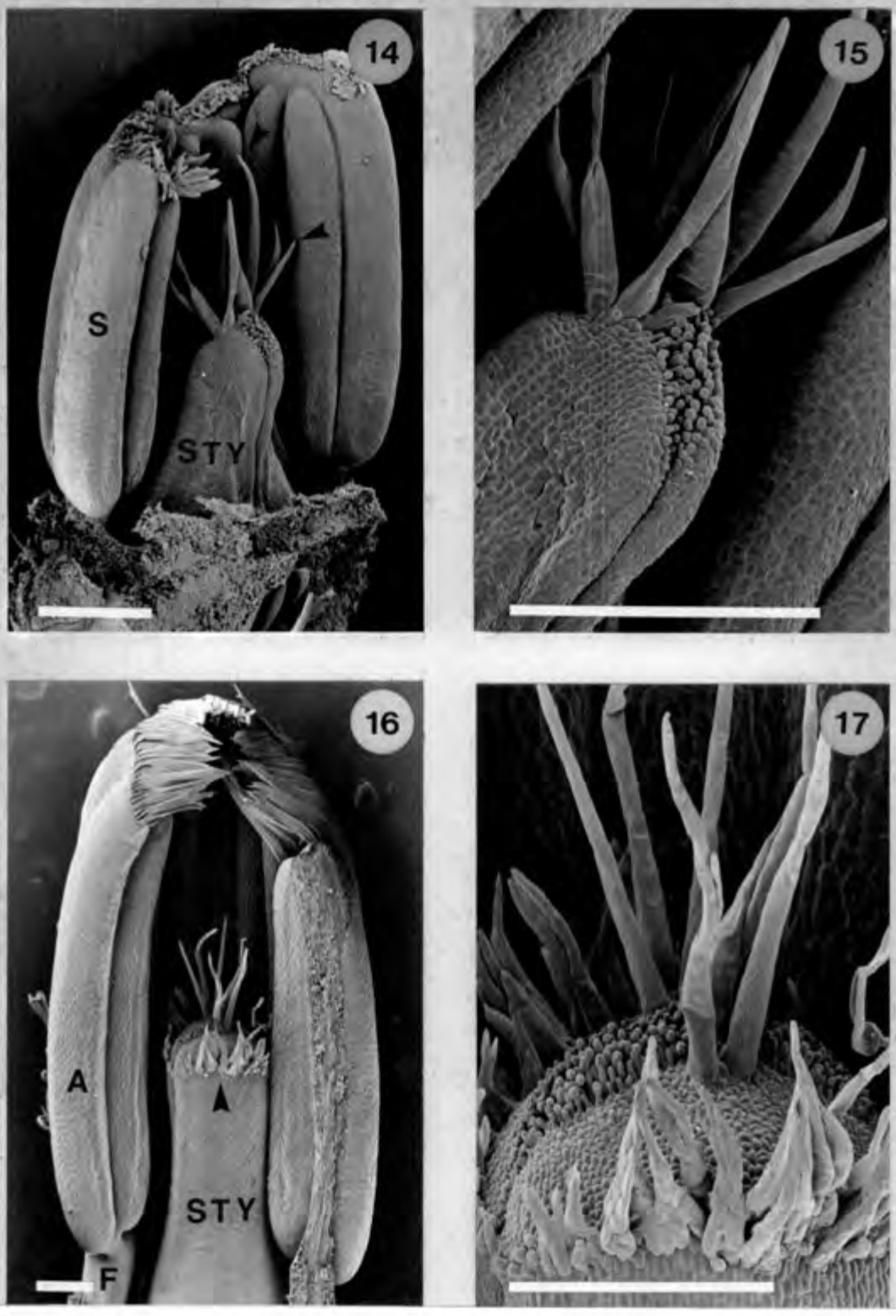
The early stages of development in this species are absent. The style is only half the length of the anther tube in the later developmental stages (Fig 14, 16); which differs from the similar sized style and anther tube found in *G.bergiana*. The dichotomy in anther size is



Figs. 4-9. Early development in *Grammatotheca bergiana* (Lobeliaceae). - 4, Young flower bud with partial removal of sepal to reveal young petals. - 5, Petals and sepals removed to reveal five glabrous stamens and initiation of two carpel primordia. - 6, Formation of independent carpels and enlargement of stamens. - 7, elongation of partially separate carpels and growth of individual stamens seen from above. - 8, Initiation of apical stylar hairs (arrow), note enlargement of apical stamen cells. Onset of stamen tube formation, note where cuticles have started to join on sides of anthers. Carpels join together close to base. - 9, Close-up of apical stylar hairs showing structure and position. SE = Sepal, P = Petal, S = Stamen, C = Carpel. - Scale bars for Figs 4-8 = 100μ for Fig. 9 = 40μ.



Figs. 10-13. Late development in *Grammatotheca bergiana* (Lobeliaceae). - 10, Elongation of apical stylar hairs (arrows) and formation of stamen tube, no hairs present at stamen apices yet. - 11, Stamen tube fully developed, Hairs present at tips of anthers, shorter stamens have longer hairs. Lower ring of stylar hairs starting to develop (arrows). - 12, enlarged style showing structure and developmental sequence of stylar hairs; apical and ring hairs (arrows). - 13, Elongation of style and filaments begin to grow, also lengthening of apical tufts. P = Petal, S = Stamen, A = Anther, F = Filament, C = Carpel, ST = Style. - Scale bars = 200μ

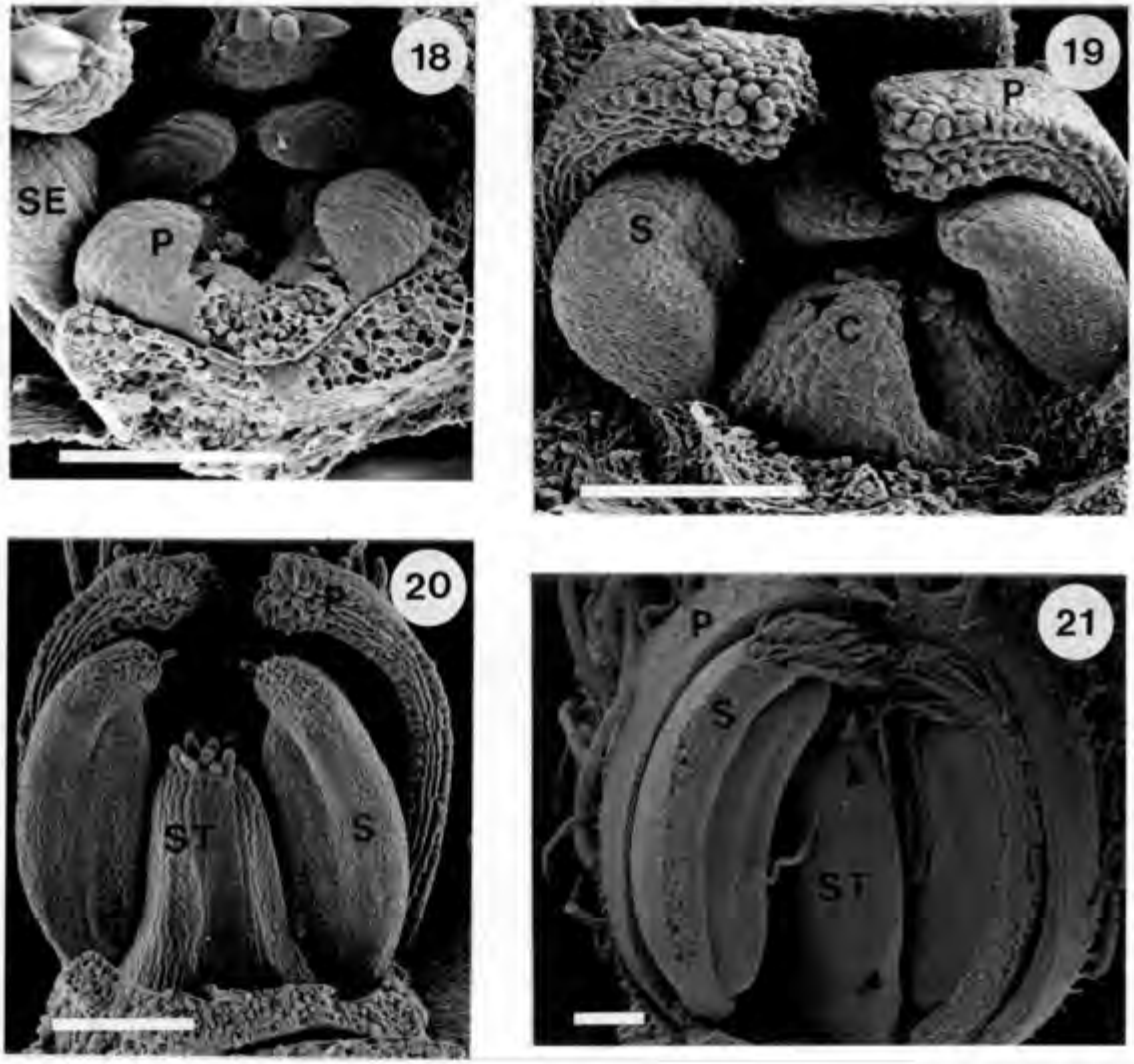


Figs. 14-17. Late development in *Lobelia coronopifolia* (Lobeliaceae). - 14, Style with very long apical hairs, (arrows), note differential stamen sizes, with tufts beginning to form at anther apices and anthers joined together to form a tube. - 15, Detailed structure of apical stylar hairs. - 16, Lower ring of hairs has been initiated below style apex (arrows), elongation of anthers and development of filaments, anther tufts well developed, further lengthening of style. - 17, Detailed structure of stylar surface and apical and ring stylar hairs. S = Stamen, A = Anther, F = Filament, STY = Style. - Scale bars Figs. 14-17 = 20μ.

evident at an early stage of development (Fig 14) as in *G.bergiana*. Apical hair development on the style precedes the hair formation at the anther apices as in *G.bergiana*. Although these apical stylar hairs are long in relation to the length of the style, they are not longer than those of *G.bergiana* (refer to scale bars for Figs 12 & 14). The lower ring of hairs develops just beneath the stylar tip with development also occurring from the centre of each style outwards (Fig 16, 17). The anther tufts are all approximately equal sizes and grow inwards to seal off the anther tube, filament extension begins once hair formation is completed (Fig 16).

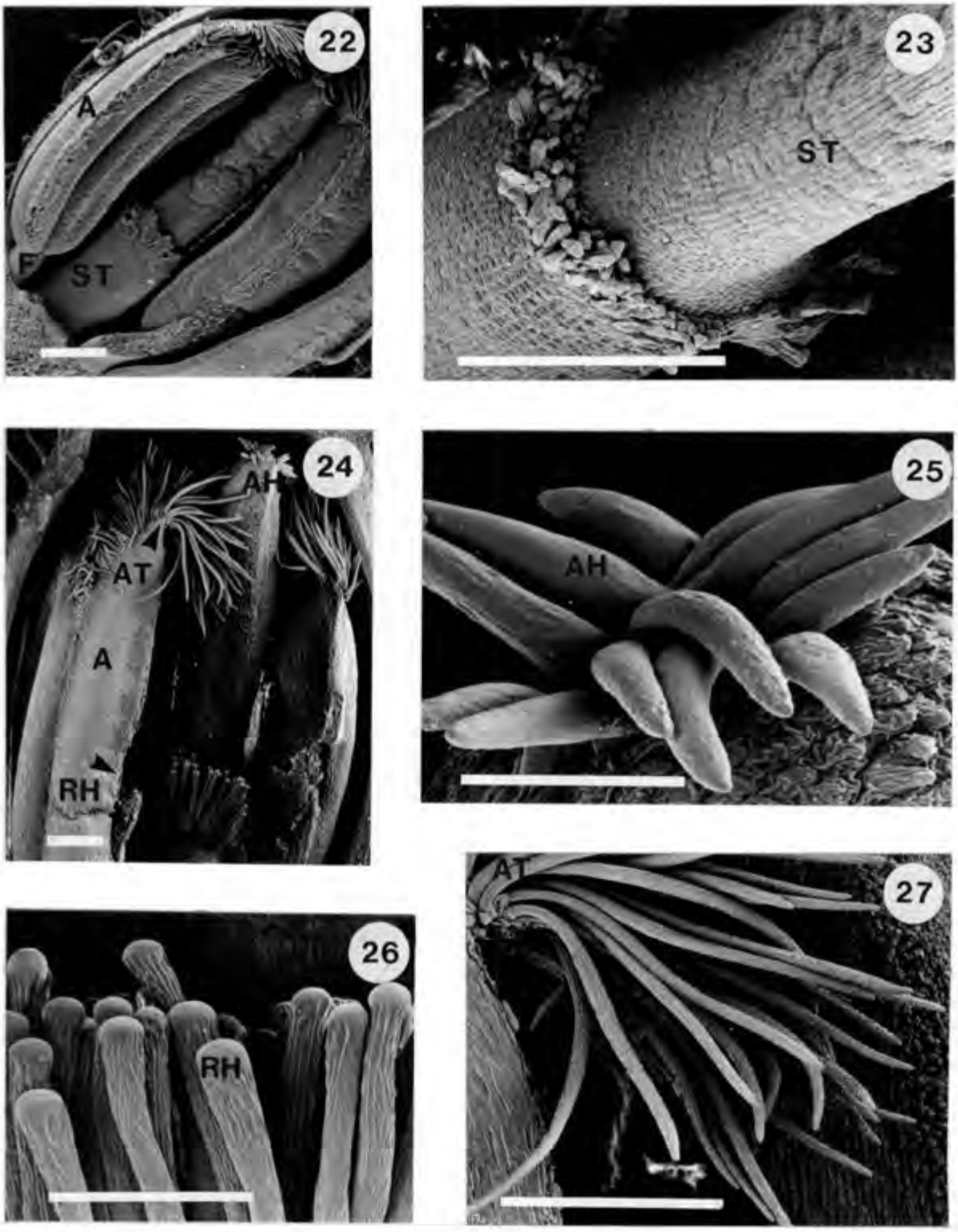
c. *Monopsis flava*

The pentamerous corolla is initiated as five independent petal primordia, which are curved inwards towards the centre of the bud and alternate with the inner whorl of stamens (Fig 18). Two separate carpel primordia develop internally to this stamen ring (Fig 19). Apical stylar hairs are initiated early in the pollen presenter ontogeny, similar to the other Lobeliaceae species (Fig 20). The style tips are slightly rounded but display no tapering as in *G.flava* and the apical stylar hairs are thus approximately all the same length. Hair development at the tips of the anthers takes place soon after stylar hair initiation (Fig 20, 21). This is followed sequentially by anther tube formation (Fig 21); which takes place in the same procedure as *G.bergiana*. The style with its apical hairs is as long as the anther tube in the intermediate developmental stages, this is similar to *G.bergiana* but differs from *L.coronopifolia*. The hairs at the anther tips elongate to seal off the anther tube (Fig 21, 22). A lower ring of hairs is simultaneously initiated near the base of the style, as apposed to just below the stigma in *L.coronopifolia* (Fig 21, 22, 23). The three types of hairs associated with secondary pollen presentation in this species differ in size and shape (Fig 24). The apical stylar hairs are short, slim and tapering, they have a slightly bumpy surface and point downwards (Fig 25). The anther beards consist of long, slender, tapering hairs; which flop into the centre of the anther tube (Fig 27). The surface of these hairs have distinct longitudinal corrugations. The ring hairs have distinct bulbous tips and all grow upwards (Fig 27). Later elongation of the style and filaments proceeds in the same way as *G.bergiana*.



Figs. 18-21. Early development of *Monopsis flava* (Lobeliaceae)

- 18, Sepals removed and ring of separate petal primordia visible, note initiation of stamen primordia alternating with petals (arrows). - 19, Sepals removed, petals partially removed, separate glabrous stamens surrounding developing carpels, note enlargement of apical carpel cells. - 20, Apical stylar hairs developing and enlarged cells at anther apices start to form hairs, stamens enlarging. - 21, Elongation of apical stylar hairs and anther tufts, filaments just starting to develop, No ring hairs present yet but cells starting to enlarge(arrows). - SE = Sepal, P = Petal, S = Stamen, C = Carpel, ST = Style. - Scale bars = 100μ



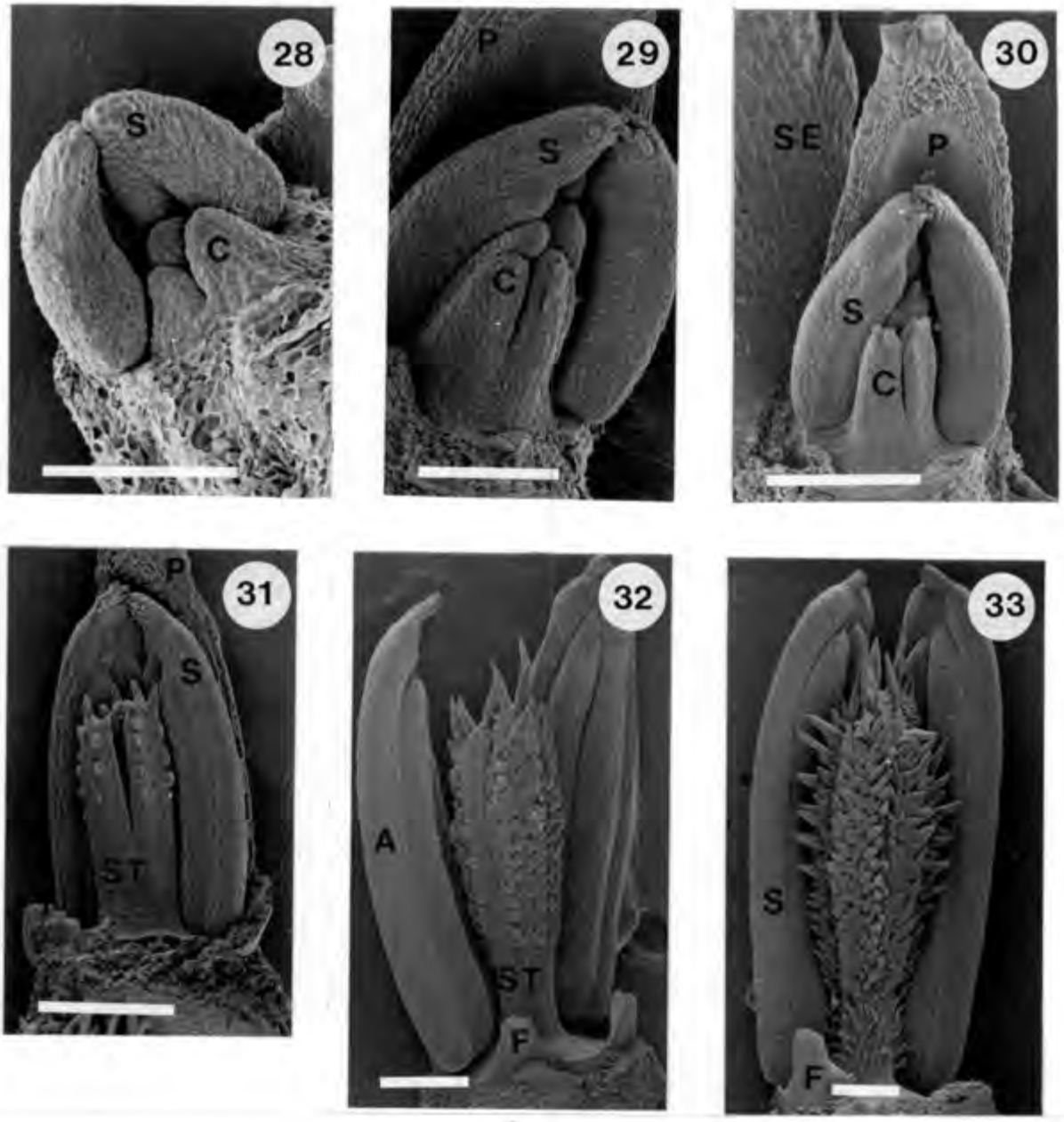
Figs. 22-27. Later development of *Monopsi flava* (Lobeliaceae).

- 22, Elongation of style and anthers, filaments starting to develop, Lower ring of stylar hairs starting to form. - 23, Stylar ring hairs all develop simultaneously. - 24, Elongation of style and extension of apical stylar hairs and anther tufts. - 25, Detailed structure of apical stylar hairs. - 26, Detailed structure of stylar ring hairs. - 27, hairs forming tufts at anther apices. F = Filament, A = anther, ST = Style, AH = Apical Hairs, AT = Anther Tufts, RH = Ring Hairs. - Scale bars for Figs. 22-24, 27 = 200μ, Fig. 25 = 50μ , Fig. 26 = 100μ

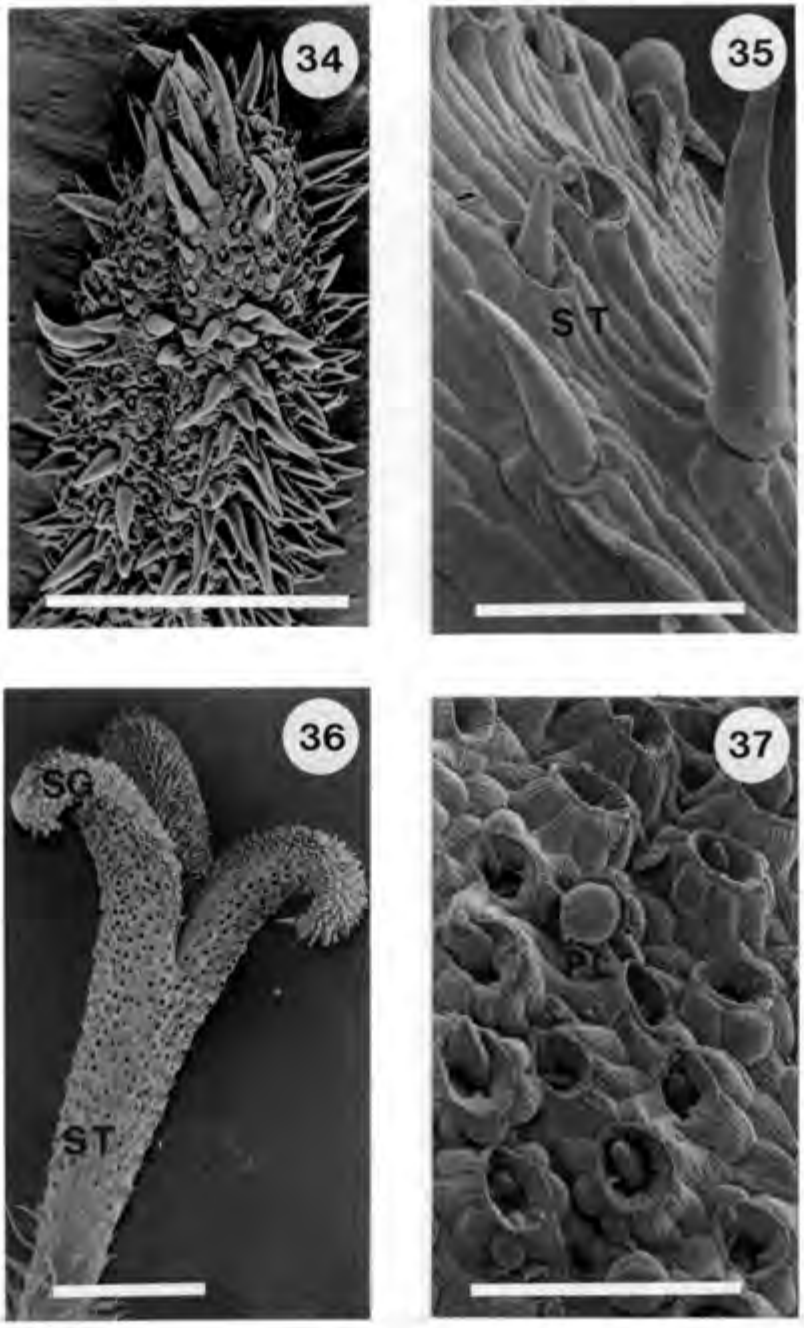
3.2.2.CAMPANULACEAE

a. *Lightfootia longifolia*

The gynoecium is initiated as three or four partially separate carpel primordia (Fig 28, 29, 30). The abaxial carpel lobe is smallest and size increases circumferentially. These carpels alternate with the already enlarged stamens, of which there can be four or five comprising the androecium (contrast with Dyer, 1975 who refers to five stamens for this genus). Apical cell enlargement is initiated early in development, however, these swollen cells do not form hairs but unite to form a tapering anther apex (Fig 29, 30, 31, 32, 33). Stylar hair primordia are initiated as rounded knobs, which develop in a sequential linear fashion from the tip to the base of the pollen presenter(Fig 30, 31, 32, 33). The developing 'stylar-brush' is characterised by vertical rows of 'older' and 'younger' hairs which alternate with each other (Fig 32, 33). These hairs all eventually reach the same size and have tapering tips. The tip hairs grow vertically, while the rest grow obliquely upwards. A second type of hair starts to develop at the base of the style, late in the developmental process (Fig 32, 33). These hairs differ from the upper stylar hairs in being widely spaced and having a wiry structure. Since they are non-retractile (Fig 46), they do not play a role in secondary pollen presentation. The filaments enlarge to form thickened triangular bases (Fig 32, 33), and lift the anthers so that their bases are in line with the base of the pollen collecting hairs at anther dehiscence. The stamens are connivent around the style until anther dehiscence after which they separate, due to shrivelling of the filaments, and expose the pollen-laden style to insects (pers. observation). The end of the male phase is initiated by the retraction of the pollen collecting hairs and the splitting open of the stigma (Fig 34, 36). Hair retraction is an irreversible osmotic process, which starts from the base of the hair until finally the tip is withdrawn into the pit; similar to the inward retraction of the fingers of a glove (Fig 34, 35, 36, 37). Since the apex hairs were the first to develop and mature it would be expected that they will retract first, however, this is not the case and retraction seems to take place randomly over the style surface. The stigmas split open when the majority, but not all, of the stylar hairs have retracted, and then recurve onto themselves (Fig 36, also pers. observation).



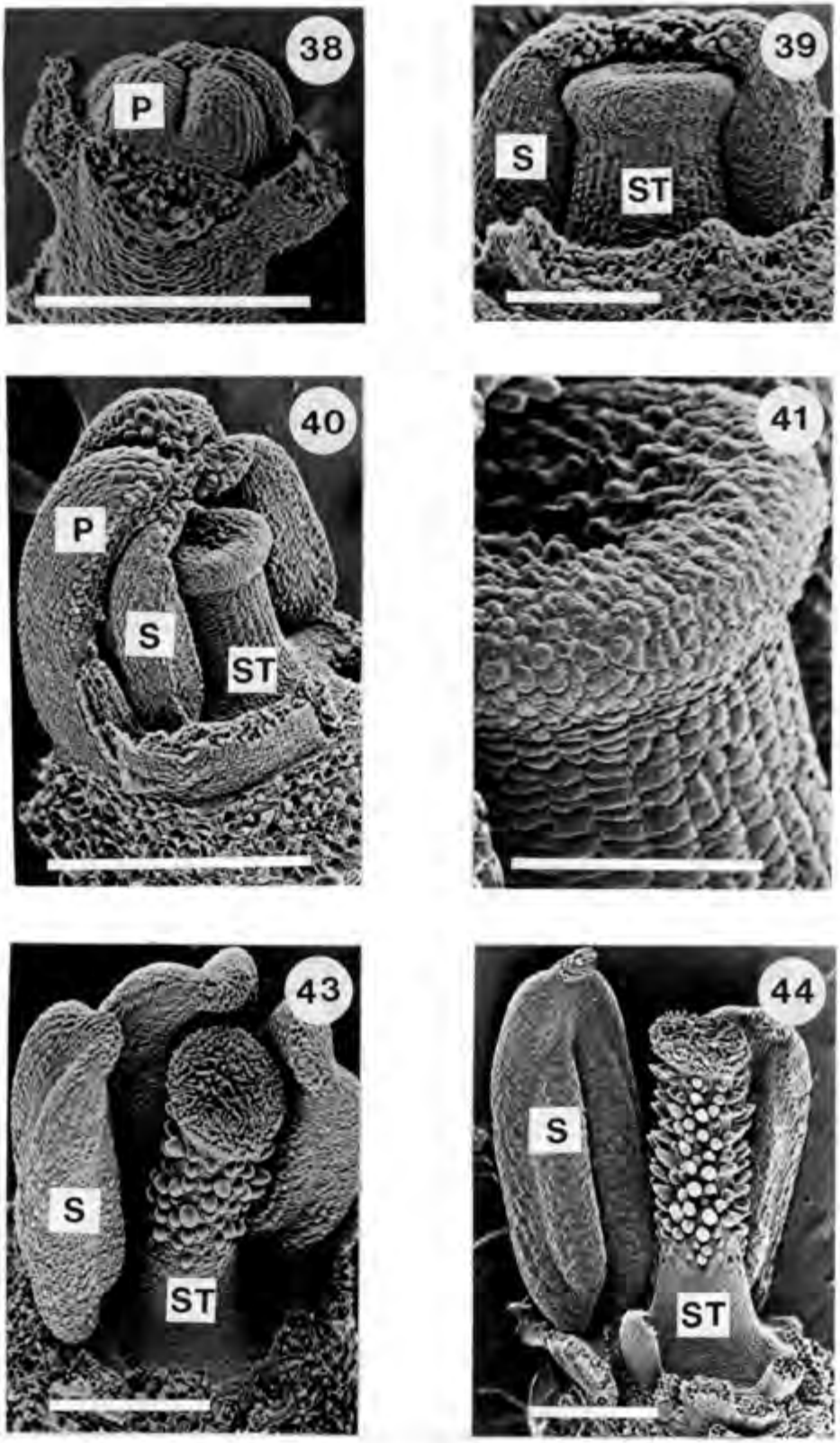
Figs. 28-33. Development in *Lightfootia longifolia* (Campanulaceae). - 28, Petals and sepals removed, Large glabrous stamens encircling the developing carpel primordia. - 29, Elongation of carpels and stamens, note swollen apical stamen cells. - 30, Initiation of apical stylar hairs, further lengthening of stamens and apical stamen cells. - 31, Linear development of stylar hairs, apical stylar hairs elongating. - 32, Style covered in developing hairs, note further extension of apical stylar hairs and tapering edge has developed at tip of stamen. - 33, Stylar pollen collecting hairs elongating, Thin, widely spaced hairs have started to develop at the base of the style, these lower hairs are not responsible for pollen presentation, note development of triangular filament bases. SE = Sepal, P = Petal, S = Stamen, A = Anther, F = Filament, C = Carpel, ST = Style. Scale bars for Figs. 30-33 = 200μ, Fig. 28, 29 = 100μ.



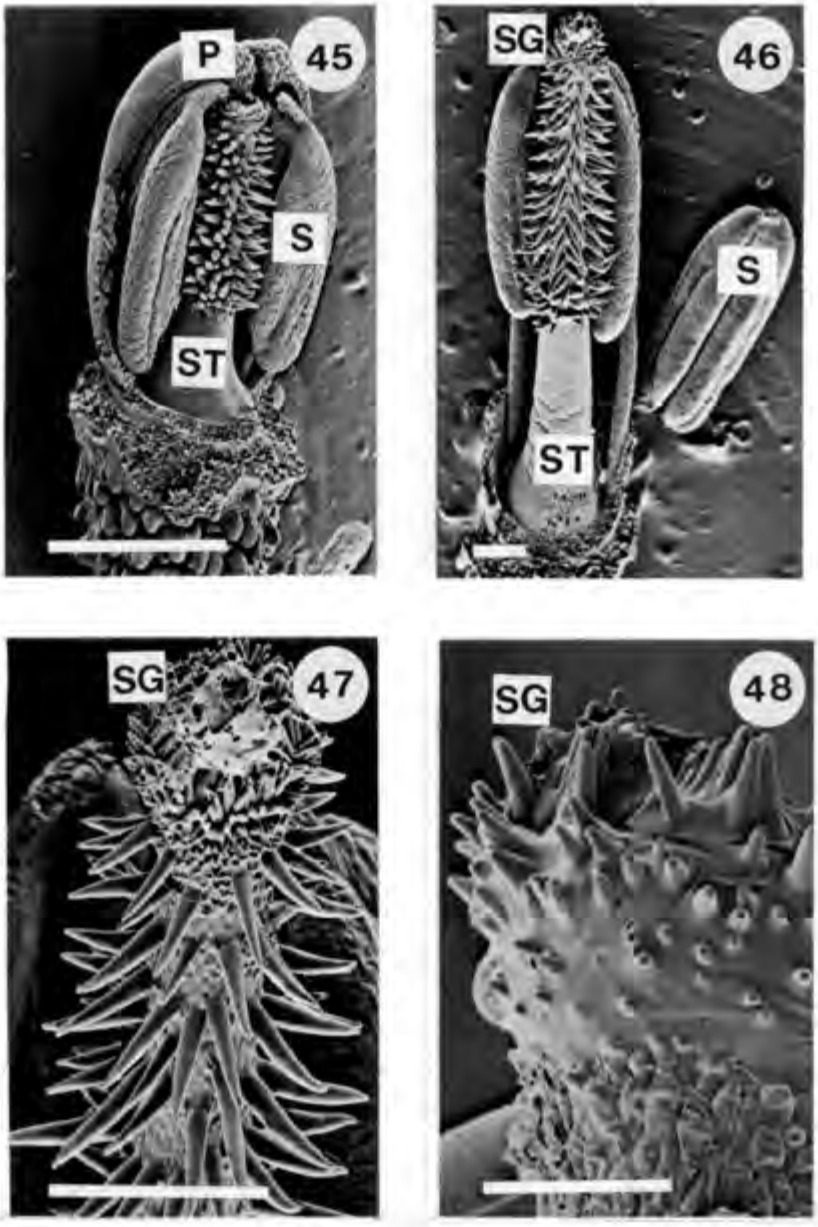
Figs. 34-37. Late development in *Lightfootia longifolia* (Campanulaceae). - 34, Retraction of unique stylar pollen collecting hairs randomly along the length of the style. - 35, Hairs retract into their bases from the bottom upwards, note hair remains intact. -36, Onset of female phase; style splits open to expose mature stigma surfaces, note virtually all pollen collecting hairs have retracted, style has a glabrous, pitted appearance. - 37, Only tips of hairs still visible in pit cavities, pollen grains roll off the style when the hairs retract. ST = Style, SG = Stigma, PL = Pollen. - Scale bars for Figs. 34, 36 = 500μ, Figs 35, 37 = 100μ.

b.*Siphocodon debilis*

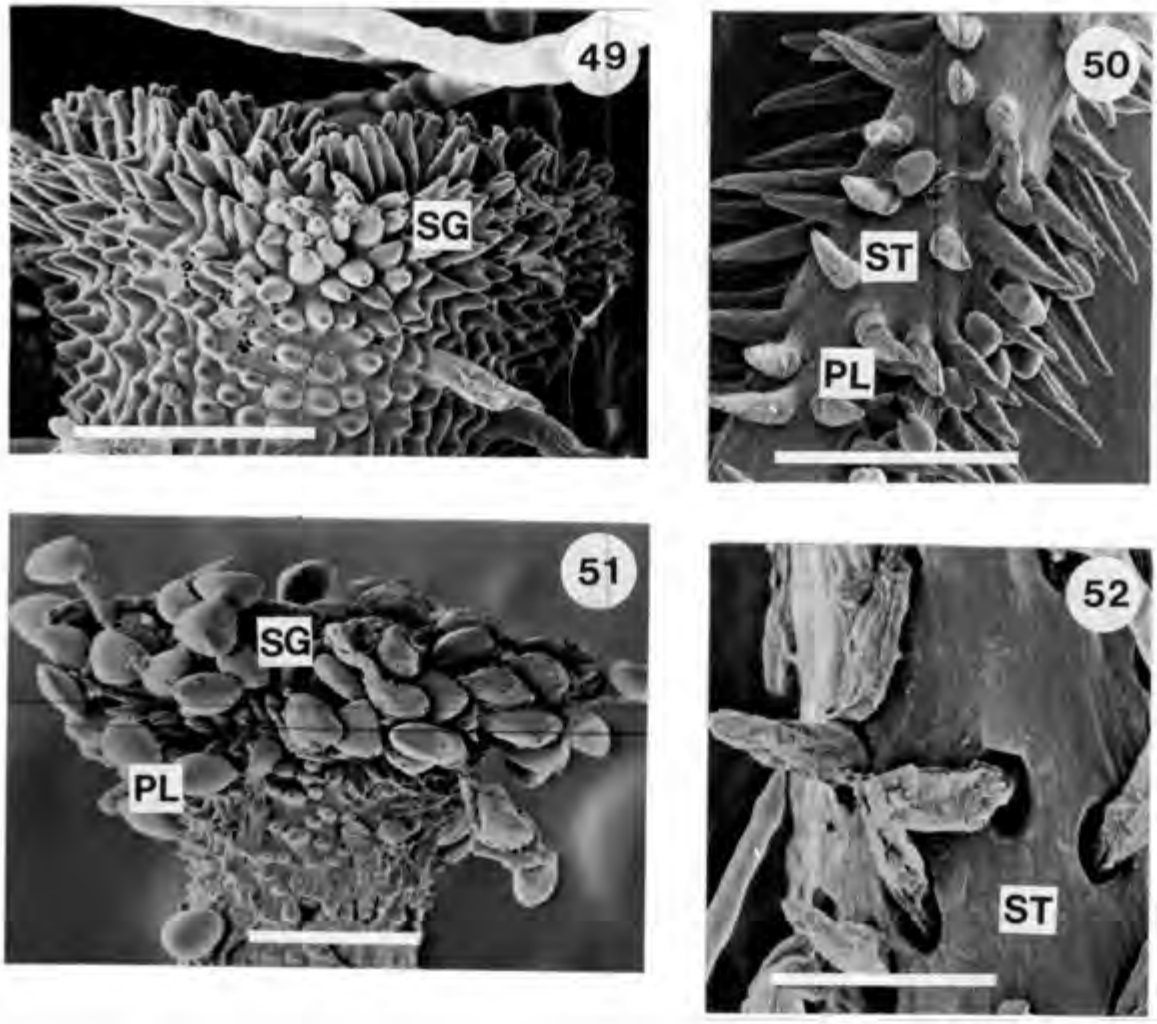
Late sympetaly is evident in the five young petals which enclose the reproductive parts of this species (Fig 38). Once the corolla is removed, the young style surrounded by connivent stamens is visible (Fig 39, 40). The tip of the style is not split as in *L.longifolia* and as Dyer (1975) suggests it should be. It is cylindrical and has a broad rim at the apex, which demarcates the position of the stigma (Fig 41). The cells constituting this apical brim are round and arranged in no particular pattern; while the stylar cells below this are linearly aligned (Fig 41). The concave stigma displays early initiation of papillae, which eventually cover its entire surface (Fig 41, 43, 44). Stamen formation is similar to that found in *L.longifolia*. Stamen apical cell enlargement takes place prior to any cell enlargement on the style or stigma (Fig 39), these enlarged cells combine to form rounded apical tips (Fig 43, 44). Stylar hair initiation occurs synchronously, after development of the stigmatic papillae, to form large bulbous structures (Fig 43), which contrasts with the sequential stylar hair development in *L.longifolia*. The filaments and style both start to elongate soon after stylar hairs have been initiated (Fig 44, 45); whereby lifting the stamens so that the anther bases are eventually aligned with the basal stylar hairs (Fig 46). The lower portion of the style remains glabrous and does not develop the long spindly hairs found in *Lightfootia longifolia*. The mature stylar hairs are widely spaced, smooth and taper at their ends, all of them point obliquely downwards (Fig 47). On closer observation of the stigma, it was discovered that the entire surface is covered by a fine membrane, which ends abruptly beneath the stigma (Fig 48). This membrane must have some elastic properties; which enable it to stretch over the elongating stigmatic papillae somewhat like 'cling-wrap'. This membraneous covering remains entire until anther dehiscence (Fig 49, 50). The style was observed to grow extensively after anther dehiscence, until it was twice the length of the dehisced anthers. The stylar pollen collecting hairs retract into their bases after anther dehiscence, although in this species the whole hair seems to shrivel before it is retracted (Fig 52). At the onset of stylar hair retraction the membrane covering the stigma starts to disintegrate, and the stigma looks as if it is covered by "cobwebs". Self pollen was found to cover the stigma of a flower which had no pollen on the style: stylar hairs had already started retracting (Fig 51).



Figs. 38-44. Early development in *Siphocodon debilis* (Campanulaceae). - 38, Sepals removed, petals enclosing the developing androecium and gynoecium. - 39, Sepals and petals removed, young stamens with enlarged apical cells, the young style has a broadened rim around its apex which demarcates the stigma. - 40, The broadened rim of the stigma extends around the entire circumference and the stigma has a distinctly concave centre. - 41, Detailed structure of stigma and stylar cells. - 43, The stylar hairs are all simultaneously initiated as rounded knobs, note the development of the stigmatic papillae, seen from above. -44, Elongation of style and stylar hairs, note developing filaments and pointed apex of anthers. P = Petal, S = Stamen, ST = Style. - Scale bars for Figs. 38, 40, 43, 44 = 200μ, for Fig. 39 = 100μ, for Fig. 41 = 50μ.



Figs. 45-48. Late development in *Siphocodon debilis* (Campanulaceae). - 45, Elongation of stylar hairs which now cover 3/4 of style. - 46, Elongation of style and development of filaments. - 47, Style covered in smooth, tapering, downward pointing stylar (PCH) hairs which are evenly spaced and equal lengths. - 48, A thin membrane covers the surface of the stigma, this membrane ends abruptly beneath the stigma, note how the stigmatic papillae continue growing without breaking this covering. P = Petal, S = Stamen, ST = Style, SG = Stigma. - Scale bars for Figs. 45-47 = 200μ, for Fig. 48 = 50μ.



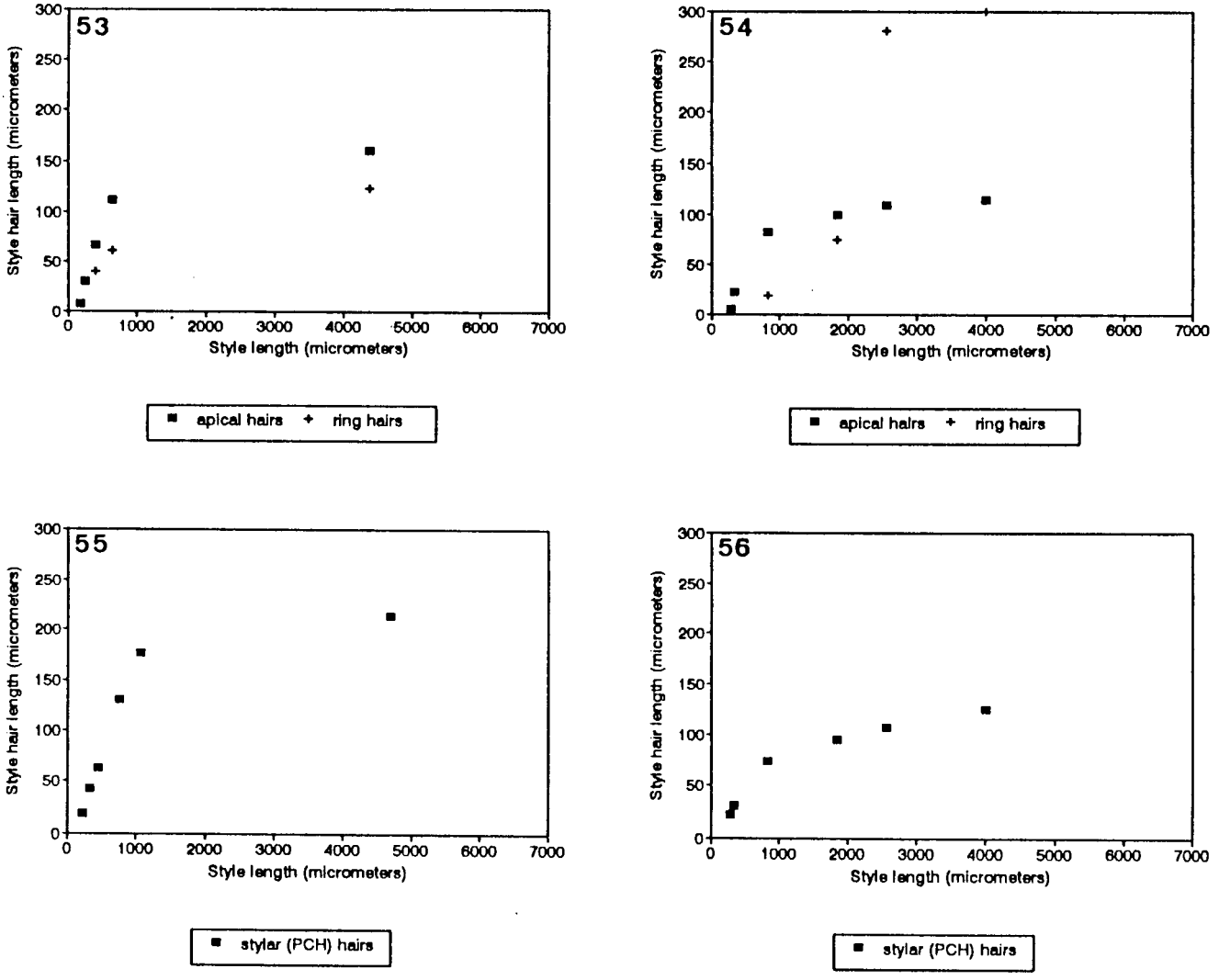
Figs. 49-52. Later development of *Siphocodon debilis* (Campanulaceae). - 49, Stigma covered in thin membrane at anther dehiscence. - 50, Downward pointing stylar hairs at anther dehiscence, note conspecific pollen grains between the hairs. - 51, Stigma covered in pollen at stage after anther dehiscence, the stigma is raised above the height of the stamens at this stage (hair retraction occurs at the end of the male phase before the onset of the female phase. - 52, Retraction of stylar hairs into base, note withered appearance of hairs. SG = Stigma, ST = Style, PL = Pollen. - Scale bars for Figs. 49-52 = 100μ

3.3.Quantitative differences in early developmental stages of the pollen presenters

Style length and apical style hair length were measured (Fig 53 - 56) to ascertain quantitative growth relationships between specific pollen presenters. Pollen presenter development is clearly dichotomised into two stages: an early stage which involves rapid elongation of the stylar hairs and a later stage which consists primarily of stylar extension, hair length having reached a constant value. Style length at anther dehiscence differs between the four species, however, further extension of the style occurs after anther dehiscence. *Lightfootia longifolia* obtains the longest hairs while the other three species obtain similar sized hairs at maturity. In both the Lobeliaceae species examined, apical hair growth is established before the lower ring of hairs is initiated and the length of these hairs varies between species. While *G.bergiana* has both apical and ring hairs reaching approximately the same size at maturity (Fig 53), the ring hairs obtain much greater lengths in *M.flava.*

3.4.Developmental sequence of pollen presenters

Using bud diameter as an indicator of age, it was found that both Lobeliaceae species followed the same sequence of developmental events: 1 - gynoecium initiation, 2 - apical hair initiation, 3 - ring/stylar hair initiation, 4 - pollen presenter maturation (anther dehiscence) (Fig 57). Similarly, the two Campanulaceae species also followed the same sequence of events but no distinct apical stylar hairs developed. The Campanulaceae buds are slightly smaller than the Lobeliaceae buds at gynoecium initiation and pollen presenter maturation. *M.flava* has apical hairs initiated relatively early compared to *G.bergiana.* There is no marked difference in the timing of ring hair development between *G.bergiana* and *M.flava,* although these structures are found at different positions on the style.



Figs. 53-56. Plots of style hair length versus style length for *Grammatotheca bergiana* (Fig. 53), *Monopsis flava* (Fig. 54), *Lightfootia longifolia* (Fig. 55) and *Siphocodon debilis* (Fig. 56). Apical and ring stylar hair were measured for the two Lobeliaceae species while only Pollen collecting hairs were measured for Campanulaceae species.

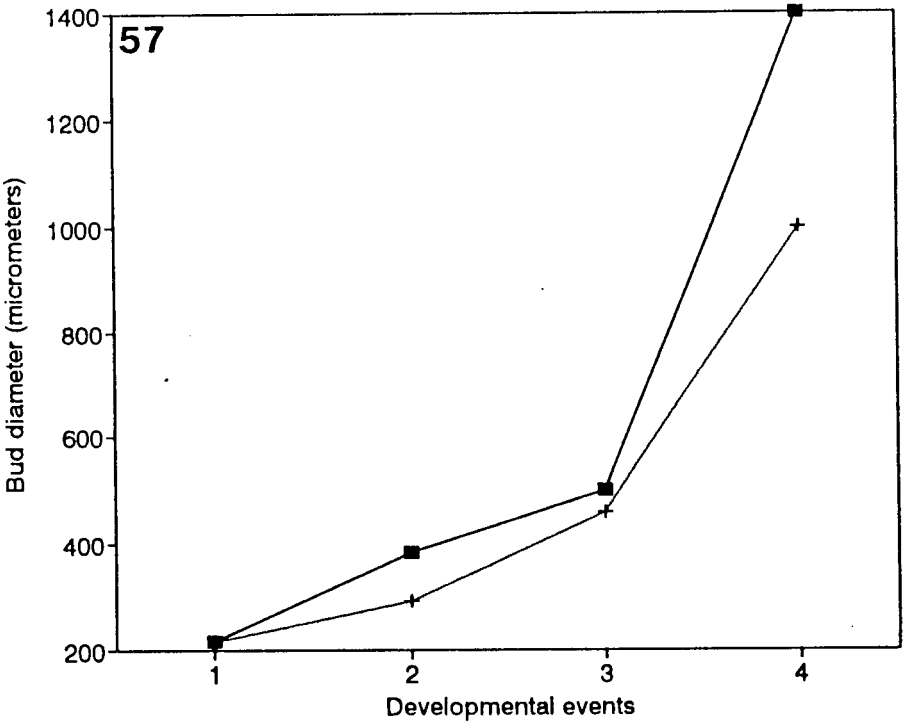


Fig. 57. Plot of developmental events involved in pollen presenter formation versus bud diameter for Lobeliaceae species: *Grammatotheca bergiana* (■) and *Monopsis flava* (+). 1 = Gynoecium initiation. 2 = Apical hair initiation. 3 = Ring hair initiation. 4 = Pollen presenter maturation (anther dehiscence).

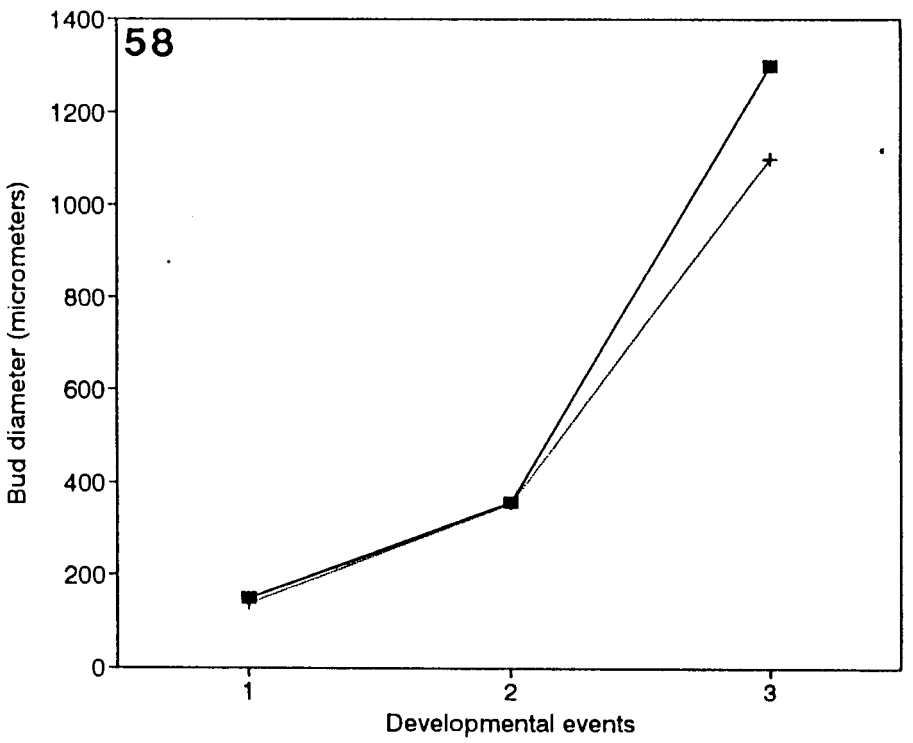


Fig. 58. Plot of developmental events involved in pollen presenter formation versus bud diameter for Campanulaceae species: *Lightfootia longifolia* (■) and *Siphocodon debilis* (+). 1 = Gynoecium initiation. 2 = Stylar hair initiation. 3 = Pollen presenter maturation (anther dehiscence).

4.DISCUSSION

Evolutionary theory is dichotomised into two opposing perspectives, concerning the role of ontogenetic change (Hufford, 1988). The first perspective embodies a strong faith in reductionism and advocates, that the same types of changes are responsible for creating both micro- and macroevolutionary levels of morphological distinction (Mayr, 1963; Stebbins, 1975). Stebbins (1975) has proposed that the two levels differ in "emphasis rather than kind". Both authors agree that transspecific evolution is nothing but an extrapolation and magnification of events, which take place within populations and species. The second, alternative, perspective is that different types of alterations in ontogenies may result in micro- and macroevolutionary patterns. Gould (1977, 1980) proposed that evolution should be considered to be a hierarchical process with complementary but different modes of change at its three major levels: variation within populations, speciation, and patterns of macroevolution. This discussion first focuses on ontogenetic modifications leading to the divergent pollen presenter forms which constitute family level distinctions (macroevolutionary patterns) and then investigates differences evident at the species level (microevolutionary patterns).

Since no phylogeny is currently available for the relationships between members of these two families, ontogenetic studies can merely provide us with phylogenetic hypotheses of the four species investigated.

4.1 Ontogenetic distinctions between Campanulaceae and Lobeliaceae (macroevolution patterns)

The ontogenesis of these pollen presenters and auxiliary structures can be divided into a number of successive developmental stages. I have diagrammatically summarised the ontogenetic sequences obtained from the SEM's of Lobeliaceae and Campanulaceae (Fig 59). A) Early androecial development is uniform in the Lobeliaceae and Campanulaceae: the androecium is initiated as five separate, glabrous, antisepalous stamens. These stamens are inserted at the base of the corolla tube, on the floral receptacle, or on top of the inferior ovary (Lammers, 1992). B) The gynoecium is initiated soon after stamen primordia have developed; both families displaying epigyny (inferior ovaries). C) Both families exhibit early enlargement of apical stamen cells, however, only in Lobeliaceae do these develop into hairs.

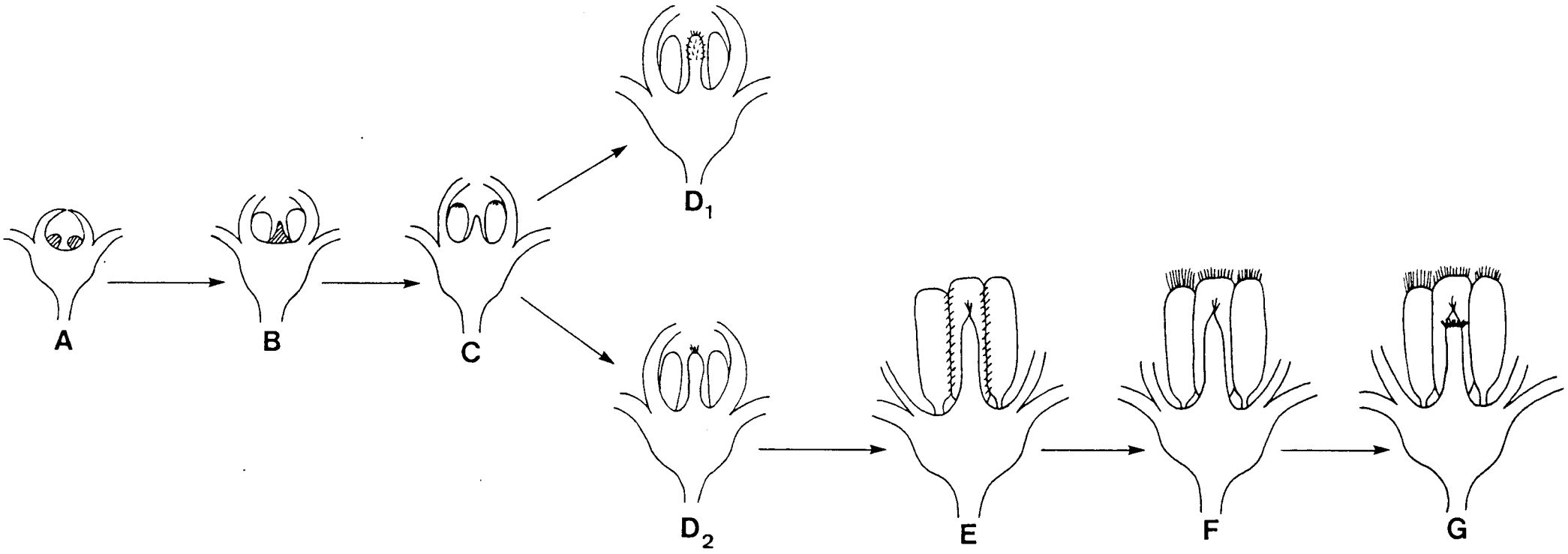


Fig. 59. Diagrammatic summary of developmental sequence involved in the ontogenesis of pollen presenters and auxiliary structures, for Campanulaceae and Lobeliaceae. A = Androecium initiation. B = Gynoecium initiation. C = Conspicuous enlargement of apical stamen cells. Development of Campanulaceae and Lobeliaceae diverges at this point. D_1 = Stylar hair initiation in Campanulaceae. D_2 = Apical stylar hairs initiated in Lobeliaceae. Campanulaceae pollen presenters are mature at this stage and able to receive pollen from the dehiscent anthers. E = Anther tube initiation in Lobeliaceae, followed or preceded, depending on which species is being considered, by F = Anther tuft formation. G = Ring hair initiation.

At this point in the sequence morphological divergence between the two families occurs. Apical stylar hairs are initiated in both Lobeliaceae species (D_2). While stylar hairs are initiated from the top, down the length of the style in the Campanulaceae species (D_1). The Campanulaceae do not develop an anther tube and pollen presenter development is completed once the stylar hairs have matured. The anther tube, which is an integral part of Lobeliaceae secondary pollen presentation; is initiated by fusion of adjacent anther cuticles (E). This is followed, or preceded, depending on which species is considered, by the development of anther tufts (F). The initiation of the lower ring of stylar hairs is the final event in the development of these pollen presenters (G).

Alberch (1985) criticizes the utilisation and interpretation of developmental "sequences" in ontogenetic studies. He argues firstly that an ontogeny cannot be described as a sequence of discrete stages. He distinguishes between "causal" and "temporal" sequences (refer to his Fig. 2), with only the former being considered to be of any phylogenetic use. I oppose this "causal" argument, since development of a certain structure is not an isolated procedure. The removal of an organ which is situated close to, but not directly associated with the initiation of a neighbouring structure, may indirectly affect its development i.e. the effect of stamen exisation on stylar development. This "effect" can, however, only be tested by careful experimental analysis. The problem of discrete stages is more complex since the continuous and usually gradual nature of ontogeny, does not always allow it to be cut in some non-arbitrary manner (Kluge and Strauss, 1985) i.e. when does a particular stage begin and end? Since the stages depicted here are morphologically distinct, the latter problem is less serious in this specific instance. Although developmental sequences are not free from these interpretational problems, they provide valuable insights into the nature of evolutionary patterns and processes. However, interpretation must be done with caution.

Understanding character polarity (which character conditions are primitive and which derived), is of critical importance to phylogenetic systematics (Mabee, 1989), since only synapomorphies can be used to recognise monophyletic groups (Hennig, 1966). Ontogeny constitutes one of two phylogenetic methods for establishing character polarity, the other being outgroup comparison. The ontogenetic criteria has been proposed as a "direct" method for assessing character polarity: polarity is recognised through direct observation of the

the development of a character in two putative sister taxa (Nelson and Platnick, 1981; Weston, 1988). This method can be contrasted with the outgroup method, which is "indirect" since polarity determination requires a prior hypothesis of higher level phylogeny and at least three taxa (Mabee, 1989). The ontogenetic criterion is stated as follows (Nelson's, 1978 biogenetic law as cited by Nelson and Platnick, 1981): "Given an ontogenetic character transformation, from a character observed to be more general to a character observed to be less general, the more general character is primitive and the less general advanced". The degree of generality of a character is directly determined by observing in how many ontogenies it appears. Thus for anther tube formation, the following conclusions can be drawn from the two distinct ontogenies:

Lobeliaceae: 0 - separate stamens initiated (A) - elongation and lateral growth (B) - fusion of anther cuticles to form anther tube (C)
Campanulaceae: 0 - separate stamens initiated(A') - elongation and lateral growth (B')

Assuming that A and A' and B and B' can be homologised, these two characters are considered more general, relative to C, since they appear in both Lobeliaceae and Campanulaceae. Thus anther tube formation is the derived form according to this hypothesis. Since this is in agreement with Lammers (1992) proposed phylogeny for these families the ontogeny criterion appears to be useful in inferring phylogenetic polarity. Kluge (1985) claims that the term "general" is ambiguous as it can be viewed in terms of strict temporal precedence or commonality. The more "general" character state could be that which arises first in ontogeny, or that which is more commonly distributed among the ontogenetic stages in the members of the study group, regardless of ontogenetic sequence. Rosen (1982) chose the former interpretation, Nelson (1978 cited by Nelson and Platnick, 1981) and de Quieroz (1985) the latter. Generality may not be the same as commonality in a universal sense but, when analysis is restricted to an ingroup (as is done with the ontogeny criterion), generality and commonality are identical (Kraus, 1988). A severe problem with Nelson's ontogeny criteria, is that paedomorphic characters (where juvenile ancestral are retained by the adult descendant); which have evolved via reverse recapitulation or terminal deletion, will be incorrectly polarised using this criterion (Kraus, 1988). Nelson's ontogeny criterion will correctly polarize characters to the extent that those characters have evolved via terminal

addition or recapitulation. Since terminal addition is only one of six possible means of evolutionarily altering ontogenies (de Quieroz, 1985, see also Kluge, 1985). The utility of the ontogenetic criteria is thus dependant on the frequency of evolutionary alterations in a particular ontogeny. While it is evident that ontogenetic theory and terminology (developmental 'sequences', 'generality' of traits) is wrought with controve2rsy, there have been few empirical studies, especially of plants, which support these arguments either way.

Assuming that the development of an anther tube in Lobeliaceae is an advanced character state, there must be some associated advantages to this structure, in comparison to the Campanulaceae anthers which shrivel up to expose the pollen coated style (Campanulaceae). I propose that the anther tube plays an active role in pollen presentation, as apposed to the assistant role which it has previously been assumed to play. The anther tufts actually present pollen which is pushed out of the anther tube and gets lodged in between these tufts, to insects. Pollen longevity may also be increased by its retention inside the protective environment of the anther tube between anther dehiscence and style elongation. The Campanulaceae contrast this by simultaneously exposing all pollen to the 'harsh' physical environment. Since the lower ring of hairs block off the base of the anther tube, this may additionally reduce loss of pollen from the bottom of the flower which is likely to occur in the Campanulaceae species. Carolin (1960), in studying the evolution of pollen presenting structures in the order Campanales, proposed that the trend was first sequential presentation and subsequent pollen protection. Since Campanulaceae are found to be far more common in dry areas than moist areas; Carolin (1960) has suggested that the family may have evolved in these areas and that protected pollen presentation of the Lobeliaceae and Goodeniaceae may have been associated with the invasion of moister areas. This narrative approach is appealing, but extremely simplistic and could be argued in either direction. In addition, the exact advantages which protection confers in a moist environment are questionable: surely the threat of pollen desiccation is greater than that of rain (Linder, pers comm.).

The position of the anther bases relative to the stylar hairs just prior to anther dehiscence, is important in determining which stylar hairs play the most important role in secondary pollen presentation. In both Lobeliaceae species investigated, the anther bases were aligned with the base of the ring hairs on the style. This alignment was also found for Lobeliaceae

species investigated by Erbar and Leins (1989) and Leins and Erbar (1990). The lower ring of hairs is thus responsible for collecting pollen while the apical hairs function as a piston; to push pollen out of the anther tube onto the apical anther tufts. This ring of hairs additionally functions to seal the bottom of the anther tube in both species of Lobeliaceae, so that pollen can only be pushed upwards and out.

Distinct differences in amount and position of stylar hairs exist between the two families. The "pump-mechanism" of pollen presentation in Lobeliaceae is associated with the development of an anther tube and a reduction of stylar hairs. The "stylar brush" (Leins and Erbar, 1990) has been replaced with the ring of hairs below the stylar apical tufts. These ring hairs have bulbous ends instead of tapering tips which occur on the apical Lobeliaceae and stylar Campanulaceae hairs. These bulbous hairs may be more efficient at supporting and subsequently moving clumps of pollen, as apposed to the tapering, widely spaced hairs of Campanulaceae which allow pollen grains to remain lodged, until they are actively removed, by insect pollinators (Nyman, 1993b). It is impossible to determine character polarity for these lower ring hairs using Nelson's law, since there is no general condition. Evolution may subsequently have proceeded from a style covered in hairs to a naked style with a ring of hairs or vice versa. The advantage of the ring of hairs in Lobeliaceae can, however, only be assessed together with the anther tube; since it is the combined functioning of these two structures which makes pollen presentation possible. It is thus possible that the advantages of pollen protection in Lobeliaceae exceed those of sequential pollen presentation in Campanulaceae. The formation of an anther tube would not have been extremely complex; since the stamens of Campanulaceae are connivent around the style and separate only once pollen is shed onto the style. Thus all that was needed was the physical connection between these anthers: the fusion of the cuticles. The retracting hairs, treated as isolated structures, are more advanced than the simple stylar hairs present in the Lobeliaceae. The function of these hairs is however still being disputed, Shetler (1979) eloquently reviews the history of these fascinating structures, and it is only recently that Nyman (1993 b) has actually demonstrated the adaptive function of these structures.

4.2.Ontogenetic distinctions between species (microevolutionary patterns)

Species level distinctions within the two families are summarised by sequence diagrams (Fig 60, 61). Within the Lobeliaceae the differences are mainly heterochronic: involving changes in developmental timing and include evidence of heterotopic character in *Monopsis*. In the Campanulaceae, differences are primarily qualitative and include the introduction of a novel character in *Siphocodon debilis*.

4.2.a.Lobeliaceae

The developmental procedure is identical for both *G.bergiana* and *M.flava* until apical hair formation on the style (Fig 60). Apical stylar hairs are initiated slightly earlier in *M.flava* (Fig 57). Apical stylar hair growth is initially rapid for both species and then levels of to a plateau (Fig 53, 54). *Monopsis* apical hairs reach a constant length sooner than *Grammatotheca* hairs which continue growing for longer and subsequently reach greater lengths (110 versus 160 micrometers respectively). Anther tube and anther tuft formation occur at alternating stages in the developmental sequence of *M.flava* and *G.bergiana*. If Nelson's hypothesis (that the general condition is ancestral) is advocated, then, the sequence of events in *L.coronopifolia* and *M.flava*: tuft development followed by tube formation, is ancestral. *G.bergiana* displays the reversed sequence of events: tube formation followed by tuft development. Heterochrony is defined by Gould (1977) as " an alteration in the timing of ontogeny such that the relative time of appearance or rate of development of a feature is either retarded or accelerated". The role of heterochrony is considered valuable for understanding: 1) changes in overall duration of development by either extension or shortening, 2) changes in rate of progression through the developmental sequence, and 3) relative time changes among initiation and cessation events within the developmental sequence (Gould, 1977; Alberch et al., 1979). Early tuft formation in *M.flava*, compared to *G.bergiana,* may subsequently be responsible for development of the extremely long anther hairs (refer to SEM's). Although it is artificial to consider the evolution of ontogenies along the lines of either heterochrony or heterotopy; identifying both can be important for establishing how ontogenies within a clade differ, and consequently produce distinct mature forms (Hufford, 1988).

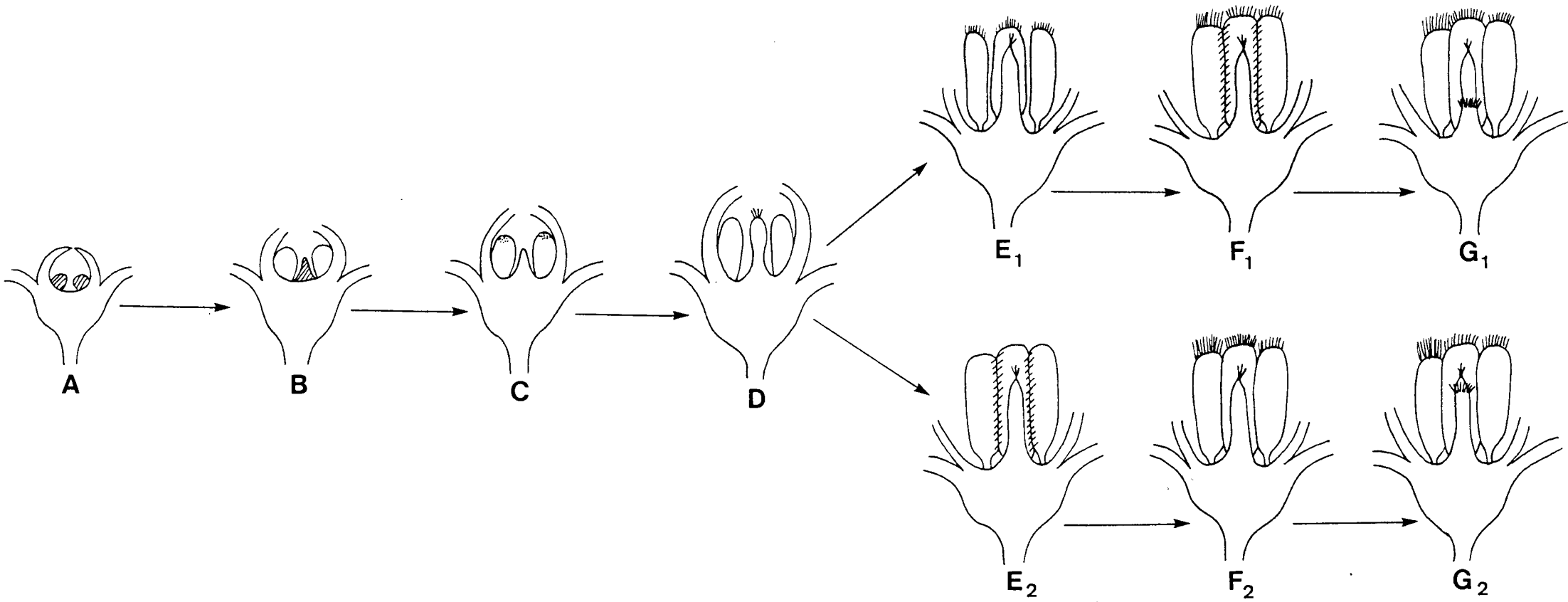


Fig. 60. Summary of developmental sequence for *Grammatotheca bergiana* and *Monopsis flava*. A = Androecium initiation. B = gynoecium initiation. C = Enlargement of apical stamen cells. D = Apical stylar hair initiation. E_1 = Anther tuft formation in *M. flava*. E_2 = Anther tube formation in *G.bergiana*. F_1 = anther tube formation in *M.flava*. F_2 = Apical tuft formation in *G.bergiana*. G_1 and G_2 = Stylar ring hair formation in *M.flava* and *G.bergiana* respectively.

Monopsis and *Grammatotheca* ring hairs develop at the same stage in the developmental sequence (Fig 57, 60), with *Monopsis* hairs being initiated fractionally earlier. *Monopsis* ring hairs exhibit a much greater growth rate and subsequently obtain greater lengths (Fig 53) than conspecific apical hairs or the ring hairs of *Grammatotheca* (Fig 54 and 53 respectively). *M.flava* displays considerable positional displacement of its ring hairs, these are found far down the length of the style in comparison to *G.bergiana* and *L.coronopifolia* which have their ring hairs positioned just below the stigma, near the style apex. Alberch (1985) has stated that heterochrony may be of limited value for interpreting various qualitative changes. Haeckel (1905 cited by Gould, 1977) used the term heterotopy to describe " a phyletic change in location (in the germinal layers) from which an organ differentiates in ontogeny - thus forming an exception to recapitulation". Sattler (1975) has dealt with this term in the botanical context and claims that it is necessary to distinguish between "postgenital" and "congenital" displacements of organs. "Postgenital" displacements are displacements in the strict sense, which result from processes of growth such as intercalary growth and zonal growth. In contrast "congenital" displacements are changes in the position of inception of organs and for this reason the phenomenon is better referred to as heterotopy. This phenomenon is known in many plant cases (refer to Sattler , 1975 for some examples). *Monopsis* ring hairs are an excellent example of "congenital displacement"(heterotopy) since thes hairs were initiated far down the length of the style. The character polarity of a heterotopy is difficult to determine since one cannot necessarily assume that the unusual position is derived. Sattler (1975) further states that heterotopy and displacement s.str. may occur phylogenetically; ie from one organism to another, or sequentially; ie within one organism between serially homologous organs or organ systems. The concept of heterotopy is useful for understanding: alteration of positional arrangement, deletion of forms from established developmental sequences, or the introduction of novel or reiterated structures into the ontogeny.

4.2.b.Campanulaceae

Both *L.longifolia* and *M.flava* follow the same sequence of developmental events (Fig 58). The developmental sequence of events from gynoecium initiation to pollen presenter maturation has been diagrammatically summarised for both these species (Fig 61).

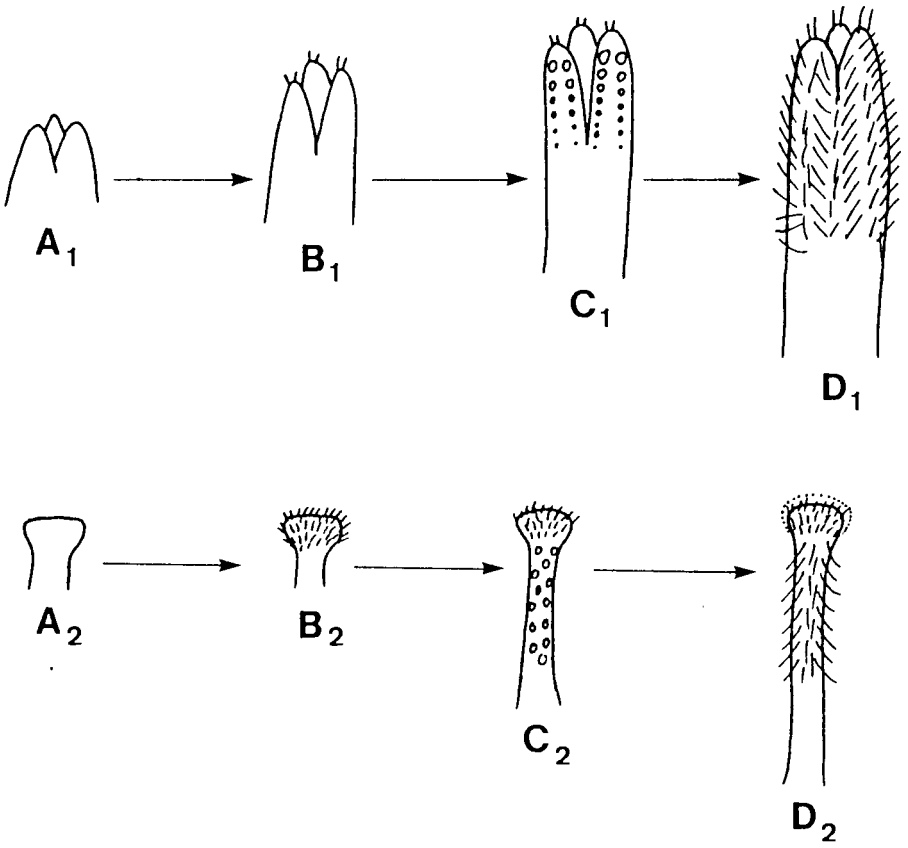


Fig. 61. Diagrammatic sequence of events for Campanulaceae pollen presenter formation. 1 = *Lightfootia longifolia* and 2 = *Siphocodon debilis*. A = Gynoecium initiation. B = Apical stylar hair initiation in *L.longifolia* and formation of stigmatic papillae in *S.debilis*. C = Stylar hair initiation: linear sequential in *L.longifolia* and simultaneous in *S.debilis*. D = Mature pollen presenter with elongated hairs which point downwards in *S.debilis* and slightly upwards in *L.longifolia*. Note the presence of a thin membraneous covering on the stigma of *S.debilis*.

The gynoecium of *L.longifolia* is initiated as three or four partially seperate stylar lobes (A_1). Each style initially develops a few apical hairs (B_1), (as in the Lobeliaceae) which is followed almost immediately by hair development along the length of the style (C_1). Unlike the Lobeliaceae, there is no structural(functional) difference between the apical and stylar hairs. It is interesting that although Campanulaceae stigmas are considered to be protected by stylar tissue (Carolin, 1960; Erbar and Leins, 1989; Leins and Erbar, 1990), they remain partially seperate and "exposed" prior to maturation of the pollen presenter.

S.debilis develops a single style with a 'naked' (unprotected by stylar tissue) stigma at its tip (A_2). This is an interesting discovery since according to Dyer (1975) " the genus *Siphocodon* has a characteristically terete style, which becomes gradually thinner upwards and the stigma consists of three lanceolate granular lobes". This apical concave stigma becomes covered in papillae (B_2). Stylar hairs are initiated simultaneously in *S.debilis* (C_2) compared to the

sequential and row-like development in *L.longifolia*. Although stylar hair formation is initiated almost simultaneously in these two species (Fig 58), the stylar hairs of *L.longifolia* elongate more rapidly and subsequently reach greater lengths than the hairs of *S.debilis*; where maximum hair length is quickly reached (Fig 55 & 56).

The mature pollen presenters differ in the direction in which the PCH's point: upwards in *L.longifolia* (D_1) and downwards in *S.debilis* (D_2). The distinction only becomes evident in the mature pollen presenters once the hairs have elongated. This difference in hair orientation is surprising since both species are thought to utilise the same pollen presentation mechanisms. The downward pointing hairs of *S.debilis* should reduce the efficiency of pollen collection, since pollen grains will not become lodged inbetween the hairs but will prohably slide off the pollen presenter. The discovery of a thin, 'elastic' membrane covering the stigma of *S.debilis* is intriguing. According to Ladd and Donaldson (1993), the pollen presenter of *Siphocodon debilis* is an exception amidst the general elongated, cylindrical structures found in most members of the Campanulaceae. "The main difference is that the stigma is a capitate tip to the style, which is exposed at anthesis and not initially enclosed by non-receptive tissue, as in the other genera". This membrane may have two, possibly alternative, functions. Firstly, to prevent self pollen from germinating on the stigma; since the stigmatic papillae are unable to pierce the membrane it may be hypothesised that pollen tubes will also not be able to penetrate it. Further more, the membrane remains intact after anther dehiscence and its disintegration may be correlated with stigma receptivity. Secondly, this membrane may function to collect and lift conspecific pollen whereby making it available to insect pollinators, similar to the pseudo-indusium found in the Goodeniaceae (Carolin, 1960). This latter hypothesis would explain the reduced function of the pollen collecting hairs and is supported by the fact that the style of *S.debilis* displays such marked elongation after anthesis. *S.debilis* may thus represent an intermediate form between the "stylar-brush" of *L.longifolia* and the "pump-mechanism" of Lobeliaceae. Both Carolin (1960) and Leins and Erbar (1990) illustrate putative phylogenetic arrangements of the different mechanisms of secondary pollen presentation and both authors suggest that the structure found in the Goodeniaceae originated from that of the Lobeliaceae which in turn is thought to have developed from the Campanulaceae (Refer to Fig 7 of Leins and Erbar, 1990). In this case the 'exposed' stigma may be considered an advanced structure which functions somewhat like

a piston and pushes pollen out of the connivent anthers to present it to potential pollinators. The structure of the stigma, which is slightly concave (dish-shaped) in the centre with the expanded rim, is reminiscent of the Goodeniaceae indusium. The structural properties of this unusual stigmatic membrane, however, need to be determined before any conclusions concerning its function are possible.

CONCLUSION

No ontogenetic studies have previously investigated the early developmental stages of Lobeliaceae and Campanulaceae pollen presenters, using SEM techniques. This study has provided intriguing information on developmental sequences and the evolution of morphological differences at both macro- (family) and micro-(species) evolutionary levels. At the macroevolutionary level ontogenetic arguments illustrate that pollen tubes are a derived trait in the Lobeliaceae. Pollen tube formation occurs only after apical stylar hairs have developed and the lower ring hairs are the last developmental event in the ontogenesis of Lobeliaceae pollen presenters. Anther tube formation is argued, from an ontogenetic perspective, to be a derived trait among the Lobeliaceae. Character polarity of Campanulaceae and Lobeliaceae stylar hairs, however, remains speculative. At the microevolutionary level, the Lobeliaceae species display both heterotopic and heterochronic characters. The Campanulaceae species display identical developmental sequences. Development within the Lobeliaceae is identical for both species prior to anther tube formation. The developmental sequence of anther tube and anther tuft formation has been reversed in *G.bergiana*. The ring hairs of *Monopsis flava* additionally display congenital displacement (heterotopy). Within the Campanulaceae, developmental sequences were identical for the two species investigated. The following morphological differences between *L.longifolia* and *S.debilis* emerged : Sequential versus simultaneous development of stylar hairs, style-enclosed versus membrane-covered stigmas, upward versus downward orientated stylar hairs. Early ontogenetic distinctions play a crucial role in the establishment of morphologically divergent pollen presenters between the Lobeliaceae and Campanulaceae and between species within these families. A similar ontogenetic investigation of *Cyphia*, which is considered an evolutionary intermediate between the Lobeliaceae and Campanulaceae, may provide useful ontogenetic information on phylogenetic relationships between these two families.

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