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Evolution of flower shape in *Veroniceae* (*Scrophulariaceae*)

C. M. Kampny and N. G. Dengler

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Key words: *Veroniceae, Scrophulariaceae.* – Flower shape, flower development, quantitative developmental character, phylogeny, evolution.

Abstract: Floral evolution in the tribe *Veroniceae* was examined using phylogenetic analysis combining 24 adult morphology and chromosome number characters with 22 qualitative and quantitative floral development characters. Taxa sampled included nine species of *Veroniceae* and as an outgroup one species each of *Digitaleae* and *Verbasceae. Veronica*, *Besseya*, and *Synthyris* formed one clade, subtended by *Pseudolysimachion* and then by the *Hebe* group; *Veronicastrum* or *Wulfenia* represent the basal-most branch of the tribe. The ancestral flowers of the *Veroniceae* may have been small with moderately short corolla tubes and lobes; long corolla tubes arose four times in the tribe and large corolla lobes twice.

Changes in flower shape and other aspects of plant reproduction evolve mainly through heterochronic changes in organ growth rates (Guerrant 1982, Kirchoff 1983, Lord & Hill 1987, Hufford 1988, Kellogg 1990, Diggle 1992). Exact flower shape is important in species in which pollinators respond to the attractive display provided usually by the corolla, and effective pollen transfer results from the fit of the pollinator to the flower, thereby exerting selection pressure. In bilaterally symmetric, tubular flowers like those of the *Scrophulariaceae*, pollen transfer is further assured by manipulation of pollinator behavior through the form of the corolla tube and its coloration or trichomes (nectar guides) (for examples see Endress 1994, Kampny 1995b). Heterochronic changes cannot be observed directly, but can be inferred by comparing related organisms for which both phylogenetic information and detailed descriptions of development are available.

Ontogenetic characters such as organ initiation sequences can be used in phylogenetic analysis in combination with adult characters (Tucker & al. 1993). Quantitative studies of floral development in the *Veroniceae* have shown that patterns of relative organ growth, especially of the corolla lobes and tube, differ significantly between genera (Kampny & al. 1993, 1994). These differences can be captured to yield character states for phylogenetic analysis (see below, Material and methods; KAMPNY 1995a). Since early growth allometries are not correlated with flower proportions at later stages and at anthesis (KAMPNY & al. 1993, 1994), they yield a number of new characters. When combined with other characters for cladistic analysis, rather than mapped on independently derived trees, they should give the most parsimonious estimate of phylogeny and flower evolution calculated from all possible information.

The *Veroniceae* are a tribe of the *Scrophulariaceae* with worldwide distribution. Their members exhibit some combinations of features that have led to varying interpretations of relationship (PENNELL 1935, THIERET 1955, YAMAZAKI 1957, HONG 1984). Species range from herbaceous annuals and perennials with cauline leaves and terminal or axillary inflorescences to small trees. Some are rosette plants with axillary inflorescences. Calyx tubes are very short in the tribe. Corolla tubes vary from long to very short, and in some cases only the lower three corolla lobes are confluent; corolla lobe proportions also differ (Fig. 1). Most *Veroniceae* have tetramerous perianths and two stamens on the upper side of the flower; exceptions with four stamens occur only in two genera (*Picrorhiza, Neopicrorhiza*). Pollen is tricolporate or -colpate with variations of reticulate surface sculpting, and seeds range from hemi-ovoid to flat to ventrally concave and show various surface sculpting (HONG 1984; YAMAZAKI 1957; THIERET 1955; PENNELL 1921, 1935; WETTSTEIN 1891).

The pre-cladistic studies of PENNELL (1935) and THIERET (1955) arranged the members of the Veroniceae into two series, one of cauline-leaved plants starting with *Veronicastrum* and leading to *Veronica* and *Hebe*, and the other of rosette plants leading from *Picrorhiza* and *Wulfenia* to *Synthyris* and *Besseya*. YAMAZAKI (1957) diagrammed a possible phylogeny of the tribe based on seed development as well as vegetative and reproductive morphology which showed *Picrorhiza, Wulfenia*, and *Veronicastrum* as more basal taxa, and *Synthyris* and *Besseya* as close relatives to *Veronica* (which included *Pseudolysimachion*), *Hebe*, and *Derwentia*.

The cladistic study of HONG (1984) used standard morphological as well as palynological characters in his manual analysis of the tribe; his placement of the genera mainly corresponded to that of YAMAZAKI (1957), but listed *Pseudolysimachion* as closest relative to *Veronica*. Processing of HONG's (1984) data matrix with the now more widely available computer programmes has yielded 352 equally parsimonious trees all differing somewhat in topology from the one chosen by HONG; their consensus tree does not resolve the placement of the more basal genera and of *Pseudolysimachion* (KAMPNY 1995a).

The *Veroniceae* were established by BENTHAM (1846) but later lumped with the *Digitaleae* (BENTHAM 1876, WETTSTEIN 1891). PENNELL (1921, 1935) re-established the *Veroniceae* as a separate tribe, with *Digitaleae* as closest relatives (THIERET 1955, HONG 1984). *Aragoa*, included in *Veroniceae* by THIERET (1955), has been assigned tribal status by itself (NILSSON & HONG 1993). The only sharply definable character helping to distinguish the *Digitalea*e from the *Veroniceae* is the septicidal capsule dehiscence in the former contrasting with the loculicidal or both loculicidal and septicidal dehiscence in *Veroniceae* (HONG 1984).

The floral development pattern of *Pseudolysimachion* reported by NOLL (1883) is more similar to that of *Veronicastrum* in showing less delay in corolla growth

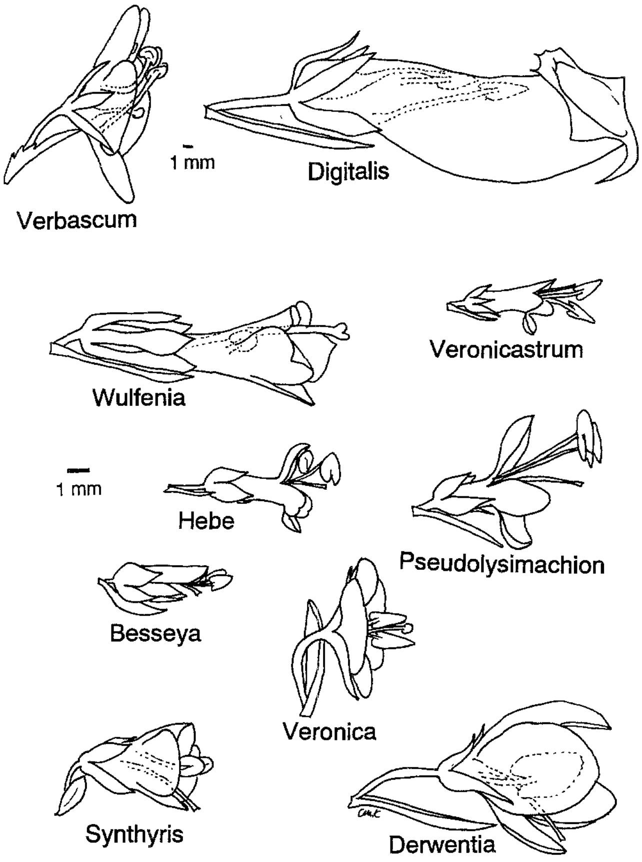


Fig. 1. Flower types of *Veroniceae*, *Digitalis* (*Digitaleae*), and *Verbascum* (*Verbasceae*), using species included in analysis of floral development as examples. *Verbascum blattaria*, calyx and corolla pentamerous, corolla tube short, lobes large; stamens five. *Digitalis grandiflora*, calyx and corolla pentamerous, corolla tube long, lobes short; stamens four. *Wulfenia carinthiaca*, calyx and corolla pentamerous, corolla tube long, lobes moderate; note stamen insertion near distal end of tube. *Veronicastrum virginicum*, calyx pentamerous, corolla tetramerous, corolla tube long, lobes short; stamen insertion near base of tube. *Hebe parviflora*, calyx and corolla tetramerous, corolla tube long, lobes short, stamen insertion in distal part of tube. *Pseudolysimachion longifolium*, calyx and corolla tetramerous, corolla tube long, lobes long. *Besseya alpina*, calyx and corolla tetramerous, corolla consists of large upper lip and small lower lip of three lobes on partial "tube"; not shown is lack of upper corolla tube, i.e. stamens are attached to receptacle. *Veronica chamaedrys*, calyx and corolla tetramerous, corolla tube very short, lobes large, stamen insertion low on corolla tube; flower of *V. serpyllifolia* similar but smaller. *Synthyris reniformis*, calyx and corolla tetramerous, corolla tube long, lobes large. *Derwentia perfoliolata*, calyx and corolla tetramerous, corolla tube moderate, lobes large

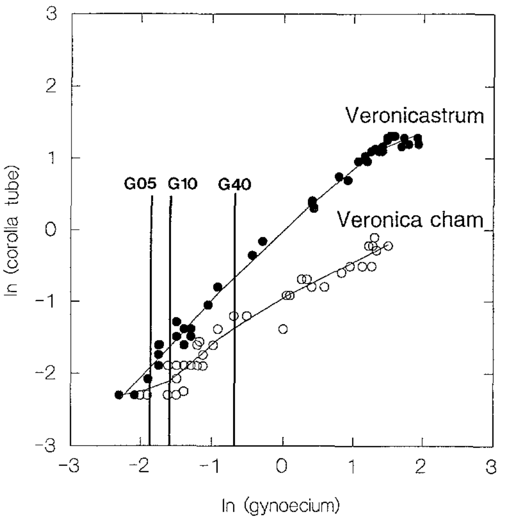
than that of *Veronica* (Payer 1857, Kampny & al. 1993). Since it was possible to extract characters from floral organ growth curves of *Veroniceae* (Kampny 1995a), this information can now be added to standard morphological characters to test whether it supports the position of *Besseya* and *Synthyris* with *Veronica* (as suggested by Yamazaki 1957 and Hong 1984) rather than with *Wulfenia* (as suggested by PENNELL 1933, 1935, and THIERET 1955), and to see whether the placement of *Pseudolysimachion* can be clarified. It would also be desirable to find additional characters that can be used in the circumscription of the tribe *Veroniceae*. Separate analysis of mature and developmental data will show which relationships are supported by each data set, and the combined analysis can give insight into the course of flower evolution in the tribe.

Material and methods

Vegetative morphology and qualitative flower features as well as pollen, seeds, and chromosome numbers were used to define 24 characters for nine species of *Veroniceae*, one of *Digitaleae*, and one of *Verbasceae* (Tables 1, 2; Appendix 1); unfortunately, garden-grown specimens of species of *Isoplexis*, *Erinus* (*Digitaleae*), and *Chelone* (*Cheloneae*) did not yield flowering material for analysis of development so that these potential outgroup species had to be omitted from the study. Most characters had two states, some three to five; they were treated as unordered (see Tables 2, 4). Vegetative and reproductive morphology including floral organ numbers were scored from herbarium specimens, usually 50 per species, borrowed from several botanic gardens (Table 1); for a list of individual specimens used see KAMPNY (1995a). Character states were established as described in Table 2 and Appendix 1. Seed and pollen samples were taken and prepared as described in KAMPNY (1995a). Pollen samples were taken from the formalin-acetic acid-alcohol (FAA) preserved material that was also used in analysis of floral development. Whole mature anthers were critical point dried in an Autosamdri 814 and placed on stubs; pollen was removed from the anthers and also placed on stubs. Specimens were coated with gold-palladium in a SEM Coating Unit PS3. Anthers, pollen, and seeds were photographed under a Hitachi S-2550 scanning electron microscope (SEM) at 10 kV. Chromosome numbers were taken from HONG (1984) and WALTERS & WEBB (1972).

Table 1. Sources of material used for mature morphology (herbarium specimens) and floral development (FAA-preserved flowers) studies. *CHR* Christchurch Herbarium, New Zealand; *JE* Jena Herbarium, Germany; *MO* Missouri Botanic Garden, USA; *NSW* New South Wales Botanic Garden, Australia; *NY* New York Botanic Garden, USA; *OAC* University of Guelph Herbarium, Canada; *OSC* Oregon State Herbarium, USA; *RM* Rocky Mountain Herbarium, USA; *TRT* Royal Ontario Museum Herbarium, Canada; *WS* Washington State Herbarium

Species	Herbarium specimens	Liquid specimens	Vouchers
Veronica chamaedrys L.	MO, NY	CANNE-HILLIKER 3024	OAC
V. serpyllifolia L.	MO, NY	CANNE-HILLIKER 3046	OAC
Synthyris reniformis (DOUGL.) BENTH.	OSC	HUFFORD 484, FREDRICKS 402,	WS
		HALSE 4584	TRT
Besseya alpina (GRAY) RYDB.	RM	HUFFORD 243	WS
Pseudolysimachion longifolium (L.) OPIZ	MO, NY	KAMPNY 226, KAMPNY 227	TRT
Hebe parviflora (VAHL) COCKAYNE & ALLAN	CHR	GARNOCK-JONES s. n.	TRT
Derwentia perfoliolata (R. BR.) RAF.	MO, NSW	NSW8502518C	NSW
Veronicastrum virginicum (L.) FARW.	MO	CANNE-HILLIKER 3053	OAC
Wulfenia carinthiaca JACQ.	JE	KAMPNY 237	TRT
Digitalis grandiflora MILLER	MO, NY	KAMPNY 235	TRT
Verbascum blattaria L.	TRT	KAMPNY 228	TRT



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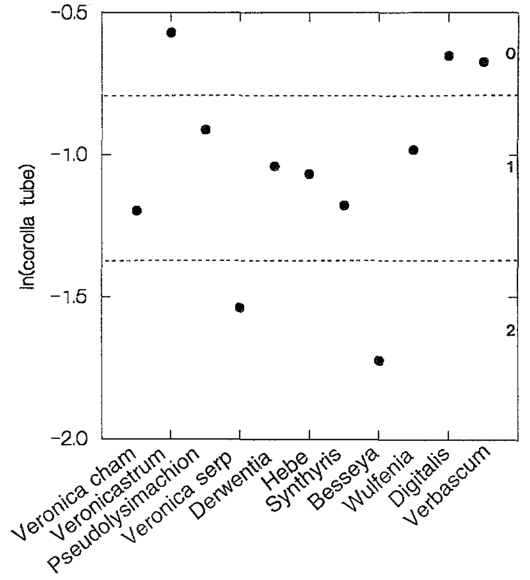

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Fig. 2. Example of allometric plots, drawn with LOWESS (locally weighted scatterplot smoothing), of corolla tubes of *Veronica chamaedrys* (circles) and *Veronicastrum virginicum* (dots); intercepts determined for gynoecium sizes of 0.05 mm (G05), 0.10 mm (G10), and 0.40 mm (G40). – Fig. 3. Example plot of result of KMEANS grouping of intercepts of corolla tube growth curves at gynoecium size 0.40 mm (G40), and assignment of character states. *Veronicastrum virginicum*, *Digitalis grandiflora*, and *Verbascum blattaria* show large intercepts, i.e. fastest growth (character state 0); *Veronica chamaedrys, Pseudolysimachion longifolium*, *Derwentia perfoliolata*, *Hebe parviflora*, *Synthyris reniformis*, and *Wulfenia carinthiaca* are intermediate (1), and *Veronica serpyllifolia* and *Besseya alpina* have small intercepts, i.e. slowest growth (2)

Twenty-two floral developmental characters orginated from SEM observations for organ initiation sequences, and from measurements under the dissecting microscope for assessment of organ growth patterns of calyx lobes, corolla lobes, corolla tube, stamen insertion height, and anther length (Tables 3, 5), as described in Kampny & al. (1993). Sources of inflorescences and vouchers are listed in Table 1. For each species, all organ types of 50 buds were measured, ranging in age from initiation to anthesis (i.e. total measurements were 50 buds × 5 organ types × 11 species = 2750). The 50 measurements per species of each organ type were plotted over gynoecium length as an indicator of bud age (Fig. 2). The species' growth patterns for each organ type were estimated with LOWESS (locally weighted scatterplot smoothing): this uses locally weighted regressions at each of the 50 data points to produce a non-parametric curve (Fig. 2). The so generated "average" growth patterns for each organ of diverse species are significantly different since intra-plant and intra-species variation is low (Kampny & al. 1994).

Character states for growth patterns for each organ type at each of three growth stages were then assigned as follows: (a) plotting the intercepts of LOWESS-traced growth curves of all species at three gynoecium sizes corresponding to open ovary (G05, 0.05 mm), ovary closing (G10, 0.10 mm), and beginning of style growth (G40, 0.40 mm) (see Fig. 2; intercepts are listed in Appendix 2) using S-plus (Statistical Sciences, Inc., 1993); (b) for each organ and gynoecium size, the intercepts of the species were divided into three groups using the clustering program KMEANS of SYSTAT (Wilkinson 1990), which iterates until the sums-of-squares between groups are maximized compared to those within groups; (c) the resulting groups were then assigned three character states of long, mid-sized, and short (Fig. 3). For a more detailed discussion of extracting characters from developmental data see KAMPNY (1995a). Organs of flowers at anthesis were also measured and the results divided into groups for assignment of character states. Thus, 22 characters (the 23rd, anther size at maturity, was uninformative and omitted) were available describing both initiation sequence and growth patterns at later stages of development (Tables 3, 5).

The character data were analyzed with Phylogenetic Analysis Using Parsimony (PAUP; SWOFFORD 1993). Three separate analyses were conducted: mature morphology was analyzed, then floral development, then the combined mature morphological and developmental data. Each analysis used the branch-and-bound search option to find all shortest trees; default settings were employed, including furthest-taxon addition sequence. Multistate characters were not reweighted to add up to one step, i.e. all character changes were equivalent. *Digitalis grandiflora* MILLER and *Verbascum blattaria* L. were designated as the outgroup to root the trees. Stability of the branches of the 46-character combination tree was tested by the bootstrap method with a branch-and-bound search, using 100 replications. Decay values of the branches were obtained by using filters to save trees equal to or shorter at one, two, three, etc. steps longer than the shortest tree, and constructing strict consensus trees from the results at each length. Character changes were plotted on trees using MacClade (MADDISON & MADDISON 1992).

Table 2. Mature morphology characters and character states

Character		Characters states
1	Habit	cauline (0), rosette (1)
2	Shoots	herbaceous (0), woody (1)
3	Leaf arrangement	helical (0), opposite (1), whorled (2)
4	Leaf blade	elongate (0), isometric (1), very elongate (2)
5	Leaf pulvinus	absent (0), present (1)
6	Inflorescence	long (0), short (1)
7	Calyx lobe number	5(0), 4(1)
8	Corolla lobe number	5(0), 4(1)
9	Stamen number	5 or 4 (0), 2 (1)
10	Anther thecae	divergent (0), parallel (1)
11	Stigma	bilobed (0), capitate (1), punctate (2)
12	Fruit	elongate (0), isometric or wide (1)
13	Fruit apex	acute (0), truncate (1)
14	Fruit apex	not notched (0), notched (1)
15	Fruit	turgid (0), flattened (1)
16	Fruit dehiscence	septicidal (0), loculicidal (1), terminal septicidal and loculicidal (2)
17	Seed shape	six-sided (0), dorsiventral (1)
18	Seed shape	hemispherical (0), flattened (1)
19	Seed surface	alveolate (0), reticulate (1), smooth (2), verrucate (3), ridged(4), wavy (5)
20	Chromosome number	x = 7, 8, 9(0); 12(1); 19, 20, 21 (2); 17 (3)
21	Pollen apertures	colporate (0), colpate (1)
22	Colpus membrane	smooth (0), granular (1)
23	Exine	reticulate (0), striate-reticulate (1)
24	Anther cells	smooth (0), ridged (1)

Table 3. Floral development characters and character states; gynoecium size G05 = 0.05 mm, G10 = 0.10 mm, G40 = 0.40 mm

Character	Character states
25(1)	Calyx initiation with abaxial lobes before (0) or after (1) adaxial lobes
26(2)	Stamen initiation after (0), simultaneous with (1), or before abaxial corolla lobe initiation (2)
27(3)	Gynoecium initiation after (0) or simultaneous with corolla initiation (1)
28(4)	Abaxial calyx lobe length at G05 large (0), medium (1), small (2)
29(5)	Abaxial calyx lobe length at G10 large (0), medium (1), small (2)
30(6)	Abaxial calyx lobe length at G40 large (0), medium (1), small (2)
31(7)	Abaxial calyx lobe length at anthesis large (0), medium (1), small (2)
32(8)	Lateral corolla lobe length at G05 large (0), medium (1), small (2)
33(9)	Lateral corolla lobe length at G10 large (0), medium (1), small (2)
34(10)	Lateral corolla lobe length at G40 large (0), medium (1), small (2)
35(11)	Lateral corolla lobe length at anthesis large (1), small (2)
36(12)	Abaxial corolla tube length at G05 large (0), medium (1), small (2)
37(13)	Abaxial corolla tube length at G10 large (0), medium (1), small (2)
38(14)	Abaxial corolla tube length at G40 large (0), medium (1), small (2)
39(15)	Abaxial corolla tube length at anthesis large (0), medium (1), small (2)
40(16)	Stamen insertion height at G05 large (0), medium (1), small (2)
41(17)	Stamen insertion height at G10 large (0), medium (1), small (2)
42(18)	Stamen insertion height at G40 large (0), medium (1), small (2)
43(19)	Stamen insertion height at anthesis large (0), medium (1), small (2)
44(20)	Anther length at G05 small (0), medium (1), large (2)
45(21)	Anther length at G10 small (0), medium (1), large (2)
46(22)	Anther length at G40 small (0), medium (1), large (2)

Table 4. Mature morphology and chromosome number character states for nine taxa of *Veroniceae* and two outgroup species (*Digitalis grandiflora, Verbascum blattaria*)

Species	Abbreviation	Characters
		0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2
		1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4
Veronica chamaedrys	Va ch	0 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 2 0 1 1 1 1
V. serpyllifolia	Va se	0 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 2 0 1 1 1 1
Synthyris reniformis	Sy re	1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 0 1
Besseya alpina	Be al	1 0 0 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 0 1
Pseudolysimachion longifolium	Ps lo	0 0 1 0 0 0 1 1 1 1 2 1 1 1 0 2 1 1 1 3 0 1 0 1
Derwentia perfoliolata	De pe	0 1 1 1 1 0 1 1 1 1 2 0 0 0 0 2 1 1 4 2 1 1 1 1
Hebe parviflora	He pa	0 1 1 2 1 0 1 1 1 1 2 0 0 0 0 2 1 1 3 2 1 1 1 1
Veronicastrum virginicum	Vm vi	0 0 2 0 0 0 0 1 1 1 2 0 0 0 0 2 1 0 1 3 0 0 0 1
Wulfenia carinthiaca	Wu ca	1 0 0 0 0 0 0 0 1 0 1 0 0 0 0 2 1 0 0 0 0 1 0 1
Digitalis grandiflora	Di gr	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Verbascum blattaria	Vb bl	0 0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 1 0 0 0 0 0

Table 5. Floral development character states for nine taxa of *Veroniceae* and two outgroup species (*Digitalis grandiflora*, *Verbascum blattaria*). For explanation of characters and states see Table 2

Species	Abbreviation	Characters																					
		0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	2	2	2
		1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2
Veronica chamaedrys	Va ch	0	2	1	1	1	1	1	2	2	1	1	2	2	1	3	2	2	1	4	2	2	2
V. serpyllifolia	Va se	0	2	1	2	2	2	1	2	2	1	0	2	2	2	3	2	2	1	4	0	1	0
Synthyris reniformis	Sy re	1	2	1	2	2	2	1	2	2	1	0	1	1	1	2	2	2	0	2	1	2	1
Besseya alpina	Be al	1	2	1	2	2	2	1	2	2	2	0	1	1	2	2	2	2	2	5	2	2	2
Derwentia perfoliolata	De pe	0	1	0	0	0	0	1	1	1	0	1	0	0	1	2	1	1	0	3	1	2	2
Hebe parviflora	He pa	0	1	0	0	0	2	2	1	1	1	0	0	0	1	2	0	0	0	2	1	2	0
Pseudolysimachion longifolium	Ps lo	0	1	0	1	1	1	1	1	1	0	1	1	1	1	2	2	2	0	2	1	2	2
Veronicastrum virginicum	Vm vi	0	1	0	0	0	1	2	0	0	0	0	0	0	0	2	2	2	1	3	0	1	1
Wulfenia carinthiaca	Wu ca	0	1	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1	0	1	2	2	1
Digitalis grandiflora	Di gr	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1
Verbascum blattaria	Vb bl	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	1	1	1	4	0	0	0

Results

Analysis of mature morphological data resulted in one shortest tree of 43 steps (character state changes) length (Fig. 4). The outgroup taxa *Digitalis grandiflora* and *Verbascum blattaria* were characterized by helical leaf arrangement, elongate leaf blades, flowers with pentamerous calyx and corolla, four or five stamens with divergent thecae, a bilobed stigma, colporate pollen, septicidal capsules, and irregularly angular alveolate seeds. Synapomorphies of the *Veroniceae* were reduction of stamen number to two, capsule dehiscence at the distal end only (simultaneously septicidal and loculicidal) and dorsiventral seeds with one convex and one flat side. All *Veroniceae* except *Wulfenia* were characterized by opposite leaf arrangement, a tetramerous corolla, a minute stigma, parallel anther thecae, and reticulate seed surfaces. Derived from this are the whorled leaves of *Veronicastrum* (and some plants of *Pseudolysimachion*) and the capitate stigmas and smooth seeds of *Veronica* and its two closest relatives. The clade above *Veronicastrum* was characterized by shorter leaf blades, a tetramerous calyx, flat seeds, and colpate pollen. Only in *Hebe parviflora* (Vahl.) Cockayne & Allen were the leaf blades very elongate. *Pseudolysimachion longifolium* (L.) Opiz and *Veronica* and allies shared only the short, truncate capsule shape with a distal notch. The placement of *Synthyris* and *Besseya* with the two species of *Veronica* was supported by short inflorescences, captitate stigmas, flat, loculicidal capsules, and smooth seeds. Monophyly of the *Hebe* clade was supported by woodiness, presence of pulvinus (see comments to characters 1 and 4 in Appendix 1), seeds verrucate (becoming ridged in *Derwentia*), chromosome number 19, 20, or 21, and pollen exine striate-reticulate, a parallelism with *Veronica*. Monophyly of *Synthyris* and *Besseya* was supported by a reversal to a helical leaf arrangement, rosette habit (both convergences with *Wulfenia*), and a chromosome number of 12.

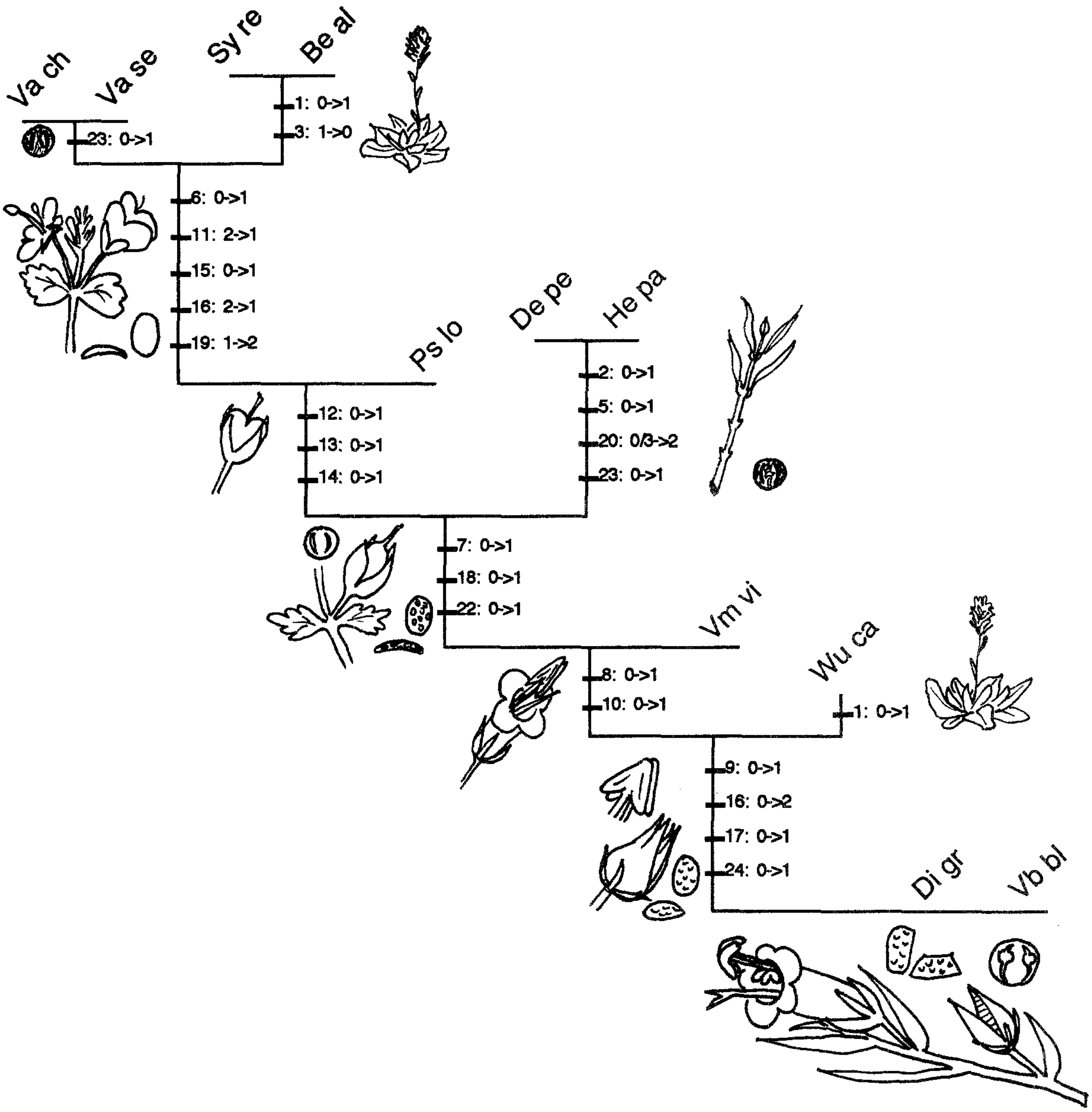

Fig. 4. Single most parsimonious tree of 24 mature morphology characters, calculated with PAUP branch-and-bound option, plotted with MacClade. Length 43 steps, consistency index 0.767, retention index 0.851; only characters that change unambiguously are shown on the branches. Species abbreviations as in Table 4; character numbers as in Table 2. Icons indicate character states: outgroup (bottom) with elongate alternate leaves, calyx and corolla pentamerous, stamens four or five with divergent thecae, stigma bilobed, capsule dehiscence septicidal, seeds polygonal, pollen colporate; *Veroniceae* with two stamens, capsule dehiscence terminally septicidal and loculicidal, seeds dorsiventral; *Wulfenia*, also *Synthyris* and *Besseya*, with rosette habit; *Veroniceae* excluding *Wulfenia* with corolla tetramerous, anther thecae parallel; *Veroniceae* excluding *Veronicastrum* and *Wulfenia* with short opposite leaves, calyx tetramerous, pollen colpate (except in *Pseudolysimachion*), seeds flat; *Hebe* clade woody, pollen striate-reticulate (also in *Veronica*), sister group with short, truncate (also in *Verbascum*), notched capsules; *Veronica*, *Synthyris*, and *Besseya* share short inflorescences, capitate stigmas, wide loculicidal capsules, and smooth seeds

The two *Veronica* species shared only the possibly plesiomorphic characters opposite leaves and chromosome numbers based on x = 7, 8, or 9, and the either convergent (with the *Hebe* group) or plesiomorphic striate-reticulate exine sculpturing.

Processing of developmental data alone resulted in six trees 76 steps long which differed in the placement of *Wulfenia* (above or below the *Hebe* group), of *Verbascum* (in outgroup or with *Veronicastrum* in ingroup), and of *Besseya* with *Veronica* or *Synthyris* (Fig. 5). These inconsistencies reflect similarities in floral organ growth patterns that must be in part homoplastic.

Mature morphological and developmental data together yielded two equally parsimonious trees of 124 steps in which *Wulfenia* and *Veronicastrum* changed places as basalmost taxa of the *Veroniceae* (Fig. 6), due to the influence of the floral development characters. The tree with *Wulfenia* at the base (Figs. 6, 7) had

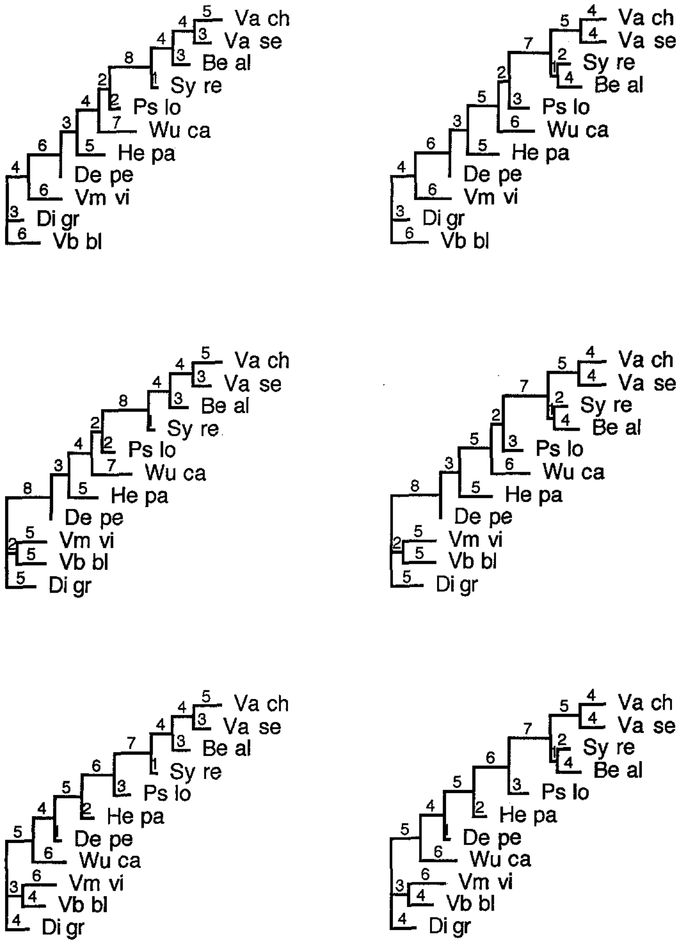

Fig. 5. Six most parsimonious trees of 22 floral development characters, calculated and plotted with PAUP branch-and-bound option. Length 76, consistency index 0.605, retention index 0.615. Species abbreviations as in Table 4. Four of the trees could not be rooted such that *Verbascum* remained in outgroup due to its slower increase in stamen insertion height, similar to that of basal *Veroniceae*

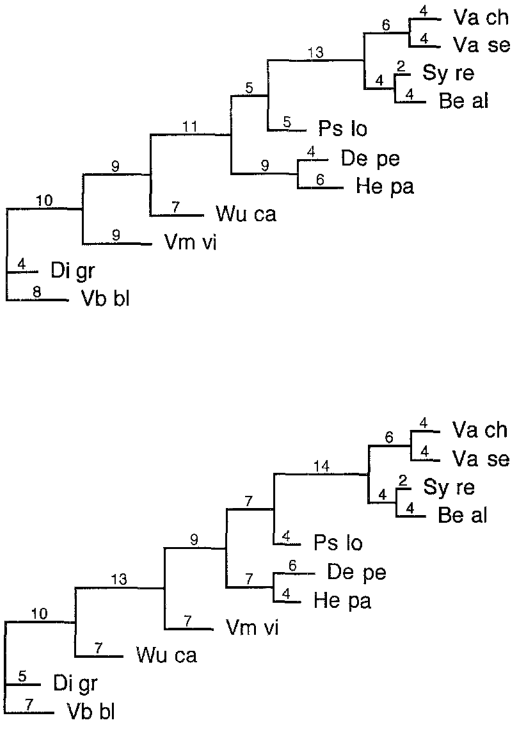

Fig. 6. Two most parsimonious trees of 46 mature morphology and floral development characters, calculated and plotted with PAUP branch-and-bound option. Length 124 steps, consistency index 0.637, retention index 0.690. Species abbreviations as in Table 4. Topology of second tree as in mature morphology tree (Fig. 3), with *Wulfenia* as most basal *Veroniceae*; character state changes detailed in Fig. 7

the same topology as the mature morphology tree (Fig. 4). Floral ontogenetic characters supported almost all branches (note especially the synapomorphies diagrammed in Fig. 7) except the monophyly of *Derwentia* and *Hebe*; possibly the members of the *Hebe* group are the endpoints of such long lineages with many changes in flower morphology that no unequivocal similarities remain. *Digitalis grandiflora* (and also other *Digitaleae* and *Scrophulariaceae* – see Discussion) shows an organ initiation sequence in which the abaxial corolla lobes precede the adaxial stamen pair. This becomes simultaneous in the *Veroniceae*, and in *Veronica*, *Besseya*, and *Synthyris* the stamen primordia precede the corolla lobes. Later developmental characters indicate a similar progression: calyx and corolla show fast growth in the outgroup species and acquire more and more delays towards the *Veronica* group (Fig. 7).

The grouping of the species of *Veronica* with *Synthyris* and *Besseya* occurred in all bootstrap trees, reflecting the number of characters supporting this clade (Fig. 8). This is also expressed in its decay value of 8, i.e. a strict consensus tree of the 254 trees of length less than or equal to 132 steps (8 steps longer than the single shortest tree of 124 steps) still retained this branching. Placement of *Pseudolysimachion* with *Veronica* and allies occurred in 73% of bootstrap replications; its decay value was 2, since it occurred in the two shortest trees and there were no trees of length 125. Placement of *Wulfenia* rather than *Veronicastrum* as sister group to the rest of *Veroniceae* had very weak support by the bootstrap consensus tree (52%); the ambiguity of the data is reflected in a decay value of 0 which reflects the presence of both options in the two most parsimonious trees.

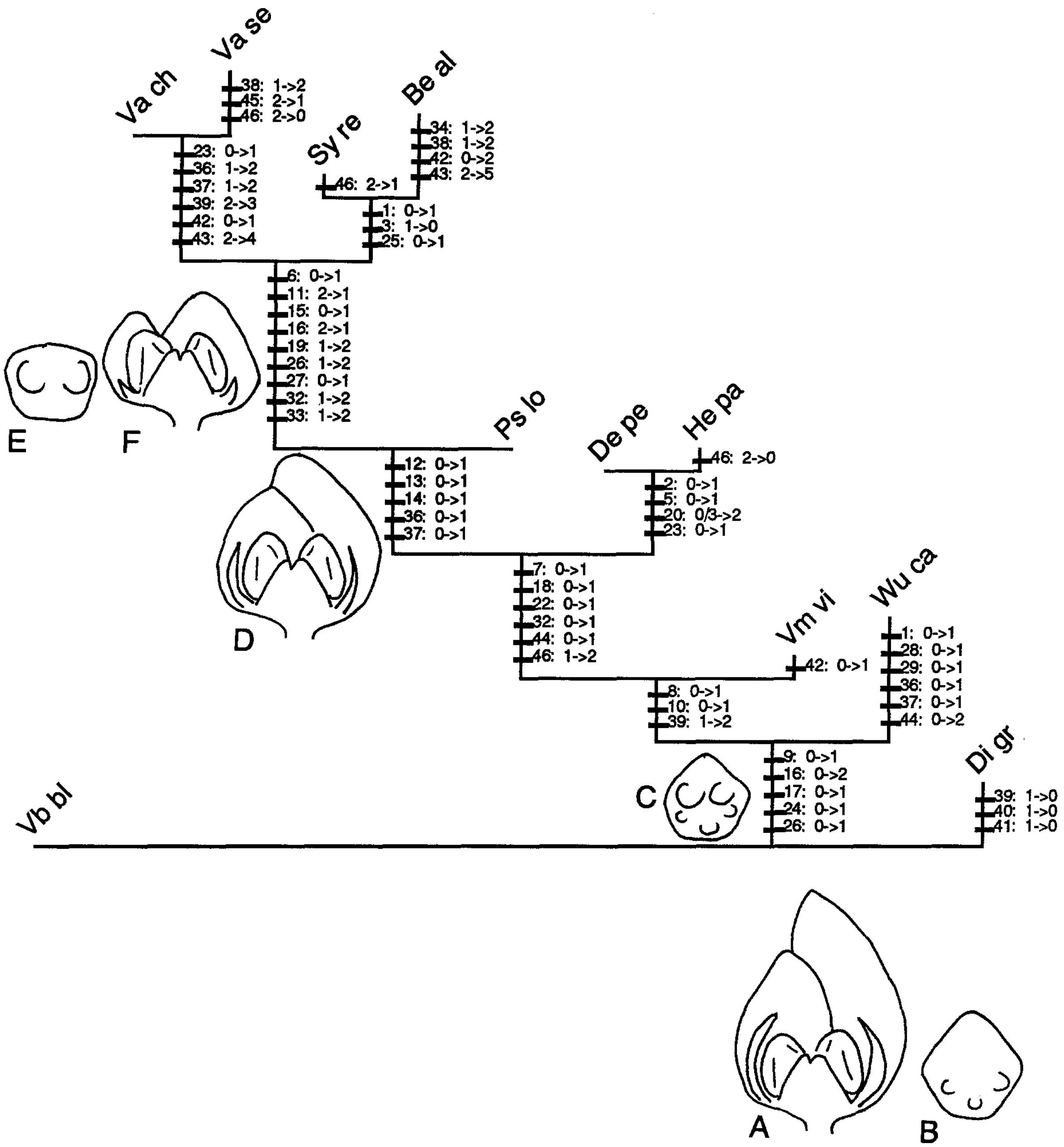

Fig. 7. One of the two most parsimonious trees of 46 mature morphology and floral development characters (cf. Fig. 6), plotted with MacClade. Only characters that change unambiguously are shown; species abbreviations as in Table 4; character numbers as in Tables 2, 3. Drawings illustrate floral development character states: A outgroup with fast calyx and corolla growth during gynoecium closing; B outgroup with three lower corolla lobes initiating before upper lobes and stamens; C *Veroniceae* with simultaneous initation of three lower corolla lobes and two stamens; D *Pseudolysimachion*, *Veronica*, and *Synthyris* clade share slower calyx and corolla growth during closing of gynoecium; E *Veronica* and *Synthyris* clade share stamen initiation preceding that of corolla lobes, as well as F, slow calyx and very slow corolla growth during closing of gynoecium

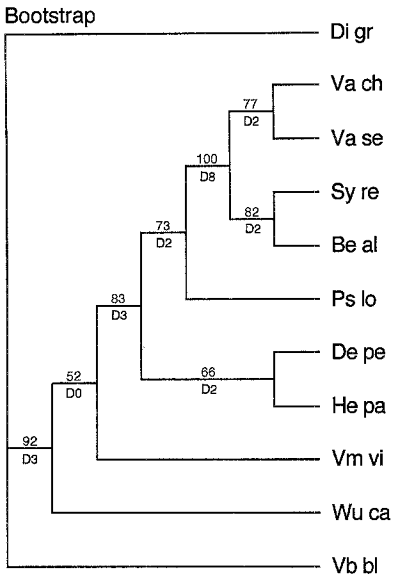

Fig. 8. Bootstrap 50% majority rule consensus tree of 46 mature and developmental characters; numbers above branches are bootstrap values of 100 replications; numbers below branches are decay values derived from strict consensus of shortest trees (DO) to trees 8 steps longer than shortest (D8)

Discussion

Phylogenetic analysis. High bootstrap and decay values indicate that *Synthyris* and *Besseya* form a monophyletic group with *Veronica* rather than with *Wulfenia*; this concurs with the hypotheses of Yamazaki (1957) and Hong (1984). *Wulfenia* shows delays in calyx and corolla tube growth similar to those occurring in *Synthyris* and *Besseya* but its corolla lobe and stamen insertion growth patterns as well as mature morphological features make it likely that it is more basal in *Veroniceae* and has evolved those growth delays and its rosette habit convergently.

Bootstrap and decay values were indicative of some support for the placement of *Pseudolysimachion* at the base of *Synthyris* and allies, not as the closest relative to *Veronica*, as was hypothesized by Hong (1984). *Pseudolysimachion* exhibits a mixture of features reminiscent of *Veronica* and *Veronicastrum* and its floral development patterns are often intermediate between those two taxa (see Table 5; also Kampny & al. 1994).

The change in initiation patterns resulting in simultaneous appearance of stamens and abaxial corolla lobes is typical for the tribe *Veroniceae*, at least for the species studied to date. In other *Scrophulariaceae*, as in *Digitalis* (additional species in Wunderlin 1992) and *Verbascum*, the abaxial corolla lobes initiate first, followed by the abaxial stamen pair and finally by the corolla lobes and stamens on the adaxial side of the floral apex (*Agalinis*, *Agalineae*; *Orthocarpus*, *Rhinantheae*; *Linaria*, *Antirrhineae*; Kampny & Canne-Hilliker 1988). Similar unidirectional initiation with between-whorl overlap was observed in some species of *Leguminosae* by Tucker (1984). The monophyly of *Veronica* with *Synthyris* and *Besseya* is supported by corolla lobes initiating after the stamens, and by simultaneous gynoecium and corolla lobe initiation. Since those two character changes co-occur, they could be only one event, namely the extreme delay in corolla appearance; the decreased growth rate of the calyx at that stage (as an indicator of age) makes it difficult to evaluate whether gynoecium and stamen initiation is accelerated. Early stamen growth is indeed accelerated, relative to the two outgroup species, in all *Veroniceae* studied here except *Veronicastrum virginicum* (L.) FARW. and *Veronica serpyllifolia* L. which may be reversals. Initiation patterns require more in-depth study by examining more species and possibly by plotting initiation against calyx size measured from scanning electron micrographs.

Flower shape evolution in *Veroniceae*. The most common flower shape in the *Scrophulariaceae* (and in the whole order *Scrophulariales*) combines a long, slightly flaring or companulate corolla tube with small or moderately sized, rounded corolla lobes; generally, calyx and corolla are pentamerous, and two pairs of stamens are present (BENTHAM 1876, WETTSTEIN 1891, PENNELL 1935). One outgroup species chosen here, *Digitalis grandiflora*, shares all these characteristics except the rounded corolla lobes; the other outgroup member, *Verbascum blattaria* (*Verbasceae*), differs in its shorter corolla tube and fertile fifth stamen (see Fig. 1). In the tribe *Veroniceae*, all these flower features have been modified.

Corolla lobes. Tapering or triangular corolla lobes seem to be unusual in *Scrophulariaceae*; most show rounded lobes as in *Verbascum*. Triangular lobes occur in *Isoplexis* and *Digitalis* of the *Digitaleae* as well as in *Picrorhiza* and *Veronicastrum* of the *Veroniceae*; the small corolla lobes with parallel-sided bases and triangular tips of *Wulfenia amherstiana* JACQ. (illustrated in LEPPER 1970) may be retained from, or reversals to, ancestral features. More rounded corolla lobes could have evolved convergently in *Wulfenia* and in the ancestor of the *Hebe-Veronica* clade (Fig. 9).

Early corolla lobe growth showed a gradual slowing within the *Veroniceae* (characters 32 and 33, Table 5). The growth rate of *Wulfenia* was similar to that of *Digitalis* and *Verbascum* but slightly slower (KAMPNY 1995a, c). In *Veronicastrum* the lobes were still marginally smaller than in *Digitalis* after initiation; after that, its lobes showed the fastest growth in the tribe until ovary closing when it began to level off (KAMPNY 1995a). The other taxa of the *Veroniceae* have slower early growth, remaining intermediate in *Hebe*, *Pseudolysimachion*, and *Derwentia* and showing the most extreme early growth delay in *Veronica*, *Synthyris*, and *Besseya* (growth curves illustrated in KAMPNY 1995a). Possibly those delays and decreases in early growth rate are indicative of ancestors with small corolla lobes: the first at the root of the *Veroniceae*, causing moderate slowing, and the second at the divergence of *Synthyris* and *Veronica*, resulting in the extreme early delay in the derived taxa (Figs. 7, 9).

In the outgroup taxa, the corolla lobes of *Verbascum* grew at the same rate as those of *Digitalis* until after ovary closing; this may be a convergence since the two genera are not closely related. Judging from qualitative accounts of corolla growth of other *Scrophulariaceae* in the literature, corolla growth patterns show considerable variation: *Agalineae* have faster early corolla lobe growth (KAMPNY & CANNE-HILLIKER 1988), while the *Rhinantheae* display an early growth delay similar to that in *Veronica* (KAMPNY & CANNE-HILLIKER 1988, ARMSTRONG & DOUGLAS 1989). Future quantitative studies of additional *Scrophulariaceae* are necessary to better resolve these issues.

Corolla tube. The corolla tubes of the *Digitaleae* range from moderate (e.g., in *Isoplexis*, illustrated in WERNER 1965) to very long, as in *Digitalis grandiflora*

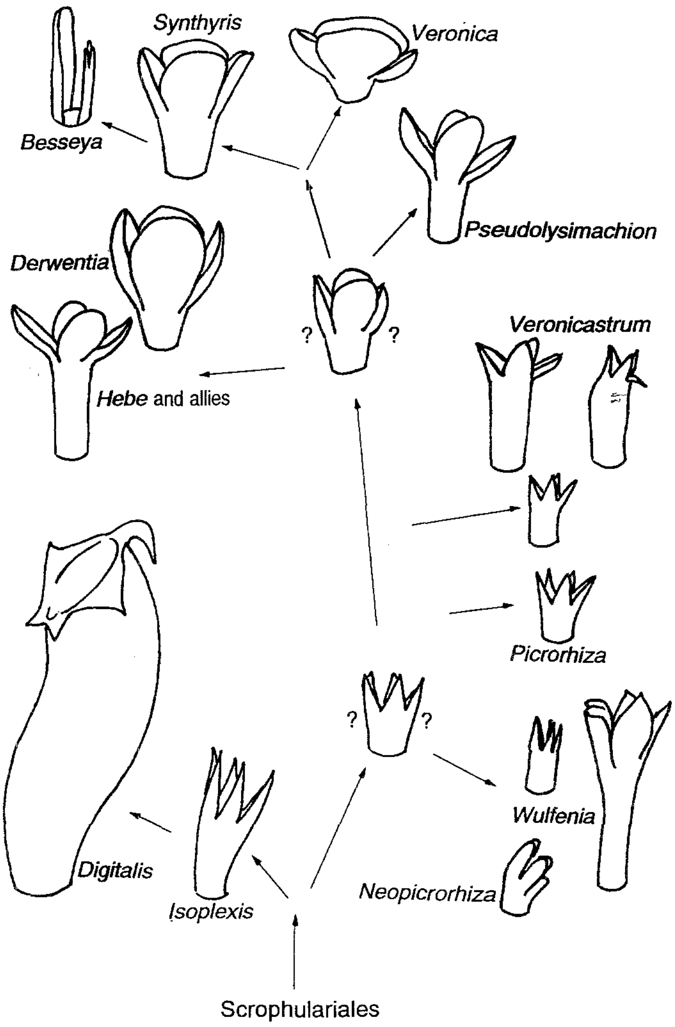

Fig. 9. Extant and possible ancestral corolla shapes mapped on cladogram in Fig. 8 (corollas schematic, not to scale; putative ancestral corolla forms indicated with question marks). Ancestor of both *Digitaleae* and *Veroniceae* arose from *Scrophulariales* (e.g., *Verbasceae*); *Isoplexis*-like flower with moderate corolla tube and tapering lobes gives rise to long-tubed *Digitalis* and putative small-flowered ancestor of *Veroniceae*; convergent reversion to long tube in *Wulfenia*, *Veronicastrum*, *Hebe*, and *Pseudolysimachion*; parallel evolution of rounded larger corolla lobes in *Wulfenia* and the ancestor of the *Hebe*-*Veronica* clade; most extreme diminuition of corolla tube in *Veronica*, loss of union of upper corolla lobe with lower ones in *Besseya*

(Fig. 9). *Digitalis* emcompasses species with a downward, then upward curvature in the corolla tube, while its closest relative *Isoplexis* (within which it is probably nested) shows only a downward curve in the corolla tube (WERNER 1965). *Isoplexis* has corolla proportions more reminiscent of *Wulfenia* and *Veronicastrum* (WERNER 1965). Other *Digitaleae* such as *Erinus* and *Campylanthus* have salverform corollas with narrow tubes, which in the latter are bent at abrupt angles. In the *Veroniceae*, corolla tube length appears to have diminished and reverted several times; moderately short corolla tubes occur in some species of *Wulfenia* (Lepper, 1984), *Veronicastrum* (Yamazaki 1957), and *Hebe* (Garnock-Jones 1993).

The corolla tube growth patterns differed in the long-tubed species of these genera investigated here (Table 5, characters 35–39; growth curves in Kampny 1995a), indicating that they are not plesiomorphies but rather reversals. The ancestral fast log-linear corolla tube growth with early increase in stamen insertion height seen in the outgroup (*Digitalis*, *Verbascum*) has changed least in *Veronicastrum virginicum*, except for a possible slight delay soon after initiation (Kampny 1995a).

Delay in increase of stamen insertion height may be an indication that *Veronicastrum virginicum* had a shorter-tubed ancestor. This delay was reminiscent of that occurring in *Veronica* and allies. The long-tubed North American *Veronicastrum virginicum* may be derived from more short-tubed ancestors similar to the Asian species *Veronicastrum kitamurae* (Ohwi) Yamazaki (illustrated in Yamazaki 1957), from which it could have retained a low stamen insertion height. *Picrorhiza* (illustrated in Hong 1984) has a nearly radial corolla with triangular lobes similar to *Veronicastrum* (but pentamerous) of only a few millimetres length, and with very long stamen filaments.

Thus it appears possible that the ancestor of the *Veroniceae* had a corolla tube of intermediate length, possibly inherited from an *Isoplexis*-like flower; this was then changed in several instances, possibly by adjusting to long-tongued pollinators through the evolution of long tubes, as in the cases of *Wulfenia*, *Veronicastrum*, and *Hebe* (Fig. 9). At the other extreme it changed to very short tubes, possibly to fit shorter-tongued pollinators, as in the case of *Veronica* (Fig. 9).

Corolla lobe number. The fusion of the upper two corolla lobes into one, resulting in a tetramerous corolla, may have been correlated with the evolution of the dense-flowered inflorescences common in the *Veroniceae*. In such bottle-brush inflorescences, the exact number of corolla lobes does not matter since the whole mass of flowers serves as advertisement to pollinators (Hufford 1992). Tetramery could have been a unique event in the ancestor of the clade giving rise to the taxa from *Veronicastrum* to *Veronica*, if the two species forming the genus *Wulfeniopsis* are regarded as close relatives of *Veronicastrum* (Hong 1984). If those two species are members of the genus *Wulfenia* as treated by Lepper (1970), complete fusion of the upper corolla lobes must have occurred twice in the tribe. *Picrorhiza* and two members of the *Hebe* group, *Detzneria* and *Chionohebe*, have reverted to pentamerous corollas, or possibly share this as a plesiomorphic character state while tetramery evolved separately in the *Neopicrorhiza*, *Veronicastrum*, *Veronica*, and *Hebe* groups (Hong 1984). Both *Digitalis* and *Isoplexis* show partially united upper corolla lobes (Wettstein 1891).

Calyx. The suppression of the fifth, upper median, calyx lobe could have happened at the base of the clade including *Veronica* and allies, *Pseudolysimachion*, and the *Hebe* group, with reversals to pentamery in several species of *Veronica* and in *Chionohebe* as well as *Paederota* and *Paederotella*. Otherwise, tetramery could have evolved several times in parallel (Hong 1984).

Calyx development showed a delay during the early stages in the terminal clade including *Pseudolysimachion*, and in *Wulfenia*, apparently a convergence in the latter case. This early delay, which was most extreme in *Synthyris*, *Besseya*, and *Veronica serpyllifolia*, is not indicative of mature calyx size: *Wulfenia* ends with long calyx lobes despite the early delay, while *Veronicastrum*, *Hebe*, and *Derwentia* have short or intermediate calyx lobes at maturity despite their fast early growth. Similarly, the species with the most extreme early delay had intermediate sized calyx lobes at maturity.

Stamens. Anther size showed a trend toward early enlargement in the *Veroniceae* which was most extreme in *Veronica chamaedrys* L. and *Besseya alpina* (GRAM) RUDB. paralleled again by *Wulfenia carinthiaca*. *Veronica serpyllifolia* had smaller anthers from the earliest stage to maturity; possibly this species self-pollinates (GRIME & al. 1988) and so has decreased its pollen production. The largest anthers in the tribe occurred in *Derwentia*, although it did not have extremely fast early growth.

The reduction of the stamens to the upper pair was probably a unique event early in the tribe's phylogeny, occurring either after the evolution of the *Picrorhiza / Neopicrorhiza* clade as postulated by HONG (1984), or occurring at the root of the tribe so that the two four-stamened genera are the result of reversal. If the latter was the case, the two occurrences of four stamens in *Veroniceae* are possibly separate events, since *Picrorhiza* shows resemblances with *Veronicastrum* with respect to flower shape and pollen type (HONG 1984) while *Neopicorrhiza* is more reminiscent of *Wulfenia* in those two respects.

Flower merosity. Merosity is generally stable at the intraspecific level in the *Scrophulariaceae*, as far as is known, but shows variability in a number of species of *Veroniceae*. No variation in calyx, corolla, or stamen number was observed in the herbarium specimens of the taxa of *Veroniceae*, *Digitalis*, and *Verbascum* included in this study (listed in KAMPNY 1995a). However, some species of *Veronica* show frequent reversals to pentamerous calyces (FISCHER 1920, SAUNDERS 1934). Pentamerous calyces and corollas also occur in the *Hebe* group (GARNOCK-JONES 1993), in which some species are so unstable that they even form flowers with six calyx and corolla lobes and trilocular ovaries (P. J. GARNOCK-JONES, pers. comm.) Trimery of calyx and corolla is common in *Besseya*, in *B. oblongifolia* PENNELL it is stable, while numbers vary within other species from two to five (HUFFORD 1992). Intraspecific variation to trimerous calyces and occasionally corollas was also observed in some strains of *Pseudolysimachion* (KAMPNY & al. 1994).

Stamen number appears to be more stable: NOLL (1883) searched for unusual numbers in *Pseudolysimachion longifolium* and only rarely found flowers with a second stamen pair before the ovary-closing stage, which even more seldom showed normal development. NOLL (1883) also described other abnormalities in *Pseudolysimachion longifolium*, such as the failure of the second stamen pair to become epipetalous, calyces and corollas with five or six lobes, or the positioning of the median calyx lobe toward the bract.

In other *Scrophulariaceae* occasional omission of the smaller median upper calyx lobe has been observed in some populations of *Agalinis tenuifolia* (VAHL.) RAF. (KAMPNY & CANNE-HILLIKER 1987). Complete fusion of the upper two corolla lobes occurs in some *Gratioleae* (PENNELL 1935); in one member of that tribe, *Mimulus guttatus* DC. intraspecific variation has been observed between partial and complete fusion (K. Ritland, pers. comm.). Reduction of stamen number to two also occurs in some genera of *Selagineae* and *Gratioleae* but in those cases it is usually the lower pair that remains present and fertile (Wettstein 1891, Pennell 1935). Despite the great variety of flower forms in the *Scrophulariaceae*, meristic variations are minor and infrequent abnormalities within most species and rarely become stabilized; in *Veroniceae* they have become phylogenetic characteristics. It is remarkable that these usually intraspecific, and sometimes intra-inflorescence, differences are determined very early in floral development, during initiation, or shortly thereafter in the case of partially fused corolla lobes.

Ancestral flower shape. It appears likely that the *Veroniceae* are derived from an *Isoplexis*-like plant which also gave rise to *Digitalis*: the latter shows the same calyx initiation sequence (upper median lobe, lower pair, upper pair) as *Wulfenia* (Wunderlin 1992) and *Veronicastrum* (Kampny & al. 1993), which was abbreviated when the tetramerous *Veroniceae* no longer initiated the upper median calyx lobe. Another member of the *Digitaleae*, *Erinus*, displays a contrasting calyx initiation pattern (upper median lobe and upper pair, lower pair) (Wunderlin 1992). There are also similarities between the corollas of some species of *Isoplexis* and *Veroniceae*, and *Digitalis* and *Veronica* have chloroplast gene (*rps2*) sequences more similar to each other than to other *Scrophulariaceae* that have been tested (DePamphilis & al. 1994).

The delays in early calyx and corolla growth seen in *Veroniceae* may be the result of derivation from small-lobed ancestors at the base of the *Hebe* branching, at the base of the *Pseudolysimachion* branching, and again at the base of *Synthyris* and allies. The parallel growth delays in *Wulfenia* could be a consequence of a miniaturization phase in its own ancestry. The flower of *Neopicrorhiza* (illustrated in Hong 1984) is small, less than 1 cm in length; it would be interesting to examine its early development to see if it too shares those delays. The possible derivation of *Veronicastrum* from short-tubed ancestors seems to have left no traces in its corolla tube development pattern, which resembles that of *Digitalis* until after the beginning of style formation (G40), unless the small delay visible near the beginning of its growth curve is such a trace. Flowering material of *Veronicastrum kitamurae* and *Picrorhiza* needs to be investigated for additional evidence.

Sources of flower shape evolution. The origin of early growth differences could lie in chance fluctuations that persist because only the flower at anthesis is under selection pressure by its pollinator. Apparently the timing and rate of phases of growth acceleration can vary but these differences are compensated later in development (Kampny & al. 1994) to comply with the requirements of stabilizing selection. A change in pollinator availability could result in flower shape changes by exerting directional selection toward one of the extremes of the variation present in a population, or by supporting the spread of a mutation happening at an opportune time. Their chance nature may be responsible for the different mechanisms of developmental change leading to the same mature results, for example the miniaturization of the *Veronica serpyllifolia* L. flower through delayed onset, decreased rate, and early cessation of growth in the various organs (Kampny 1995a).

Little is known about pollinators of *Veroniceae*. Generally, long-tubed flowers seem to be pollinated by bees (Pennell 1935). Short-tubed *Veronica* flowers show a trend from bee toward fly pollination (PENNELL 1935) or selfing (GRIME & al. 1988). Bees forage on long-tubed *Pseudolysimachion* (at least when in cultivation in Canada; pers. obs.) and also syrphid flies (NOLL 1883). Species of *Hebe* and *Parahebe* show a variation of corolla tube lengths; short-tubed lowland species are pollinated by bees, while diptera, beetles, and moths have been observed on the generally long-tubed montane species (DELPH 1990) that may also be predominantly autogamous (GARNOCK-JONES 1976). The narrow tubes of some *Digitaleae* are probably associated with pollination by *Lepidoptera*. The flowers of *Digitalis* are pollinated by bumble bees (e.g., BEST & BIERZYCHUDEK 1982).

Growth patterns as indicators of previous changes. The three types of changes predicted by ALBERCH & al. (1979) – onset, rate, offset of growth – can occur at any time during development, leading often to several growth phases of different duration and rate (KAMPNY & al. 1993, 1994; KAMPNY 1995a). Since new growth patterns may not always obliterate all of the ancestral ones, and convergent evolution of similar shapes may not necessarily occur by the same mechanism, phyogeny can leave indications of past developmental patterns in an organism's ontogeny.

Calyx and anther lengths displayed the least number of growth rate changes and the same overall growth pattern, probably due to being less influenced by pollinator constraints. The numerous growth rate changes of the corolla measurements may echo many previous adjustment steps. The patterns of the corolla lobes appeared to be the most valuable for estimating phylogeny; possibly they had not developed as many homoplasies as the corolla tube and stamen insertion height.

Conclusions

Quantitative intraspecific and interspecific comparisons (KAMPNY & al. 1994, KAMPNY 1995a) have shown that evolution of floral form does not occur through a Haeckelian compression of older stages by terminally added new stages; organ initiation sequences can be altered, and growth rates can change anytime during development. Flowers show mosaic development, with different growth patterns in the various organ types; each organ can change individually, resulting in a variety of shapes. Bilateral symmetry and united parts (corolla tube, epipetalous stamens) are added factors which increase the possibilities of flower configuration greatly, as demonstrated by the flowers of the *Scrophulariaceae*. The *Veroniceae* are an example of the flexibility of floral development in such a group, and the ease and frequency of changes in growth patterns and mature forms.

Floral development features together with other characters indicate that there are three main flower types in the *Veroniceae*, as exemplified by the different growth patterns of *Wulfenia*, *Veronicastrum*, and *Veronica*. Progressive changes seem to have led to the more and more apomorphic (derived) initiation and early growth features from *Hebe* and *Derwentia* to *Pseudolysimachion* to the clade of *Synthyris* and *Besseya* and finally *Veronica*. The consistency of those patterns needs to be tested with evidence from additional taxa, material of which is often not easily grown or collected as several of the genera are only found in the Himalayas and adjacent areas. It would also be desirable to test the phylogenetic hypothesis resulting from developmental and mature morphology with evidence from an independent source such as DNA sequences.

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Appendix 1. Comments on the character states of mature morphology data of *Veroniceae*, *Digitalis*, and *Verbascum*.

1. Habit cauline or rosette: *Wulfenia*, *Synthyris*, and *Besseya* have extremely short internodes so that their leaves form rosettes; inflorescences are axillary, i.e. the main shoot does not "bolt", and cauline leaves are never present. *Digitalis* and *Verbascum* show a rosette-like habit as seedlings but later the stems elongate so that cauline leaves are present, and the inflorescences are terminal.

2. Shoots herbaceous or woody: *Digitalis* is a perennial or biennial herb, but it belongs to a clade with shrubby habit (*Isoplexis*). Most *Veroniceae* are perennial herbs which may be comparatively tender as in *Veronica* or tough and thick-stemmed as in *Veronicastrum*. *Hebe parviflora* is obviously woody, occurring as pendent shrubs and small trees up to 5m, but *Derwentia perfoliolata* is a subshrub to shrub with a tough rootstock with growth rings and herblike shoots with large xylem cylinders, i.e. not very different from a large tough perennial. Garnock-Jones (1993) in his cladistic analysis of the *Hebe* complex treated woodiness as a synapomorphy of the group.

3. Leaf arrangement helical, opposite, or whorled: In the species with cauline leaves, their arrangement was consistent except in *Pseudolysimachion longifolium*, in which the leaf arrangement was opposite in the majority of plants observed; approximately 40% of the plants had whorled leaves, which in one case turned to alternate near the inflorescence. In *Pseudolysimachion spicatum* (L.) Opiz all plants have opposite leaves but in about one third of them the upper leaves are arranged helically. It is difficult to determine the leaf arrangement in rosette plants. In the biennial *Digitalis grandiflora* and *Verbascum blattaria* the cauline leaves following the first year's rosette are helical, as are the bracts on the peduncles of *Wulfenia carinthiaca*. FAA-preserved rhizome material was available for *Synthyris reniformis* showing a helical arrangement.

4. Leaf blades elongate or isometric: Leaf length/width ratios were measured for representative leaves (if available) for each specimen observed, and averaged per species ($N > 25$). Leaf blades were scored as elongate with length/width ratios from 3.02 to 4.21; *Hebe parviflora* has very long narrow leaves with an average ratio of 10.03. Leaf blades were scored as isometric with length/width ratios of 0.88 to 1.62.

5. Pulvinus absent or present: Garnock-Jones (1993) listed this feature as a synapomorphy of the *Hebe* group; it may be only visible in fresh material, not on herbarium specimens (although Hong 1984 mentioned the pulvinus persisting after the leaf falls), but it was included in this analysis to support the monophyly of the group.

6. Inflorescence long or short: Length of inflorescence was measured on as many undamaged inflorescences beginning anthesis as were available, and averaged per species

(N > 10). Long inflorescences had averages from 7.4 to 12.57 cm, short inflorescences from 1.13 to 4.8 cm.

7. Calyx lobe number five or four. This feature did not show any variability within species.

8. Corolla lobe number five or four. This feature did not show any variability within species. In *Digitalis grandiflora* the upper corolla lobes are often too shallow to be countable; also, late in floral development a supernumerary median point appears between the upper corolla lobes. Still, *Digitalis* was scored as five-lobed since five corolla primordia are initiated in the buds.

9. Stamens five or four, or two: *Verbascum blattaria* has five pollen-bearing stamens; in *Digitalis grandiflora*, five stamen primordia appear in the bud, the abaxial pair largest and the adaxial median one the smallest. These size differences persist throughout development, but the growth of the smallest primordium is soon arrested altogether and no trace of it is visible in the mature flower. Only two stamen primordia are initiated in the species of *Veroniceae* included in this study [except in rare cases in which a second stamen pair is initiated in *Pseudolysimachion longifolium*, but this usually does not develop normally (NOLL 1883); no such flowers were observed in this study].

10. Thecae divergent or parallel: *Verbascum blattaria* has confluent thecae which at maturity diverge at 180 degrees. In *Digitalis grandiflora* and *Wulfenia carinthiaca*, the thecae diverge but at an obtuse angle. All other *Veroniceae* have parallel thecae (except one specimen of *Hebe parviflora* and of *Pseudolysimachion spicatum* which had divergent thecae, possibly mutations).

11. Stigma two-lobed, capitate, or punctate: The stigmas of *Digitalis* and *Verbascum* are deeply bilobed; in *Wulfenia* the stigmas are also bilobed, but not as deeply, and strongly thickened. In *Veronica serpyllifolia*, *Synthyris*, and *Besseya* the stigmas are capitate; in *V. chamaedrys* they range from slightly capitate to capitate. *Veronicastrum virginicum* and *Derwentia perfoliolata* have punctate stigmas; in the *Hebe* and *Pseudolysimachion* species the stigmas vary from punctate to slightly thickened.

12. Fruit elongate, or isometric or wider: The fruits of *Veroniceae* range from about 2.5 times as long as wide to much wider than high (in species of *Veronica*). The isometry of the globose fruit of *Verbascum* is a convergence.

13. Fruit apex acute, or truncate: This character showed no intraspecific variation except in *Hebe parviflora* and to some extent *Derwentia perfoliolata*; the fruits in those species had usually acute apices and occasionally truncate ones.

14. Fruit apex not notched, or notched: In the species with apical notches, the fruit is shortest at the septum , and the locules extend upward into lobes. This feature did not vary within species except in *Pseudolysimachion*; in the two species studied here, the fruit were usually notched but unnotched fruit also occurred.

15. Fruit turgid, or compressed: This feature showed intraspecific variation in *Hebe parviflora* and *Besseya alpina*. The direction of compression was usually at right angles to the septum (i.e. a very narrow septum) except in *Hebe parviflora*, in the fruits of which the compression was parallel to the septum (i.e. a septum as wide as the fruit).

16. Fruit dehiscence septicidal, or loculicidal, or both and terminal: The ripe capsules can split septicidally, along the septum; loculicidally, along the middle of each locule; or both septicidally and loculicidally opening the terminal (distal) part of the capsule (in *Pseudolysimachion*, both loculicidal and terminal dehiscence occurred).

17. Seed shape six-sided, or dorsiventral: The seeds of *Verbascum blattaria* and *Digitalis grandiflora* were box-shaped or irregularly polygonal while those of the *Veroniceae* were dorsiventral (illustrated in KAMPNY 1995a).

18. Seed shape hemispherical, or flattened: In *Pseudolysimachion longifolium* the seeds were hemispherical, while in *P. spicatum* they were shallowly convex on the upper side

and shallowly concave on the other, similar to those of *Veronica serpyllifolia*. The seeds of *Synthyris reniformis* were flattened above and concave on the lower side; this type of seed also occurs in some sections of *Veronica* (WALTERS & WEBB 1972).

19. Seed surface alveolate, or reticulate, or smooth, or verrucate, or ridged: For SEM illustrations see KAMPNY (1995a).

20. Chromosome number x = 7, 8, 9, or 12, or 17, or 19, 20, 21: Only chromosome number and not chromosome morphology was used for this character. Studies of the morphology of the chromosomes, like the one performed by LEPPER (1970) for *Wulfenia*, would help to elucidate whether equal chromosome numbers are homologous.

21. Pollen colporate, or colpate: Colporate pollen can be recognized by the protruding pore membranes and the semicircles formed by the exine around the pores; in colpate pollen the colpus membrane may be flat or bulging (illustrated in KAMPNY 1995a, HONG 1984).

22. Colpus membrane smooth, or granular: This small irregular sculpturing of the colpus membrane can delimit the pore in colporate pollen.

23. Exine reticulate, or striate-reticulate: Both types occur in species of *Veronica* and of the *Hebe* group (HONG 1984), indicating parallelisms and/or reversals.

24. Anther cells smooth or ridged: This feature needs more study, especially among other species of *Digitalis* as well as *Isoplexis* and *Erinus*, to see whether it helps delimit *Veroniceae*; *Verbascum* has ridged anther cells, indicating that other *Scrophulariaceae* anthers also bear this sculpturing.

Appendix 2. Intercepts of floral organ growth curves at gynoecium length 0.05 mm (G05), 0.10 mm (G10), and 0.40 mm (G40); species abbreviations as in Table 4

Measurement	Species	G05	G10	G40
Abaxial calyx lobe length				
	Va ch	– 1.0835552	– 0.7314979	0.42995843
	Vm vi	– 0.7903699	– 0.3722947	0.18743035
	Ps lo	– 1.0445939	– 0.5710210	0.32693952
	Va se	– 1.5310727	– 1.0318838	0.04087466
	De pe	– 0.9347607	– 0.4151576	0.66709965
	He pa	– 0.7683036	– 0.4137362	– 0.10873452
	Sy re	– 1.5699477	– 1.2410618	– 0.04242880
	Be al	– 1.5445218	– 1.1852813	– 0.04789883
	Wu ca	– 1.2163750	– 0.7468379	0.49048558
	Di gr	– 0.7368569	– 0.2289959	0.85293561
	Vb bl	– 0.8704640	– 0.4551702	0.54137343
Lateral corolla lobe length				
	Va ch	– 2.052047	– 1.856169	– 0.6813591
	Vm vi	– 1.730754	– 1.247315	– 0.2639874
	Ps lo	– 1.900840	– 1.604338	– 0.2360246
	Va se	– 2.110790	– 1.867678	– 0.7985761
	De pe	– 1.956126	– 1.700599	– 0.3649867
	He pa	– 1.894708	– 1.630135	– 0.7643097
	Sy re	– 2.156950	– 2.026238	– 0.9700141
	Be al	– 2.070169	– 1.958808	– 1.3996526
	Wu ca	– 1.824080	– 1.589918	– 0.9600509
	Di gr	– 1.719593	– 1.444136	– 0.7894085
	Vb bl	– 1.760798	– 1.508747	– 0.4562608

Appendix 2 (continued)

Measurement	Species	G05	G10	G40
Stamen insertion height				
	Va ch	– 2.303000	– 2.303000	– 1.848591
	Vm vi	– 2.303000	– 2.246336	– 1.903814
	Ps lo	– 2.246802	– 2.126985	– 1.408967
	Va se	– 2.302999	– 2.216409	– 1.844679
	De pe	– 2.140689	– 2.007497	– 1.281216
	He pa	– 2.005956	– 1.779823	– 1.351049
	Sy re	– 2.261894	– 2.204673	– 1.424075
	Be al	– 2.303000	– 2.303000	– 2.303000
	Wu ca	– 2.182555	– 2.032589	– 1.227370
	Di gr	– 2.018659	– 1.783944	– 1.194895
	Vb bl	– 2.103302	– 1.985053	– 1.675041
Anther length				
	Va ch	– 1.455978	– 1.188549	– 0.2690345
	Vm vi	– 1.732715	– 1.396832	– 0.4805813
	Ps lo	– 1.608798	– 1.264715	– 0.2266827
	Va se	– 1.665098	– 1.368844	– 0.6104324
	De pe	– 1.585346	– 1.221249	– 0.1838237
	He pa	– 1.582830	– 1.226491	– 0.6811044
	Sy re	– 1.527353	– 1.232492	– 0.3521968
	Be al	– 1.421637	– 1.106785	– 0.1181983
	Wu ca	– 1.371273	– 1.122624	– 0.4887085
	Di gr	– 1.705244	– 1.309317	– 0.4589759
	Vb bl	– 1.768783	– 1.600139	– 0.9017253
Abaxial corolla tube length				
	Va ch	– 2.303000	– 2.276428	– 1.1972816
	Vm vi	– 2.059511	– 1.666506	– 0.5687844
	Ps lo	– 2.137360	– 1.946366	– 0.9121582
	Va se	– 2.303000	– 2.224317	– 1.5379121
	De pe	– 2.047671	– 1.851783	– 1.0397881
	He pa	– 1.898568	– 1.615679	– 1.0672688
	Sy re	– 2.113956	– 1.965617	– 1.1779387
	Be al	– 2.183171	– 2.068289	– 1.7215909
	Wu ca	– 2.177598	– 2.048474	– 0.9816218
	Di gr	– 1.962439	– 1.675746	– 0.6509307
	Vb bl	– 1.921106	– 1.674037	– 0.6727100

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Addresses of the authors: C. M. Kampny, Department of Botany, University of Florida, Gainesville, Florida, 32611, USA. – N. G. Dengler, Department of Botany, 25 Willcocks St., University of Toronto, Toronto, ON M5S3B2, Canada.

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