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DIVERSITY AND EVOLUTION OF SEEDLING *BAUPLÄNE* IN *PELARGONIUM* (GERANIACEAE)

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ABSTRACT

The genus *Pelargonium* (Geraniaceae) exhibits tremendous variation in growth form. We apply a broadly defined concept of *Bauplan* in our study of growth form, architectural and anatomical features of early seedling development in the type subgenus. We analyze variation in these features within a phylogenetic framework based on sequence comparisons of internal transcribed spacer regions (ITS) of nuclear ribosomal DNA.

Preliminary ITS sequence comparisons show strong support for two major clades. One major clade contains two subgroups, one consisting of three previously recognized sections of more or less woody shrubs and subshrubs (sections *Pelargonium*, *Glaucophyllum* and *Campylia*), and a second clade of five sections consisting of geophytes and branched stem-succulents (sections *Hoarea*, *Ligularia*, *Polyactium*, *Cortusina* and *Otidia*). The shrubby subgroup is characterized by relatively high levels of variation in architectural determinants such as internode elongation and branching. All members of this subgroup exhibit similar patterns of tissue production by the vascular cambium wherein secondary xylem consists predominantly of lignified tracheary elements and fibers. The stem-succulent clade shows less variation in some architectural determinants such as internode length and branching. The location of the initial onset of axis thickening is variable among species. In all sections except *Ligularia*, axis thickening is correlated with the onset of cambial activity wherein both secondary xylem and phloem are composed of few conducting cells scattered among abundant storage parenchyma. Section *Ligularia* is characterized by cambial activity similar to that in the shrubby clade; epicotylar thickening in this section arises through expansion and division of cortical cells.

The second major clade within the type subgenus contains the monotypic section *Isopetalum*, and sections *Peristera* and *Reniformia*. Members of these sections range from shrubs that are woody at the base to herbaceous scramblers. Species examined exhibit variation in branching but limited internodal elongation. These seedlings were thin stemmed and showed relatively limited activity of a vascular cambium, which produced secondary xylem consisting of lignified tracheary elements and fibers. The occurrence of a vascular cambium producing largely lignified tracheary elements and fibers in all members of the subgenus except the stem-succulent clade suggests that this type of secondary xylem is basal within the subgenus, and that section *Ligularia* has either retained or reevolved the basal state.

Key words: *Bauplan*, Geraniaceae, ITS sequences, *Pelargonium*, plant anatomy, plant development, seedlings, systematics.

INTRODUCTION

Bauplan, the German word for design or architectural plan, has been used in biology to refer to the organizational, structural “ground plan” of morphology characteristic of a group of organisms (see Hall 1992). Frequently the concept is restricted to common features shared by members of phyla or classes, with

Baupläne at lower levels of classification being *Unterbaupläne*, or sub-*Baupläne*. Unfortunately, discussions of *Bauplan* or sub-*Bauplan* rarely state the criteria used to circumscribe any given *Bauplan*, although some workers have described *Bauplan* as the “set of homologous characters defining a group” (e.g., Schwenk 1994/95).

Woodger (1945) proposed a formal definition of

Bauplan based on recognition of the “complete set of parts of a life” for which correspondence (i.e., structural homology) could be determined. His view was explicitly hierarchical and evolutionary: *Baupläne* could be constructed at any taxonomic level and nested within levels, and “If two lives exhibit the same *Bauplan*, there is an ancestor common to both which exhibits a *Bauplan* which is exhibited by all three lives” (Woodger 1945). Similar views of *Bauplan* have been articulated by others such as Verraes (1981), who emphasized the necessity of including development in delimiting a *Bauplan*.

Practical delimitation of the *Bauplan* for a group of organisms requires explicit statements of the morphological and developmental determinants involved. Woodger (1945) emphasized that the organism, not the character, is the meaningful unit of a *Bauplan*. All characters evolve as part of an integrated whole, and frequently it is the conflicting demands between optimization of a particular feature versus the need for function of the whole that results in organismic integration and limits the independent evolution of parts (see also Wake, Roth, and Wake 1983; Hall 1992).

If *Baupläne* are to be constructed from “the complete set of parts of a life,” they must include both apomorphic and plesiomorphic features. Synapomorphic characters alone are rarely sufficient for reconstructing organismal morphology at the hierarchical level at which they are synapomorphic, whereas considering only the plesiomorphic characters that have traditionally defined *Baupläne* ignores the unique features of each group. Furthermore, many features of plants, such as internode lengths or branch number, are essential determinants of morphology, yet are much too variable to be considered apomorphic at any level. Ideally, *Baupläne* would include statements of the amount of variation associated with each character at multiple levels, as detection of both variant and invariant characters at each level may ultimately lead to recognition of which components of *Bauplan* are historically stable underlying patterns, and which have potentially higher levels of evolvability (Arnold 1989; Wagner 1995).

In plants, the body is generally constructed of similar subunits, e.g., metamers and modules, repeated according to an underlying “architectural model” (see Hallé, Oldeman, and Tomlinson 1978). Complete description of a plant *Bauplan* must include both the individuating features (see Wagner 1989) of these metamers or modules where they exist (e.g., heteroblasty) and statements of the rules of organization underlying the pattern arising from assemblages of these subunits (see, e.g. Nijhout 1991). This type of integrated approach may allow for detection of emergent architectural properties arising from correlated evolution of suites of characters that otherwise would be missed by

a sole emphasis on unique features of the individual units.

For comparison of *Baupläne* within an evolutionary context, it is critical to obtain a phylogenetic framework of the groups in question so that one can examine the *Baupläne* for monophyletic groups. *Baupläne* can then be determined for specific nodes or hierarchical levels within the study group. In terminal groups such as species, the *Bauplan* will be a statement of morphology of the group. For deeper branches within the phylogeny, the *Bauplan* will include the aspects of morphology held in common by the taxa in the clade, while excluding autapomorphic features of a particular taxon. The evolution of the *Bauplan* can then be expressed in terms of clade-specific patterns of loss and gain of characters, the generation or restriction of specific character states, their inherent variation, and ultimately elucidation of “rules” underlying their integration.

In the following study, we compare patterns of seedling development among species and sectional groups in the genus *Pelargonium* as a first contribution to the study of evolution of *Baupläne* in this group. We also present a preliminary phylogenetic framework for a portion of the genus based on DNA sequence comparisons of the ITS region. *Pelargonium* is an excellent group in which to explore the evolution of architectural *Baupläne* because it is extremely diverse in morphology, particularly in whole plant growth form. The genus is also very important in horticulture, with the “garden geranium” (*P. ×hortorum*) being one of the most widely grown ornamental plants in the world, and as an important source of essential oils in the perfume trade.

The family Geraniaceae *sensu stricto* (delimited by an unusual combination of ovary and fruit characters) comprises the five genera *Erodium*, *Geranium*, *Monsonia*, *Sarcocaulon* and *Pelargonium*, and is strongly supported as a monophyletic group by sequence comparisons of the chloroplast gene *rbcL* (encoding the large subunit of ribulose biphosphate carboxylase/oxygenase) (Price and Palmer 1993). The closest relative to the family found to date from *rbcL* sequence comparisons is a small Andean genus of rosette plants, *Hypseocharis*, which differs from the Geraniaceae *sensu stricto* in having a capsular fruit with five multi-seeded units rather than a schizocarpous fruit with five one-seeded units. The *rbcL* sequence comparisons have provided strong evidence that *Pelargonium* is a monophyletic group which comprises a basal sister-group to the other four genera in the family (Price and Palmer 1993). The genus *Pelargonium* contains approximately 250 species (Albers, Gibby, and Austmann 1992), of which 80% are generally restricted to the winter rainfall region of the southwest coast of South Africa, particularly the Cape Province area

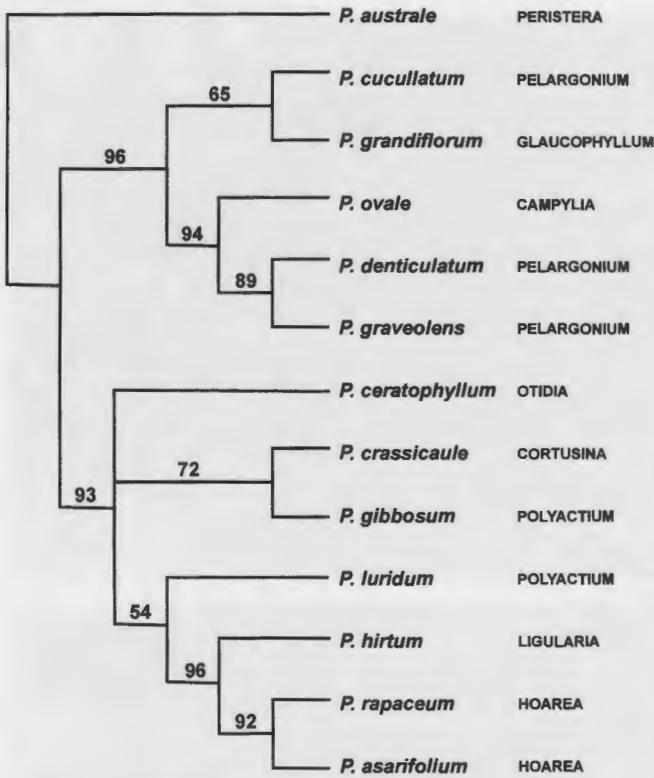


Fig. 1. Bootstrap consensus tree based on maximum parsimony analysis of internal transcribed spacer regions (ITS) of nuclear ribosomal RNA of the type subgenus of *Pelargonium*. Bootstrap values out of 100 replicates are given.

(van der Walt and Vorster 1988). At least 30 species are restricted to areas outside of South Africa. These occur primarily in the region from eastern Africa north to the Arabian Peninsula, Iraq, and Turkey. There are also a small number of species on Madagascar, on two isolated islands in the Atlantic Ocean, and in Australasia.

The genus *Pelargonium* has been the subject of intensive systematic study over the last two decades by J. J. A. van der Walt and colleagues, resulting in a greatly improved knowledge of species delimitation in the genus and the publication of the elegantly illustrated multivolume series of species treatments, *Pelargoniums of Southern Africa* (van der Walt 1977, 1981; van der Walt and Vorster 1988), and detailed monographs of several sections (e.g., van der Walt 1985; van der Walt and Van Zyl 1988). Thirteen sections were recognized in the genus in the provisional treatment of van der Walt (1990), and the additional section *Reniformia* was delimited by Dreyer et al. (1992). Recent comparative studies of morphology, cytology, secondary product chemistry, and *rbcL* sequences conducted in a collaborative effort by a number of research groups in South Africa, Europe, and the United States have resulted in a number of proposed changes in the sectional classification of the genus, particularly with respect to the delimitation of the highly unnatural section *Ligularia* (see for example Albers et al. 1992; van der Walt et al. 1995).

Ongoing phylogenetic comparisons across the sections of *Pelargonium* using *rbcL* sequence comparisons suggest that the genus is divided into two previ-

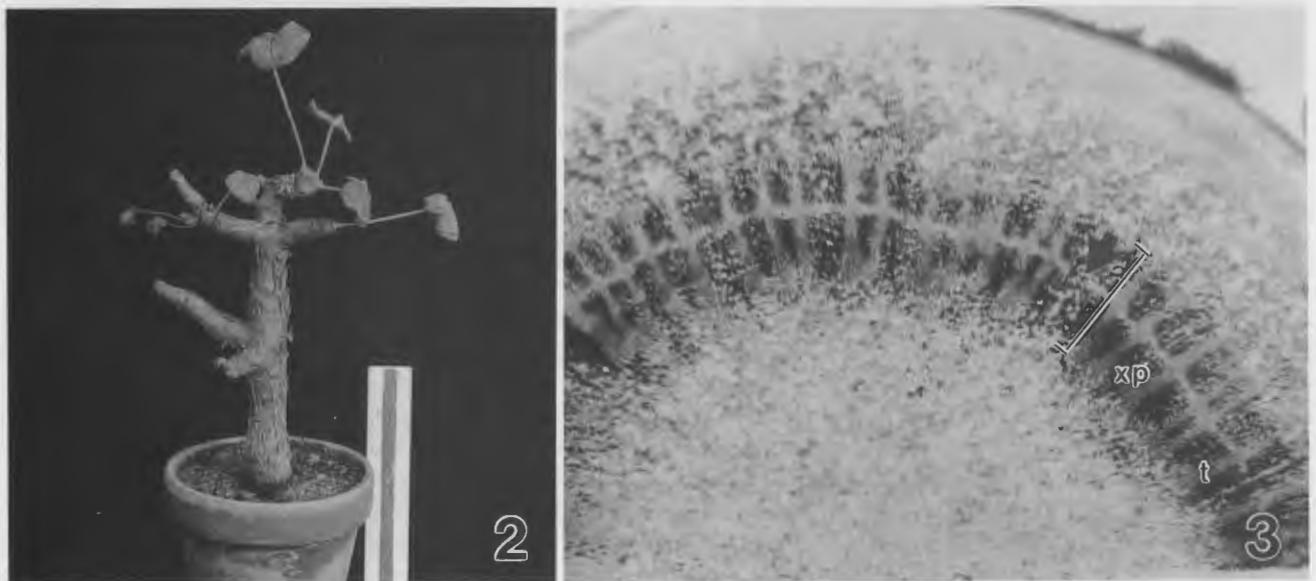


Fig. 2-3. *Pelargonium cotyledonis* (section *Isopetalum*).—2. Whole plant.—3. Hand transection of branch stained with IKI and phloroglucinol; blackened regions indicate presence of starch in fresh tissue. Bracket demarcates extent of secondary phloem and xylem; arrow = cambial zone. Note broad bands of secondary parenchyma (*xp*) derived from interfascicular cambium alternating with narrow bands of tracheary elements and fibers (*t*).

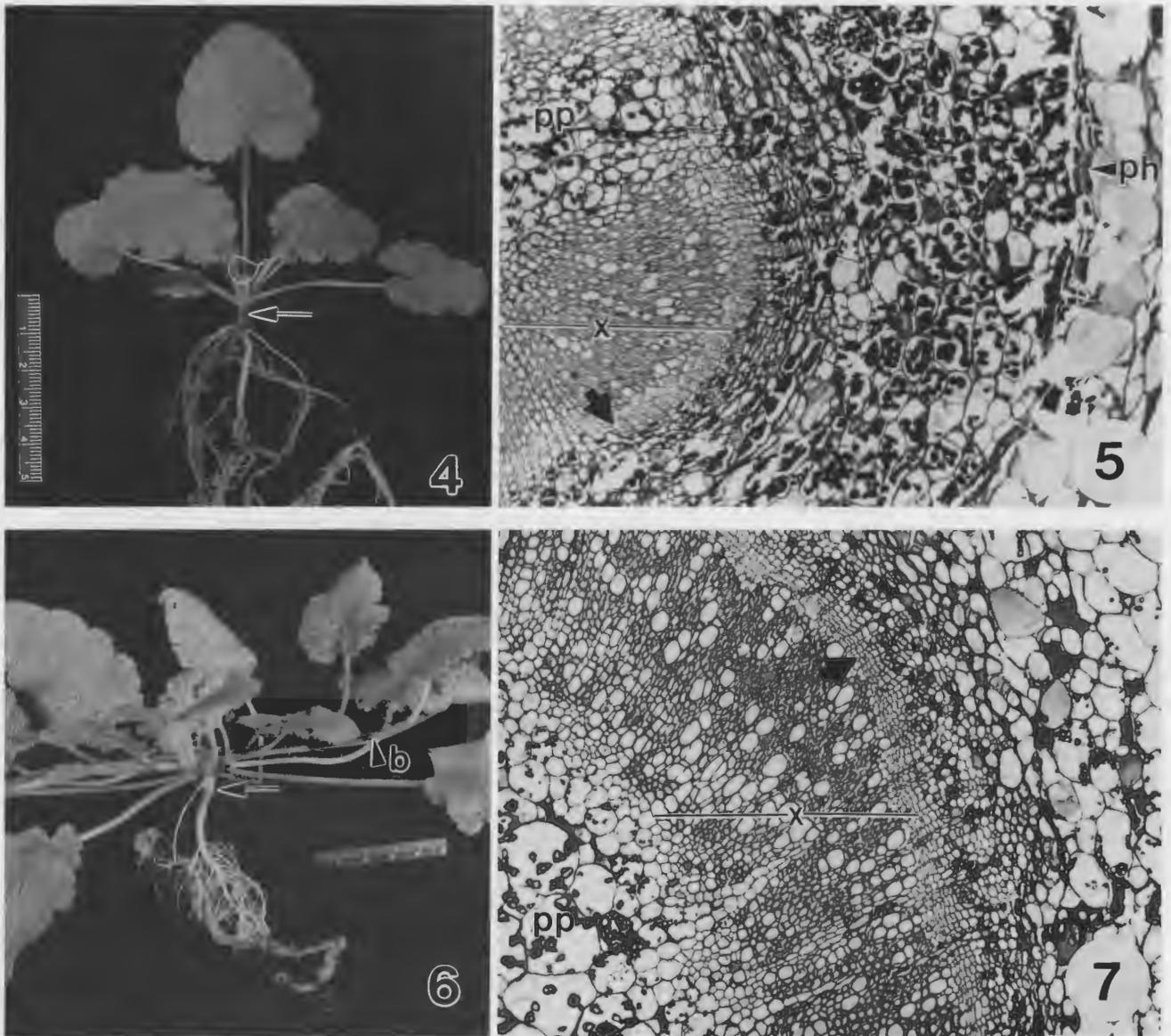


Fig. 4–7. Members of the type subgenus serving as an outgroup to the remaining sections.—4, 6. Seedlings two months old; arrow = hypocotyl; ruler = 5 cm.—5, 7. Transverse sections through upper regions of hypocotyl of seedlings two months old; x = secondary xylem, predominantly tracheary elements and fibers; arrow = cambial zone; pp = primary parenchyma of pith (see Esau, 1977, p. 494 for description of typical vascular arrangement in hypocotyls of dicotyledons).—4–5. *Pelargonium odoratissimum* (section *Reniformia*).—5. ph = phellogen; 100 \times .—6, 7. *P. chamaedryfolium* (section *Peristera*).—7. 82.5 \times .

ously unrecognized subgeneric groups (Price and Palmer 1993; Price, pers. comm.) One subgenus includes the horticultural geranium (*P. \times hortorum*) and its relatives while the second subgenus includes section *Pelargonium* (which includes the commercial scented geraniums) and a number of highly speciose sections which exhibit a wide range of vegetative morphology, including annual and perennial herbs, stem succulents, and geophytes.

The type subgenus includes eleven currently recognized sections and the great majority (over 200) of the species in the genus. Resolution of relationships among these sections was very limited from *rbcL* se-

quence comparisons, so we have undertaken preliminary sequence comparisons of the internal transcribed spacer regions (ITS) between the 18S and 26S nuclear ribosomal RNA genes. The sequences of these spacer regions exhibit a rate of base substitution approximately 5–6 times greater than for *rbcL* within *Pelargonium* and thus give considerably more resolution among closely related sectional or species groups, but cannot be used to compare subgenera within the genus because of difficulty in aligning the sequences.

RbcL sequence comparisons have suggested a major division of the type subgenus into two groups of sections (see Fig. 28). The first of these subgroups in-

Table 1. Characteristics of two month old seedlings of species studied.

Sections	Seedling Characters					Site of axis thickening
	Hypocotyl elongation above ground (mm)	Length of internode #5 (mm)	Width of internode #5 (mm)	# branches per plant at internodes 1-10		
Clade I (shrubs woody at base to herbaceous scramblers)	range of species means	<2.0 ± 0 to	2.0 ± 0 to	0	none or root	
		14.0 ± 1.4	2.21 ± 0.1			
Clade IIa (woody shrubs and subshrubs)	(P. chamaedryfolium) range of species means	9.25 ± 10.7	4.49 ± 1.4	5.25 ± 4.6	none or root	
		<2 ± 1.7 to 9.67 ± 8.9	<2.0 ± 0 to 3.7 ± 2.1	0 to 9.67 ± 0.8	none	
Clade IIb (stem succulents)	(P. tabulare-1 individual at 2 mo.) range of species means	5.0	8.52	8.0	none	
		0	<2.0 ± 0 to 3.0 ± 1.4	2.52 ± 0.3 to 3.23 ± 1.1	3.67 ± 4.7 to 7.00 ± 0	
Clade IIb (stem succulents)	range of species means	4.60 ± 0.6 to 7.00 ± 4.2	<2.0 ± 0	0 to 1.50 ± 2.1	root/hypocotyl/epicotyl	
		0	<2.0 ± 0	<2 to 4.15 ± 1.2	hypocotyl	
Clade IIb (stem succulents)	range of species means	14.00 ± 3.6 to 17.50 ± 4.7	<2.0 ± 0	0 to 1.00 ± 1.7	epicotyl	

cludes sections *Isopetalum*, *Peristera* and *Reniformia* and will be referred to as Clade I for purposes of discussion. The monotypic section *Isopetalum* consists of the unusual species *P. cotyledonis*, which is a sparsely branched subshrub with relatively succulent stems (van der Walt and Vorster 1988). Section *Peristera* includes a group of sprawling, thin-stemmed, herbaceous plants, which range from annuals to short lived perennials. The newly described section *Reniformia* includes a group of eight or more evergreen species which can be either biennial to perennial herbs or woody subshrubs (Albers et al. 1992; Dreyer et al. 1992), several of which form root tubers. In the ITS comparisons presented here, Clade I has been used as the outgroup (represented by *P. australe* from section *Peristera*).

The ITS sequence comparisons provide support for two major clades among the remaining eight sections of the subgenus, supported by bootstrap values of 96 and 93% respectively (Fig. 1). One clade, referred to as Clade IIa, comprises the three currently recognized sections *Campylia*, *Glaucophyllum* and *Pelargonium*. Our preliminary results suggest that section *Pelargonium* (represented by *P. cucullatum*, *P. denticulatum* and *P. graveolens*) is a paraphyletic (and quite likely polyphyletic) group as currently circumscribed, while section *Campylia* (represented by *P. ovale*) and section *Glaucophyllum* (represented by *P. grandiflorum*) appear to be derived from within it.

The second major clade, referred to as Clade IIb, contains the sections *Ligularia sens. str.*, *Otidia*, *Polyactium*, *Cortusina*, and *Hoarea*. Of these, our preliminary ITS comparisons only provide support for section *Hoarea* as a clear-cut monophyletic group, supported by a bootstrap value of 92%. Section *Ligularia* has traditionally been delimited in a very broad sense, including a very heterogeneous group of species in terms of morphology and cytology (see Albers et al. 1992). It is currently being divided into a number of new sections (see Albers et al. 1995; van der Walt et al. 1995). A representative of section *Ligularia sens. str.* (*P. hirtum*) is placed as a sister-group to section *Hoarea* in our analysis, with a bootstrap value of 96% for the branch linking the two groups.

MATERIALS AND METHODS

Elucidation of the *Bauplan* in *Pelargonium* requires dissection of growth forms into component parts. Rather than focusing directly on complex morphological patterns of older plants, which arise in part from phenotypic plasticity, we focus on early stages of seedling development. We examined 3-5 individual seedlings at 1-4 months of age in 2-4 species per section in the type subgenus (Appendix 1).

Plants were grown from seed in the greenhouse at

the University of Connecticut to provide as uniform an environment as possible over the observation period. Characters measured emphasized early development, including the mode of germination; degree of elongation and width increase of internodes, and frequency of branching, as indicators of early "shrubbiness"; and the degree and location of stem thickening as indicators of the onset of succulence. Leaf shape variation was not considered in the present analysis. Distal regions of hypocotyls were fixed in FAA (Berlin and Miksche 1976), dehydrated through a graded ethanol series, embedded in JB-4 embedding medium (PolySciences, Warrington, PA), and sectioned at 5 μ m. Sections were stained with Periodic Acid-Schiff's reagent (PAS) to detect starch granules, and counterstained with toluidine blue (O'Brien and McCully 1981).

RESULTS AND DISCUSSION

We begin by considering morphological variation in Clade I of the type subgenus that includes *Peristera*, *Reniformia* and *Isopetalum*, i.e., the outgroup to the remaining sections of the type subgenus (see Fig. 28). The island endemic *P. cotyledonis* (section *Isopetalum*) is a stem succulent forming a sparsely branched "shrublet" (van der Walt and Vorster 1988; Fig. 2). The plant is very rare under natural conditions, but has been widely vegetatively propagated in cultivation. It is apparently self-incompatible (van der Walt and Vorster 1988) and thus we have not had seeds available from which to study early development. Hand sections through branches suggest that stem succulence arises through proliferation of pith and cortical parenchyma, and to a lesser extent, secondary tissues (Fig. 3). Unstained parenchyma tissue appears highly vacuolate, indicating water storage, and both pith and inner cortical parenchyma cells contain starch, as indicated by positive reaction to IKI. Secondary xylem is present and consists of thin, radial files of tracheary elements alternating with recognizable radial files of parenchyma cells that stain darkly in the presence of IKI.

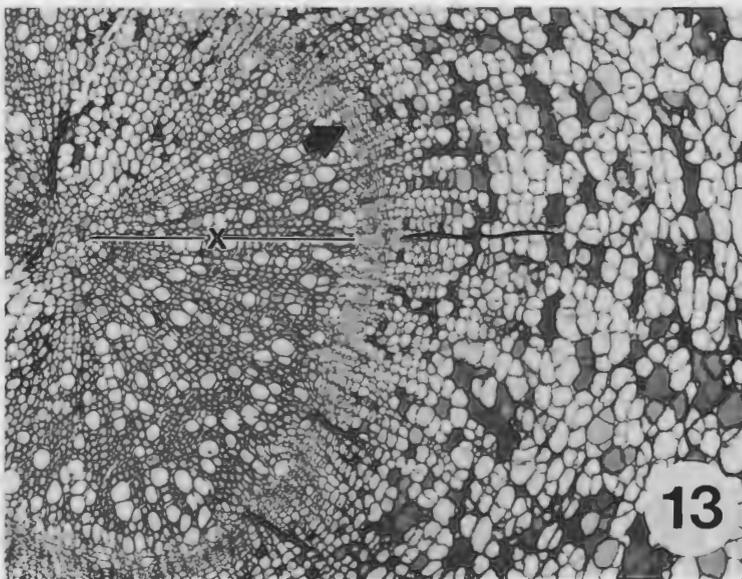
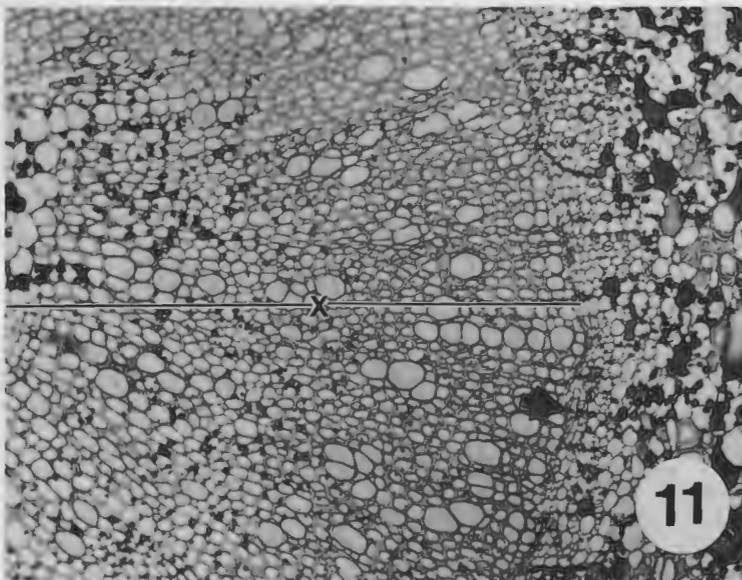
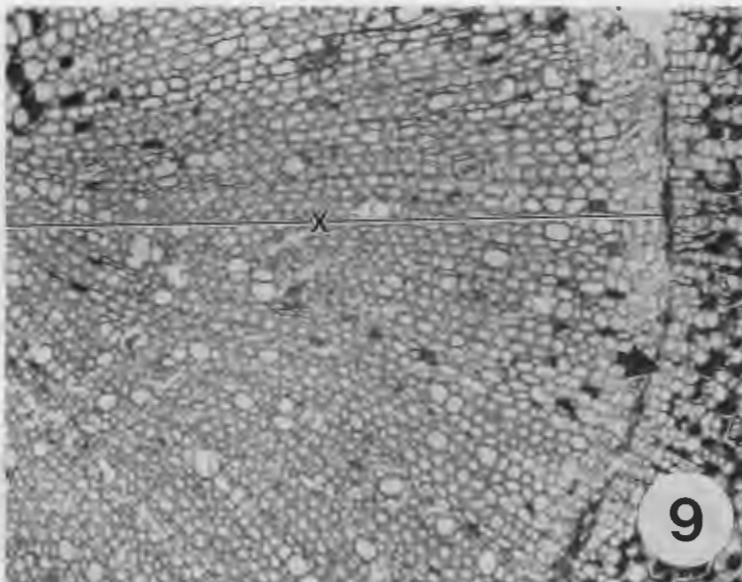
The species of section *Reniformia* have similar floral and pollen morphology and an unusual chromosome base number for the genus ($x = 8$) (Albers et al. 1992; Dreyer et al. 1992), thus they are considered to comprise a monophyletic group. The section includes subshrubs described as herbaceous, or subshrubs woody at the base, e.g., *P. abrotanifolium* (van der Walt 1977, p. 1) and *P. exstipulatum* (van der Walt 1981, p. 61).

Some of the herbaceous species have tuberous roots (e.g., *P. sidoides*; see van der Walt and Vorster 1988, p. 129). In the seedlings examined in this section, hypocotyl and internodal elongation vary among species, and in some species, vary among individuals as well. All seedlings were thin-stemmed with relatively short internodes (e.g., *P. odoratissimum*, Fig. 4, Table 1). Histological sections through the hypocotyls of the species examined showed limited activity of a continuous vascular cambium. Inner xylary derivatives of the cambium differentiated largely as thick-walled fibers and tracheary elements in *P. odoratissimum* (Fig. 5); the secondary xylem of the hypocotyl of *P. exstipulatum* contained more axial parenchyma and few fibers (data not shown). In all taxa examined, inner cortical cells did not enlarge, but contained starch granules. These cells were separated from outer cortical cells at this stage by the origin of a phellogen (Fig. 5, ph).

Section *Peristera* is a poorly understood group of generally small-flowered herbaceous species with a wide distribution from southern and eastern Africa and Madagascar west to the island of Tristan de Cunha and to Australia and New Zealand. Under natural conditions the plants may behave as facultative annuals, while under cultivation the plants can readily perennate, and some produce root tubers (van der Walt and Van Zyl 1988; observations of the authors). The members of this section do not become obviously woody at the base and tend to have a low-sprawling, highly branched habit (e.g., *P. chamaedryfolium*, Fig. 6, Table 1). Many species are relatively thin stemmed. Histological sections through the hypocotyl of *P. chamaedryfolium* revealed the presence of a vascular cambium, although production of secondary xylem and phloem was not extensive. Secondary xylem consisted of predominantly lignified tracheary elements and fibers (Fig. 7). Hypocotyls of this species had a prominent pith of unligified parenchyma cells containing starch granules.

Hence, within this group of sections, growth forms range from annuals to perennials, and from prostrate scramblers to shrubs that are somewhat woody at the base (Fig. 28). The pronounced stem succulence in *P. cotyledonis* is unusual in Clade I. Although studies of additional species are needed to document complete diversity of seedling growth forms, our preliminary observations suggest that seedling hypocotyls consistently exhibit a typical bifacial vascular cambium initially limited in its activity, even in species that sub-

Fig. 8–13. Members of the clade containing sections *Pelargonium*, *Glaucophyllum*, and *Campylia*.—8,10,12. Seedlings; arrow = hypocotyl; ruler = 5 cm.—9,11,13. Transverse sections through upper hypocotyl; x = secondary xylem, predominantly tracheary elements and fibers; arrow = cambial zone.—8. *Pelargonium cucullatum* subsp. *tabulare* (section *Pelargonium*) two months old.—9. *P. cucullatum* subsp. *tabulare* three months old; 87.5 \times .—10–11. *P. tabulare* (section *Glaucophyllum*) four months old.—11. 78 \times . 12–13. *P. ovale* (section *Campylia*) two months old.—13. 78 \times ; bracket = secondary phloem.



sequently become somewhat woody at the base, such as *P. exstipulatum*, or succulent (*P. cotyledonis*). Secondary xylem is composed largely of tracheary elements and fibers, except in *P. cotyledonis*. Differences in hypocotyl thickness in this group result primarily from differences in thickness of pith and cortical tissues (Jones, unpublished data).

The second major clade, Clade IIa, consists of more or less woody shrubs and subshrubs in sections *Pelargonium*, *Glaucophyllum* and the tuftlike subshrubs in section *Campylia*. Section *Pelargonium* as currently circumscribed comprises branched woody or partially woody shrubs that are moisture loving and distributed in the southern and southwestern Cape Region of South Africa under winter rainfall conditions, with a few species occurring in summer rainfall regimes (van der Walt 1985). Seedlings of section *Pelargonium* are all initially orthotropic (e.g., *P. cucullatum* subsp. *tabulare*, Fig. 8) but some species lose their orthotropic orientation when the newer internodes elongate and become plagiotropic and sprawling (e.g., *P. capitatum*, van der Walt 1977, p. 7). Initial seedling development is characterized by substantial variation among species in hypocotyl elongation above ground, internodal elongation, and degree of branching (Table 1). Sections through the two-month-old hypocotyls in all species examined revealed an active, continuous, bifacial vascular cambium producing largely fibers and tracheary elements centripetally, and a small pith (Fig. 9). (Details of wood anatomy of mature stems of several species in section *Pelargonium* are presented in van der Walt et al. 1987). The cortex consists of relatively small cells containing numerous starch granules.

Section *Glaucophyllum* as currently circumscribed comprises small shrubby plants with leathery glaucous leaves which are commonly xerophytes from the winter rainfall area. A close relationship between sections *Pelargonium* and *Glaucophyllum* has been suggested previously based on chromosome numbers, floral morphology and hybridization (van der Walt 1985; van der Walt et al. 1990). Members of section *Glaucophyllum* also were similar to members of section *Pelargonium* in seedling architecture, showing strong orthotropic growth coupled with variation in above ground hypocotyl elongation, internodal elongation and branching (Fig. 10). As in *Pelargonium*, members of section *Glaucophyllum* exhibited an active, continuous bifacial vascular cambium producing inner derivatives which

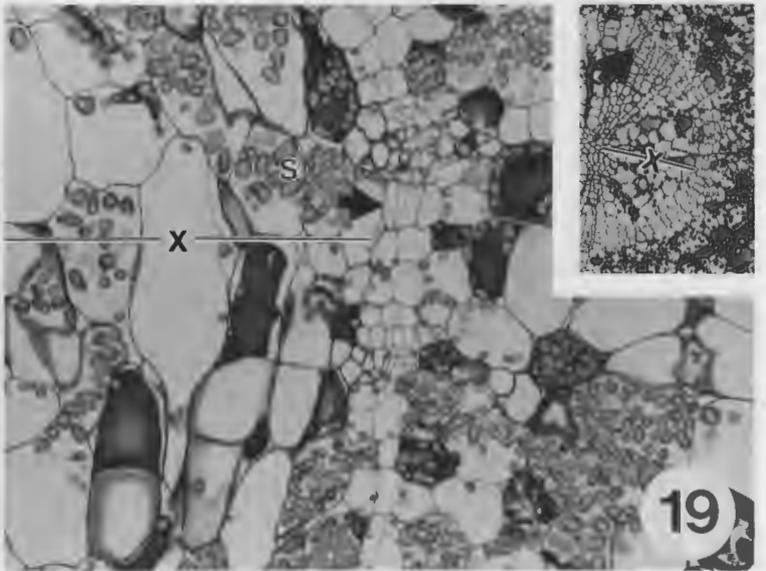
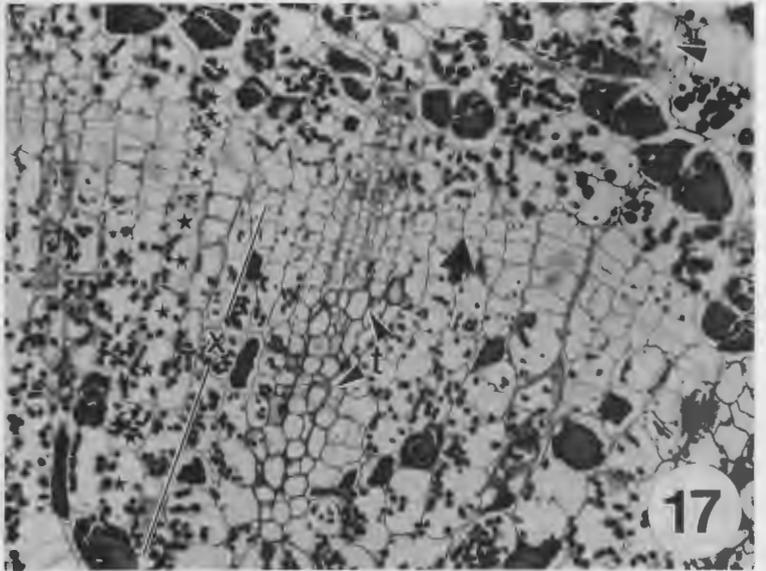
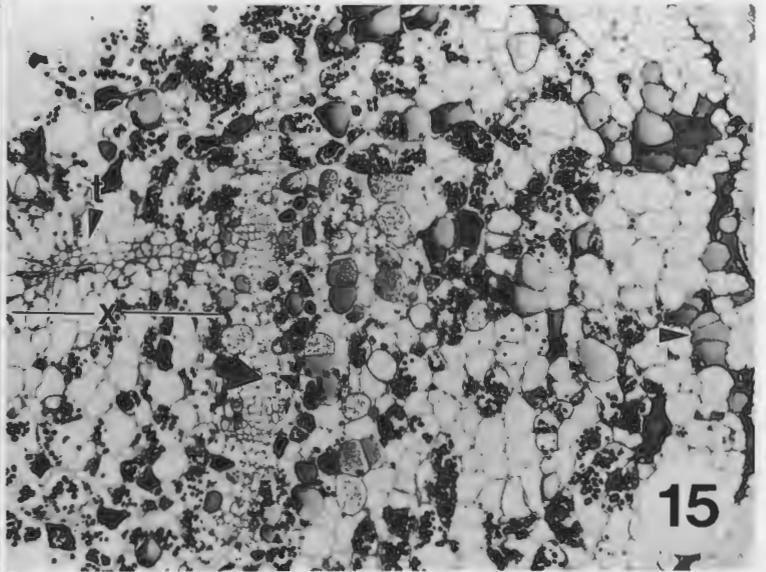
differentiated predominantly as tracheary elements and fibers (Fig. 11). Hypocotyls of *P. tabulare* also contained a small amount of pith parenchyma (not shown).

Section *Campylia* apparently arises from within the clade comprising sections *Pelargonium* and *Glaucophyllum* (Fig. 1). Because the species of section *Campylia* are similar in floral morphology and differ from the rest of the clade in having a derived chromosome base number ($x = 10$ as compared to $x = 11$ in sections *Pelargonium* and *Glaucophyllum* and most of the subgenus; see Albers and van der Walt 1984; van der Walt and Van Zyl 1988; van der Walt and Roux 1991), the section is considered to be a derived, natural group. Members of this section are tuft-like subshrubs without underground tubers of any kind, inhabiting sandy regions in the winter rainfall area of southwestern Cape Province. Seedlings of all species of *Campylia* examined were nearly invariant in morphology, showing no hypocotyl elongation above the surface of the soil, very short internodes and some variation in branching (Table 1; Fig. 12). Histological sections of the upper region of the hypocotyls revealed a continuous vascular cambium. Accumulation of secondary xylem in seedlings was not extensive; that which was formed contained predominantly tracheary elements (Fig. 13). Based on the linear arrangement of cells exterior to the vascular cambium and the presence of scattered sieve tube members in this tissue, the production of secondary phloem in species of section *Campylia* was more extensive than that in sections *Pelargonium* and *Glaucophyllum*. Very little pith tissue was present in the hypocotyl; cortical thickness contributed significantly to the thickness of the stem.

Within Clade IIa as a whole, hypocotyl elongation, internodal growth and the degree of branching are quite variable (Fig. 28). The vascular cambium becomes continuous and active at an early point in seedling hypocotyl development, with the majority of inner derivatives differentiating as lignified tracheary elements or fibers. Within section *Campylia*, however, the variation in several determinants of seedling architecture is reduced relative to the remaining sections. This lack of morphological variation is coupled with a lower amount of early activity of the vascular cambium and thus a reduced accumulation of xylary conducting tissue in the hypocotyl.

The last major clade (Clade IIb), consisting of five

Fig. 14–19. Members of section *Otidia*.—14,16,18. Seedlings; arrow = hypocotyl; ruler = 5 cm.—15,17,19. Transverse sections through upper hypocotyl; x = secondary xylem consisting predominantly of parenchyma derived from vascular cambium; t = tracheary elements; arrow = cambial zone.—14–15. *Pelargonium laxum* two months old.—15. 44 \times , arrowhead = recently divided cortical cells.—16. *P. carnosum* four months old.—17. *P. carnosum* two months old. Note linear files of cambial derivative cells (stars) extending both centripetally and centrifugally from cambial zone; arrowhead = recently divided cortical cells; 90 \times .—18–19. *P. crithmifolium* four months old.—19. s = starch granules; 90 \times .—Insert: Transection of one month old seedling of *P. crithmifolium*. Note linear files of secondary xylem parenchyma cells; 37.5 \times .



sections, includes growth forms ranging from geophytes to sparsely or highly branched stem succulents. Despite diversity of growth form, all members of this clade share one feature in common: early onset of axis thickening originating in the root, the hypocotyl, or uniformly along the root/hypocotyl/epicotyl axis.

Section *Otidia* as currently circumscribed contains stem succulent species with thick, photosynthetic stems and deciduous leaves (van der Walt and Vorster 1988). Seedling growth in this section was characterized by variation in hypocotyl growth above ground, limited internodal elongation, very limited branching, and early onset of stem thickening (Table 1; Figs. 14, 16, 18). In *P. laxum*, as in all members of this section, stem thickening was externally apparent from one month of age and was initiated in either the epicotyl or hypocotyl, but soon extended along the main axis. Sections through hypocotyls revealed that thickening occurred both through accumulation of secondary parenchyma produced both centrifugally and centripetally, and through scattered divisions of cortical parenchyma cells (Fig. 15). Parenchyma cells in both secondary xylem and phloem frequently increased in size and functioned in storage of starch, water, and other compounds. That these parenchyma cells were originally derived from the vascular cambium was evidenced by the arrangement of cells in linear files apparent in transverse section. In some species, such as *P. carnosum*, parenchyma derivatives did not enlarge significantly and the integrity of cell files of both tracheary elements and parenchyma cells was maintained in slightly older seedlings (Fig. 17, note cells marked with stars). However, in other species, such as *P. crithmifolium*, differential enlargement of secondary parenchyma cells was so extensive that radial files of cells were impossible to detect in older seedlings (Fig. 19). However, sections through 3–4 week old seedling hypocotyls revealed that these parenchyma cells arose from the early activity of a continuous vascular cambium producing linear files of xylem cells (insert).

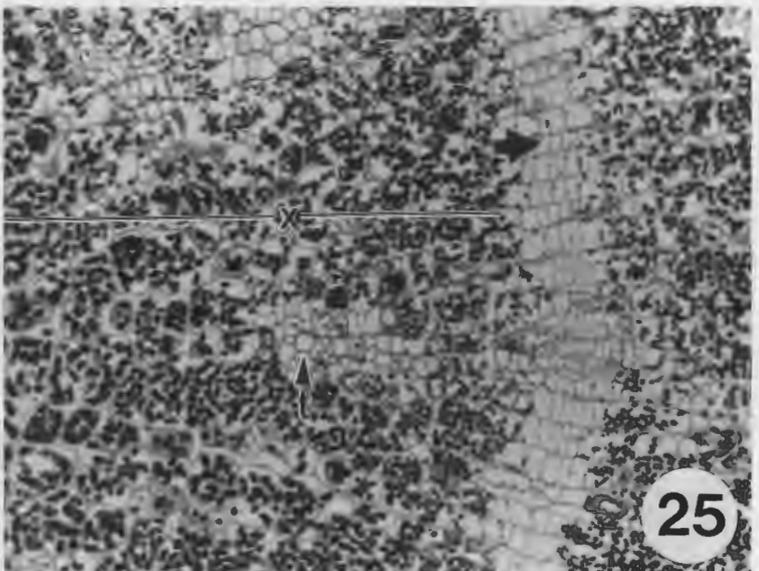
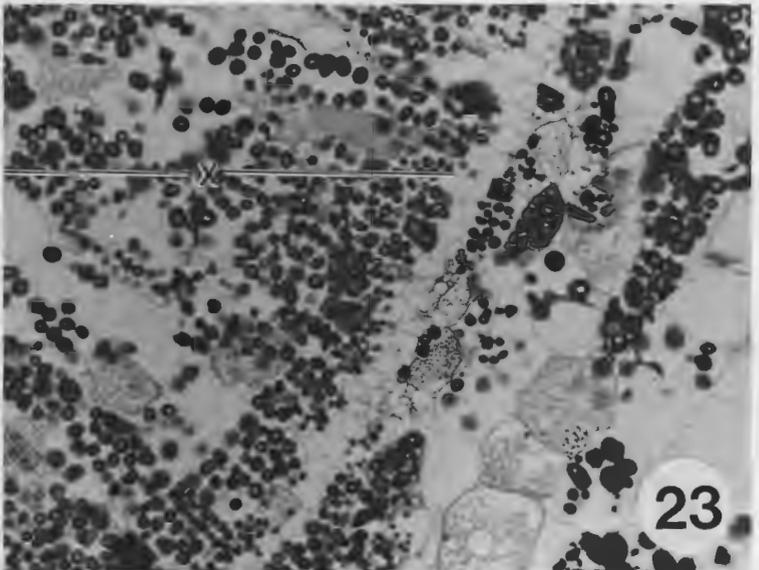
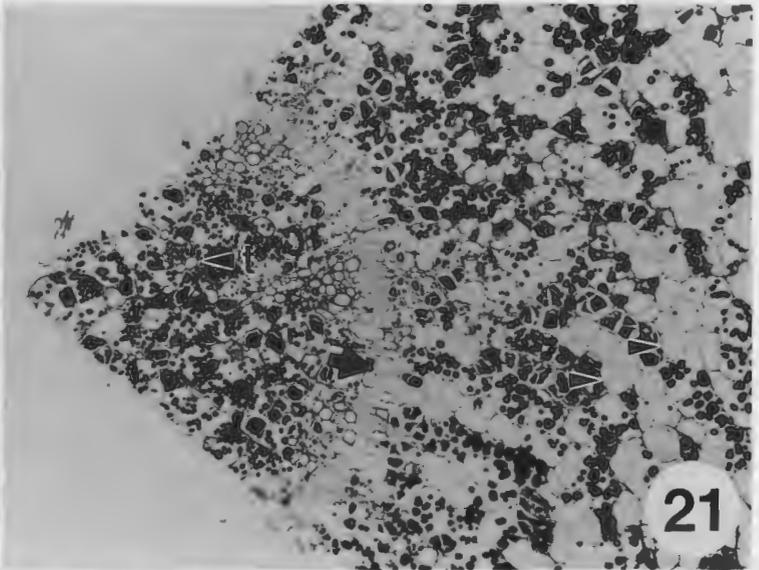
Section *Cortusina* also is characterized by stem succulence, and seedling morphology in this group showed consistent early thickening of the hypocotyl, variable hypocotyl elongation above ground, and limited internodal elongation (Table 1). This section exhibited variation in the distribution of thickening growth, with some members having initial axis thickening localized in the hypocotyl, and others with early thickening distributed over a broader region of the

stem (see, e.g. *P. xerophyton*, Fig. 20). Some species of section *Cortusina* displayed no seedling branching, while others were quite branched by four months of age. Cambial activity was similar to that in members of section *Otidia*, showing extensive activity in hypocotyls, with the majority of derivatives differentiating as parenchyma cells that store starch or water (Fig. 21). As in section *Otidia*, stem thickening in this group was further enhanced by radial anticlinal divisions in cortical parenchyma cells.

Members of section *Polyactium* display very divergent morphologies, ranging from geophytes to stem succulents (van der Walt and Vorster 1988). Despite diversity in mature plant morphology, seedling morphology of all species of section *Polyactium* examined was similar to that of the other two sections in the cluster, showing initial thickening in the hypocotyl or root. Even *P. gibbosum*, a spindly, scrambling "shrub" with elongate internodes and swollen nodes, formed a tight rosette and a swollen hypocotyl during its first year (Fig. 22). Internode elongation began in the second year, following dormancy. Also consistent with sections *Otidia* and *Cortusina*, the hypocotyl of *P. gibbosum* had an active vascular cambium producing predominantly secondary parenchyma cells that function in storage of water and starch (Fig. 23). Enlargement of cambial derivatives was not extensive; consequently, linear cell files were apparent.

Members of section *Hoarea* are characterized by thick accumulations of bark sheaths that surround subterranean tubers (van der Walt and Vorster 1988) and reduced numbers of petals and stamens in some species. Members of section *Hoarea* uniformly exhibited no appreciable hypocotyl elongation above the surface of the soil and no internodal elongation. This lack of extension of above-ground growth was coupled with an early onset of hypocotylar thickening (Fig. 24; Table 1). Histological sections through the hypocotyl revealed patterns of internal anatomy consistent among members of this section. Activity of the vascular cambium was extensive, and resulted in the production of numerous, relatively small parenchyma cells containing abundant starch granules. These derivatives underwent little enlargement, thus maintaining radial files of cells. Tracheary elements were infrequent and scattered within this secondary tissue (Fig. 25). Extensive numbers of cells were produced centrifugally as well; these cells were also predominantly starch-storing parenchyma cells. The outermost parenchyma cells

Fig. 20–25. Members of sections *Cortusina*, *Polyactium* and *Hoarea*.—20, 22, 24. Seedlings; arrow = hypocotyl; ruler = 5 cm.—21, 23, 25. Transverse sections through upper hypocotyl; x = secondary xylem consisting predominantly of parenchyma derived from vascular cambium; t = tracheary elements; arrow = cambial zone.—20–21. *P. xerophyton* (section *Cortusina*) two months old.—21. Arrowhead = recently divided cortical cells; 44×.—22–23. *P. gibbosum* (section *Polyactium*) four months old.—23. 86×. 24–25. *P. incrassatum* (section *Hoarea*) four months old.—25. Note integrity of radial files of cells and scattered tracheary elements; 87.5×.



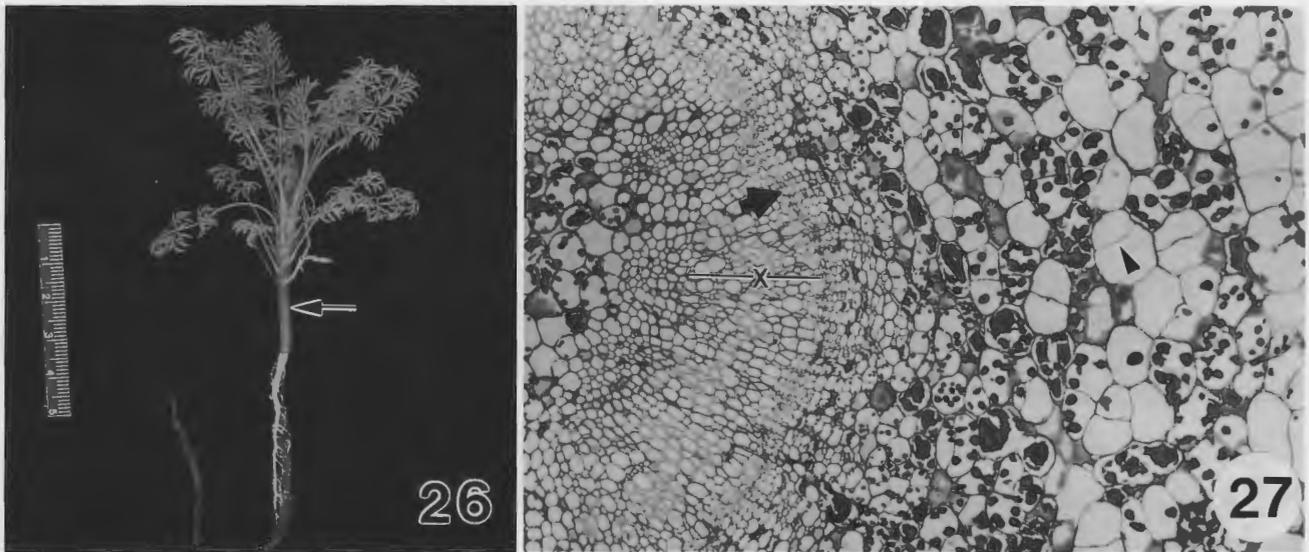


Fig. 26–27. Member of section *Ligularia*.—26. Seedling of *P. oreophilum* two months old, note cotyledon attached on stem opposite and just above arrow; arrow = hypocotyl; ruler = 5 cm.—27. Transverse section through upper hypocotyl; x = secondary xylem; arrowhead = recently divided cortical cell; 83 \times .

eventually lose their starch grains, expand, undergo radial anticlinal divisions and perhaps function in water storage (not shown). Numerous periderms arose from within these tissues.

Members of section *Ligularia sens. str.* are characterized by some degree of stem thickening in the aerial plant body. All seedlings examined showed considerable hypocotyl elongation above ground, but little internode elongation subsequently (Fig. 26; Table 1). Very few seedlings branched within the first four months of growth. Histological sections through the upper hypocotyl revealed a continuous vascular cambium of limited activity. Secondary xylary tissue consisted predominantly of lignified tracheary elements and fibers (Fig. 27). Little pith parenchyma was present in hypocotyls; cortical cells expanded considerably, contributing to the limited increase in hypocotyl thickness. Stem thickening was more pronounced in the epicotyl, resulting in a strongly obconic main axis (easily visualized when leaves were removed from the stem). Interestingly, thickening in the upper hypocotyl and epicotyl arose through divisions and enlargement of primary-walled cortical cells, rather than through pronounced cambial activity typical of other members of this clade.

The members of the stem-succulent Clade IIb exhibit variation in the degree of hypocotyl elongation above ground, and in branching, but all seedlings show little elongation of internodes. This clade is also characterized by some degree of axis thickening, but there is variation in the location, timing of onset, and function of specific tissues in the thickened regions (Fig. 28). In all sections but *Ligularia sens. str.*, the vascular cambium is active early in development of the seed-

ling, and the majority of xylary derivatives function largely in storage of starch, as in section *Hoarea*, or in storage of both starch and water, as in sections *Oti-dia*, *Polyactium* and *Cortusina*. Members of both sections *Hoarea* and *Ligularia* present interesting morphological divergence from the remaining sections. Section *Hoarea* exhibits less variation in architectural determinants, with no elongation of the hypocotyl above ground, no internodal growth, and limited branching. This lack of variation in architecture is associated with a more restricted pattern of cambial activity, localized in the hypocotyl and producing small, parenchymatous interior derivatives that function in starch storage. Section *Ligularia* also shows relatively little variation in seedling architecture, but the mode of stem thickening involving predominantly expansion of cortical cells is apparently restricted to this clade.

GENERAL COMMENTS ON THE EVOLUTION OF BAUPLÄNE IN THE TYPE SUBGENUS OF PELARGONIUM

The three clades within the type subgenus of *Pelargonium* differ in the degree of activity of the vascular cambium and the types of derivatives produced. Two separate lineages exhibit stem succulence, but the degree of involvement of the derivatives of the vascular cambium in succulence differs between the lineages. In *P. cotyledonis* (section *Isopetalum*), the only succulent member of the basal clade (Clade I), succulence in mature stems arises predominantly through the production of parenchyma cells derived from primary tissues; the vascular cambium shows limited activity and its inner derivatives differentiate as alternating bands of tracheary elements and parenchyma

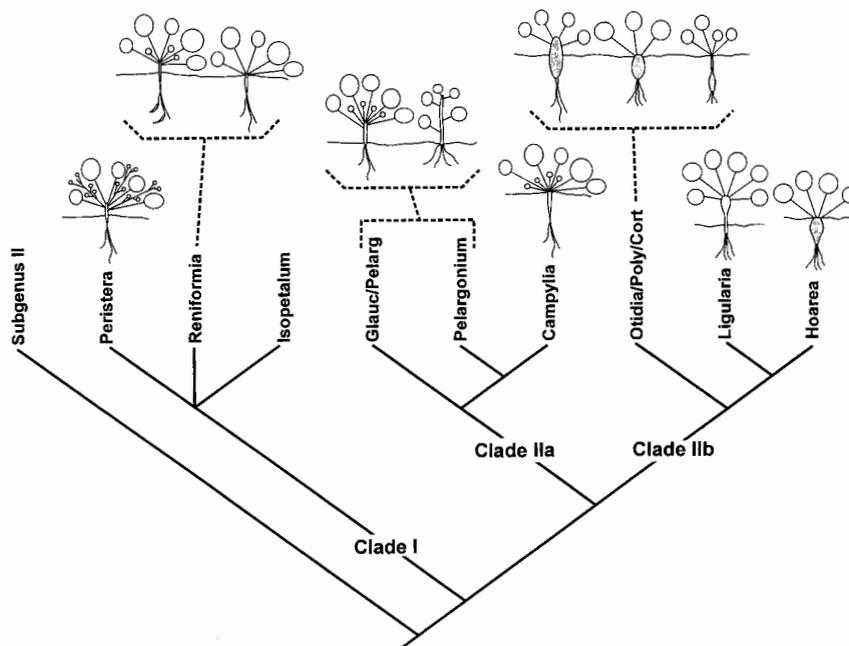


Fig. 28. Cladogram showing sectional relationships based on ITS sequence data presented in Fig. 1. Schematic representations of seedling *Baupläne* are shown for each section or section complex. Bracketed diagrams reflect variation within each indicated group. Shading indicates axes thickened via accumulation of secondary xylem parenchyma. Members of section *Ligularia* exhibit stem thickening due to expansion of primary tissues, i.e., pith and cortical parenchyma cells. In the remaining clades with unshaded axes, a vascular cambium is present and active to varying degrees in producing secondary xylem composed of tracheary elements and fibers.

cells. In the stem succulent clade (Clade IIb), with the exception of *Ligularia sens. str.*, primary and secondary tissues contribute to the succulent nature of the stem, but the contribution of secondary parenchyma to stem thickening predominates; the vascular cambium produces few tracheary elements. These anatomical observations, coupled with the molecular data supporting a close affinity between *P. cotyledonis* and section *Reniformia*, suggest that succulence in *P. cotyledonis* has arisen independently of that in Clade IIb.

In the shrubby Clade IIa, the vascular cambium is also quite active at an early point in development, but its centripetal derivatives are largely tracheary elements and fibers. These observations, coupled with similarity to types of secondary xylary derivatives in sections *Peristera* and *Reniformia*, suggest that a possible key innovation in Clade IIb was the switch in the differentiation of inner cambial derivatives from a predominance of thick-walled tracheary elements and fibers to a predominance of enlarged, thin-walled parenchyma cells. If such a switch occurred prior to the evolution of section *Ligularia sens. str.*, then the latter section represents a reversal to the more ancestral type of secondary xylem tissue. The limited production of lignified xylary derivatives, coupled with enhanced centrifugal production of secondary phloem, is similar to the mode of cambial activity observed in section *Campyilia*, and may represent parallel evolution of these cambial traits in those sections.

Our simultaneous consideration of growth form, ar-

chitecture, and internal structure are preliminary steps toward construction of *Baupläne* in *Pelargonium*. We offer the approach as a context for organizing information from comparative morphological and developmental studies into an evolutionary framework. In emphasizing interaction among variant and invariant characters, as well as their individuating features, this view ultimately will allow detection of clade-specific patterns of character variation and association. For example, in Clade IIa, section *Campyilia* appears to be a well-supported monophyletic group with a consistent growth form of a "tufted subshrub." This growth form arises in all species through a suite of developmental characters that are largely invariant and thus may represent evolution through restriction or constraint on variation in growth form relative to variation in foliar and floral features. In contrast, the group from which section *Campyilia* presumably arose, i.e., the *Pelargonium/Glaucophyllum* complex, exhibits high diversity in the expression of shrubbiness which becomes apparent early in development. In these sections, the early onset of secondary growth involving highly lignified xylem cells is correlated with considerable woodiness at the base of mature plants, increases in plant height, and the early expression of branching in some species.

A similar pattern of restriction in *Bauplan* variation characterizes the monophyletic section *Hoarea*. The entire section is geophytic. In this section, the geophytic habit is associated with invariant features such

as early onset of cambial activity in the hypocotyl, rosette epicotylar growth with no internodal elongation, and very limited branching until flowering. In addition, members of this section are deciduous during the dry season, and leaves and flowers are often not borne simultaneously. Within *Hoarea*, relatively invariant architectural features are associated with great diversity in foliar and floral morphology, which has supported the recognition of over 80 species in this section, rendering it the most speciose section in the genus. It is tempting to conclude that the evolution of the geophytic habit has facilitated radiation of *Hoarea*. However, there are other geophytic species in *Pelargonium* that do not share the particular suite of features described above for section *Hoarea*. For example, *P. antidyentericum*, placed in subgenus II, possesses an underground tuber, but has an extended shoot with elongate internodes and deciduous leaves. Thus it is not simply possession of a geophytic habit or tubers that has led to the successful radiation of section *Hoarea*, but rather possession of the particular suite of traits characteristic of this group. Only by specifically listing all features, their states, and the variation in these states, will such character suites be recognized.

Our approach to *Baupläne* de-emphasizes individualizing or unique aspects of individual characters and instead emphasizes organizational "rules" underlying whole plant architecture. Although our detection of such rules in *Pelargonium* is elementary at this point, we see these rules of interaction ultimately providing links into how diversity is generated or stabilized. By understanding the degree to which the rules themselves determine the degree of variation in features, we will be closer to explaining evolutionary patterns of changes in *Baupläne* that have occurred in the past, and we will be in a much better position to predict which components of *Bauplan* exhibit greater clade-specific "evolvability" and are more likely to vary in the near future.

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Appendix 1. Species of *Pelargonium* examined in architectural and anatomical studies.

Section	Species	Source*
<i>Isopetalum</i>	<i>P. cotyledonis</i> (L.) L'Hérit.	Arid Lands ^{1,4}
<i>Reniformia</i>	<i>P. exstipulatum</i> (Cav.) L'Hérit.	Silverhill Seeds ² —Ladismith area, Cape
	<i>P. odoratissimum</i> (L.) L'Hérit.	Chiltern Seeds ³
	<i>P. reniforme</i> Curt.	Chiltern Seeds
<i>Peristera</i>	<i>P. australe</i> Willd.	selfed; original seed provided by R. Price
	<i>P. chamaedryfolium</i> Jacq.	Silverhill Seeds—ex hort.
	<i>P. grossularioides</i> (L.) L'Hérit.	selfed; original seed provided by Chiltern Seeds
<i>Pelargonium</i>	<i>P. senecioides</i> L'Hérit.	selfed; plant provided by Arid Lands
	<i>P. betulinum</i> (L.) L'Hérit.	Silverhill Seeds—ex hort.
	<i>P. capitatum</i> (L.) L'Hérit.	Silverhill Seeds—west coast of SW Cape, N. of Cape Town
	<i>P. cucullatum</i> subsp. <i>tabulare</i> Volschenk	Silverhill Seeds—SW Cape and Cape peninsula
	<i>P. scabrum</i> (Burm. f.) L'Hérit.	Silverhill Seeds—Citrusdal
<i>Glaucophyllum</i>	<i>P. tabulare</i> (L.) L'Hérit.	Silverhill Seeds—Table Mt. in Cape Town
<i>Campylia</i>	<i>P. elegans</i> (Andr.) Willd.	Silverhill Seeds—ex hort.
	<i>P. ovale</i> (Burm. f.) L'Hérit.	Silverhill Seeds—Franschoek Mts.
	<i>P. tricolor</i> Curt.	Silverhill Seeds—Swartberg Mts.
<i>Otidia</i>	<i>P. carnosum</i> (L.) L'Hérit.	selfed; original seed provided by Chiltern Seeds
	<i>P. crithmifolium</i> J.E. Sm.	selfed from plant in UConn collection
	<i>P. klinghardtense</i> Knuth	Silverhill Seeds—ex hort.
<i>Cortusina</i>	<i>P. laxum</i> (Sweet) G. Don	selfed; plant provided by Arid Lands
	<i>P. cortusifolium</i> L'Hérit.	selfed; plant provided by Arid Lands
	<i>P. echinatum</i> Curt.	Silverhill Seeds—ex hort.
	<i>P. xerophyton</i> Schltr. ex Knuth	selfed from plant collected by C. Craib—upper slopes of Achab SE Berg on the Bushmanland
<i>Polyactium</i>	<i>P. gibbosum</i> (L.) L'Hérit.	selfed from plant in UConn collection
	<i>P. triste</i> (L.) L'Hérit.	Silverhill Seeds—S.W. Cape and Namaqualand
<i>Hoarea</i>	<i>P. appendiculatum</i> (L.f.) Willd.	Silverhill Seeds—ex hort.
	<i>P. asarifolium</i> (Sweet) G. Don	Silverhill Seeds—Somerset West near Cape Town
	<i>P. auritum</i> (L.) Willd.	
	<i>P. incrassatum</i> (Andr.) Sims	Silverhill Seeds—Namaqualand
<i>Ligularia</i>	<i>P. rapaceum</i> (L.) L'Hérit.	Charles Craib (from collection of the late R. Parsley)
	<i>P. crassipes</i> Harv.	selfed; plant provided by Arid Lands
	<i>P. fulgidum</i> (L.) L'Hérit.	Silverhill Seeds—St. Helena Bay
	<i>P. hirtum</i> (Burm. f.) Jacq.	Silverhill Seeds—Lion's head, Cape Town
	<i>P. hystrix</i> Harv.	Silverhill Seeds—ex hort.
	<i>P. oreophilum</i> Schltr.	selfed; plant provided by Arid Lands

* Voucher specimens from each seed source will be deposited in the UConn Herbarium as plants flower.

¹ Arid Lands Greenhouses, Tucson, Arizona, USA.

² Silverhill Seeds, Kenilworth, Republic of South Africa

³ Chiltern Seeds, Ulverston, Cumbria, England; original location of seed not available.

⁴ Not started from seed.