Replacement of Species Along Altitude Gradients: The Role of Branch Architecture

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\textbf{Background and aims} Plant species typical of cold and warm habitats differ in a suite of morpho-physiological traits, although their evolutionary routes have been poorly explored. Here, it is advocated that traits typical of different climate regimes can be largely driven by contrasting branch architectures. This is explored within \textit{Saxifraga}. First, an investigation was carried out to determine whether series \textit{Ceratophyllae} (lateral inflorescences) is segregated to lowlands compared with \textit{Pentadactylis} (terminal inflorescences). Then, two altitudinal vicariants, \textit{S. trifurcata} (lowland, with lateral inflorescences) and \textit{S. canaliculata} (highland, with apical inflorescences), were selected. It was hypothesized that apical flowering of \textit{S. canaliculata} constrains its growth period, bringing with it traits typical of short growth season plants, and conversely for \textit{S. trifurcata}.

\textbf{Methods} The hypothesis was tested by measuring plant compactness and organ pre-formation in seven populations of these species along an altitude gradient.

\textbf{Key Results} Most variables differed among species. Morphological variables at all scales support that the architecture of \textit{S. canaliculata} generates a more compact habit. A higher number of primordia and earlier inflorescence pre-formation in \textit{S. canaliculata} indicate that it begins organogenesis earlier. Data on organogenesis suggest that the different timing of inflorescence initiation may be the origin of the contrasting architectures. Within species, shoot compactness increased, and the length of lateral primordia decreased, as altitude increased. All other metrics were similar among locations of the same species at contrasting altitudes.

\textbf{Conclusions} The hypotheses linking elevational segregation of species, architecture and pheno-morphological traits were validated at broad (gen. \textit{Saxifraga}) and local (altitudinal vicariants) scales. This supports the initial idea that shoot architecture may to a large extent condition high altitude adaptive syndrome.

\textbf{Key words:} \textit{Saxifraga,} meristem activity, altitude, architecture, phenology, organogenesis.

\section*{INTRODUCTION}

Plant species thriving at habitats that differ in harshness or seasonality of climate show conspicuous phenotypic syndromes that demonstrate the adjustment of the plant body to contrasting thermal regimes. Comparisons of plant species living at low vs. high altitudes in temperate latitudes are illustrative examples. At canopy level, plant species from high altitudes tend to develop creeping and prostrate habits, with the living biomass close to the soil surface (Körner, 1999). This habit confers more resistance to high seasonal and daily air temperature amplitudes and strong winds (Körner and Cochrane, 1983). Also, the compaction of organs in condensed spaces protects against variable and harsh climates, modulating the microclimate to a point where the climate experienced by actual plant organs is buffered to a high degree (Körner, 1999). Apart from thermal amplitudes, the shortness of the growth season is perhaps the most relevant constraint for life at high altitudes, so plants have developed long-lasting organogenetic periods that facilitate fast extension of pre-formed organs at the onset of the short growth season (Diggle, 1997; Meloche and Diggle, 2003; Cox, 2005).

Considerable documentation on the above patterns contrasts with an almost total lack of knowledge on the evolutionary routes for a given genotype to generate a syndrome for adaptation to a short growth season. Architectural approaches may provide an insight in this regard. Simple architectural features impact on variation in phenomorphology and in other life-history traits (Diggle, 1999). For example, changes in the location of reproductive modules within shoots result in altered vegetative morphology (Wiltshire \textit{et al.}, 1994; Comes and Kadereit, 1996). Diggle (1997) found that the architecture of high altitude plants and their ability to respond to environmental variation was highly dependent on pre-formation dynamics. She suggested that the pattern of development was crucial to understand plant adaptation to cold environments, and to gain knowledge of ecosystem processes in cold habitats.

The location and timing of bud differentiation with regard to reproduction and vegetative growth are poorly plastic as compared with continuous characters such as many leaf traits. It may be argued that this lack of plasticity downgrades their evolutionary role; however, the facts that (a) variation in bud differentiation is of a genotypic nature, and is under the control of a set of organ identity genes (Zik and Irish, 2003), and (b) architecture indeed shows variation among genotypes of the same species, and is also responsive to the environment to a certain extent (McIntyre and Best, 1975; Sachs and Novoplansky 1995; Burgess \textit{et al.}, 2007), support the potential evolutionary significance: inheritable variation occurs frequently.
enough to produce evolutionary divergences. Only the adaptive relevance of that variation needs to be unravelled.

The above has received close attention from an agronomic perspective, as a tool to select cultivars that evolved contrasting flowering phenologies or vegetative morphologies (see, for example, Jones, 1992; Segura et al., 2008). In addition, the location of reproductive and vegetative modules within the branch has significant consequences for fitness components as diverse as performance of offspring or light-capture efficiency (Fleming, 2005; Lundgren and Sultan, 2005; Prusinkiewicz et al., 2007). Extensive knowledge of plant architecture and its developmental, morphological and genetic correlates has been accumulated since the middle of the 20th century (Hallé et al., 1978; Sussex and Kerk, 2001; Barthelmy and Caraglio, 2007). Extending the developmental approach to the problem of the altitudinal segregation of wild species can provide insightful advances. To our knowledge, the use of the architectural approach to understand the segregation of closely related species across altitudes has not been explicitly addressed in detail.

In the present work, it is suggested that relatively subtle differences in branch design and vegetative vs. reproductive differentiation of buds can have far-reaching consequences for typical traits related to adaptation to altitude, such as plant compactness or bud pre-formation. This idea is explored making use of two series of Mediterranean-Macaronesian Saxifraga species within Sect. Saxifraga, Subsect. Triplinervium (namely ser. Ceratophyllae and ser. Pentadactylis). The five species of Ceratophyllae and the 15 of ser. Pentadactylis differ mainly in their shoot architecture (Vargas, 1991). Pentadactylis species show determinate growth, with the apical meristem developing a terminate inflorescence, and vegetative new shoots developing from axillary meristems of the previous-year shoot. In contrast, in Ceratophyllae species, the apical meristem develops a new vegetative shoot, while axillary meristems produce either lateral inflorescences or vegetative shoots. This architectural trait led to their classification in separate series within the subsection Triplinervium (see Fig. 1, and Vargas, 1991).

The relevance of shoot architecture for altitudinal segregation and trait variation of these species is investigated in this study, making use of two distinct approaches. First, the hypothesis that the location of inflorescences within shoots can predict altitudinal segregation of Ceratophyllae and Pentadactylis species is tested. In order to do this, altitude data were collected for as many reliable and precise field locations of most Iberian species were obtained from the Anthos project (http://www.anthos.es/), from the Jaca Herbarium (http://www.ipe.csc.es/floragon/) and from the authors’ own records for S. trifurcata and S. canaliculata. Additional altitude data for locations of Macaronesian species (S. maderensis and S. portosanctana) and S. pedemontana were obtained from the Global Biodiversity Information Facility (http://www.gbif.org/). Data for S. wahlenbergii were obtained upon request from the herbarium of the University of Vienna, and from Flora of Slovakia records. All reliable records were considered, and qualified by personal communications when necessary. Data are summarized in Table 2.

Correlates of the altitudinal segregation: a detailed study

Saxifraga trifurcata (ser. Ceratophyllae) and S. canaliculata (ser. Pentadactylis) are endemic to the north of the Iberian Peninsula and colonize the same type of micro-habitat: fissures and small holes in limestone rocks, where some sort of organic matter has been previously deposited. In the area under study, S. trifurcata inhabits lowland rocky sites, ranging from 0 to 1300 m. At approx. 1300–1400 m, S. canaliculata replaces the lowland species, reaching about.
Thus, both species behave as typical altitudinal vicariants, with a very narrow contact zone at approx. 1300–1400 m. In this contact zone, infrequent hybrids are occasionally sighted (Saxifraga × faucicola, Díaz-González et al., 1990). Both species are close morphologically and phylogenetically (see Fig. 1, Castroviejo et al., 1990; Vargas, 1991). Both are evergreen chamaephytes that exhibit continuous growth: a short spring growth flush followed by a neoformation period that expands throughout the season for as long as environmental conditions allow, which means a longer neoformation period for the lowland than for the highland vicariant (R. Milla et al., pers. obs.). Individuals are long-lived, polycarpic and are able to reach sexual maturity at the age of 2 years (Vargas, 1994). Leaves are disposed in a helicoid pattern, and, by the end of the growing season, tend to accumulate at the distal nodes in a rosette-like pattern (Fig. 1). Branching is acrotonic, with lateral shoots and/or lateral inflorescences developing chiefly from the distal axillary buds of previous-year shoots. Also, in non-reproductive axes, branching tends to be monopodial, with the apical shoot developing more intensely than lateral shoots. The branching pattern of reproductive branches differs among the species (see below, and Introduction). Their hermaphro-dite flowers are displayed in panicles within inflorescences. Fruits are dry capsules containing a large number of small, wind-dispersed, seeds. Few morphological differences distinguish the species: S. trifurcata possess larger and more lobed leaves than S. canaliculata. Also, S. canaliculata’s foliage is highly sticky, as compared
with that of *S. trifurcata*. Thirdly, the most outstanding difference between species is that their shoot development pattern differs; *S. trifurcata* possesses lateral inflorescences (i.e. typical of the ser. *Ceratophyllae*) and *S. canaliculata* has terminal inflorescences (ser. *Pentadactylis*; see Introduction). The latter trait constitutes the most reliable discontinuity between species from a taxonomic viewpoint (Vargas, 1991).
Study site and populations

The study area was located in the north of the Iberian Peninsula, Asturias province. The climate of the lowlands of this region is oceanic humid type, with mild winters and warm summers, which turn colder and subtly more humid as the altitude increases (Table 3). Typical Mediterranean summer drought is absent. The area has very abrupt topography, with altitudes ranging from 0 to 2640 m a.s.l. in only 40 km. In this area, four populations of the lowland *S. trifurcata* and three of the highland *S. canaliculata* were located growing along a wide altitude gradient from the sea shores of the Cantabrian sea to the summits of the ‘Picos de Europa’ mountain range (Table 3). The altitude gradient mirrors a growth season length gradient, ranging from 12 months in the lowest population of *S. trifurcata*, to 4 months in the highest population of *S. canaliculata* (number of months with mean monthly temperature ≥5°C, Wright et al. 2005). The highest study population of *S. trifurcata* and the lowest of *S. canaliculata* co-exist, i.e. they belong to the narrow contact zone at 1300–1400 m. All populations were numerous and typical of the limestone fissure habitats of both species. Three sampling campaigns were conducted to gather the data necessary to develop the response variables as detailed in Table 1 (column 4).

Measurement of canopy, branch and leaf traits

Canopy, branch and leaf traits were measured to test the following prediction (detailed in Table 1): if *S. canaliculata* has apical inflorescences, once the plant blooms the apical dominance is interrupted, leading to vegetative branching and a more compact and shorter canopy at all scales, an advisable growth habit in high altitude environments. In mid-August 2006 (end of the shoot growth season), the canopy height (*h*, cm) of 200 randomly selected plants per study population was measured to the nearest 0.5 cm. Major and minor diameters, and canopy height of 10 average-sized individuals per population were also measured. The canopy volume (*V*<sub>canopy</sub>, cm<sup>3</sup>) of each plant was estimated, assuming an ellipsoid shape, as:

\[
V_{\text{canopy}} = h\pi (D/2)(d/2)
\]

where *h* is canopy height (cm), and *D* and *d* are major and minor canopy diameters (cm), respectively. The whole canopy of each plant was then cut and taken to the laboratory. Once in the laboratory, two 2-year-old reproductive branches per plant were sampled, and a branching index (*B*) was computed as the number of lateral vegetative current-year shoots per branch.

From each branch, one mature green leaf was harvested and soaked in a Petri dish overnight at 4°C to reach full hydration (Garnier et al., 2001). Once fully hydrated, the leaves were scanned and lamina thickness was measured to the nearest 0.01 mm using a dial thickness gauge (Mitutoyo Co., Aurora, IL). The leaves were subsequently oven dried at 70°C until they reached constant weight, and then weighed to the nearest 0.01 mg. Scanned leaves were processed with ImageJ software (http://rsb.info.nih.gov/ij) to measure leaf area and length. Leaf volume (*V*<sub>leaf</sub>, m<sup>3</sup>) was computed as leaf area × leaf thickness; and, subsequently, leaf density or compactness (*C*<sub>leaf</sub>, g cm<sup>-2</sup>) was calculated as mass per volume:

\[
C_{\text{leaf}} = M_{\text{leaf}} / V_{\text{leaf}}
\]

The term leaf compactness is used throughout, instead of the more usual leaf density, to maintain the same terminology for all three measures of compactness of the vegetative biomass. All other leaves of each plant’s canopy were cut, oven-dried and weighed to obtain the dry mass of current-year leaves of the whole individual (*M*<sub>canopy</sub>). Then, an index of canopy compactness (*C*<sub>canopy</sub>, g m<sup>-2</sup>) was computed as:

\[
C_{\text{canopy}} = M_{\text{canopy}} / V_{\text{canopy}}
\]

Shoot compactness, pre-formation of shoot organs and internode elongation pattern

Measurement of these traits was motivated by the above-mentioned prediction and the following one (Table 1): if *S. canaliculata* has apical inflorescences, once the plant blooms the apical dominance is interrupted, leading to a shorter growth period but a longer time for bud preformation for the next season, a relevant trait in high altitude environments.

During the mid-August sampling, one 2-year-old branch was harvested from each of 10 well-developed reproductive individuals per population. Sampling by mid-August, when

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**Table 3. Location and climate of study sites**

<table>
<thead>
<tr>
<th>Site (UTM coordinates)</th>
<th>Species</th>
<th>Altitude (m a.s.l.)</th>
<th>Mean air temperature (°C)</th>
<th>Rainfall (mm year&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Growing season length (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30T 353 4807</td>
<td><em>S. trifurcata</em></td>
<td>100</td>
<td>14.7</td>
<td>1248</td>
<td>12</td>
</tr>
<tr>
<td>30T 328 4794</td>
<td><em>S. trifurcata</em></td>
<td>540</td>
<td>11.5</td>
<td>1293</td>
<td>12</td>
</tr>
<tr>
<td>30T 337 4791</td>
<td><em>S. trifurcata</em></td>
<td>1064</td>
<td>8.2</td>
<td>1602</td>
<td>8</td>
</tr>
<tr>
<td>30T 338 4789</td>
<td><em>S. trifurcata</em> + <em>S. canaliculata</em></td>
<td>1360</td>
<td>6.9</td>
<td>1629</td>
<td>6</td>
</tr>
<tr>
<td>30T 338 4789</td>
<td><em>S. canaliculata</em></td>
<td>1430</td>
<td>6.7</td>
<td>1642</td>
<td>6</td>
</tr>
<tr>
<td>30T 339 4787</td>
<td><em>S. canaliculata</em></td>
<td>1872</td>
<td>3.9</td>
<td>1676</td>
<td>4</td>
</tr>
</tbody>
</table>
most current-year leaves are expanded and fruits are mature, aimed to characterize the compactness of full-grown current-year shoots, and the organogenesis of the next-season organs at that stage. Efforts were made to obtain analogous shoot structures for comparison between species. In *S. trifurcata*, the apical vegetative shoot of reproductive branches was the study unit. However, in *S. canaliculata*, the apical vegetative shoot of non-reproductive branches ≥2 years after branching was selected. Reproductive branches of this species produce small, lateral and poorly developed vegetative shoots that are less likely to reproduce the following year (R. Milla *et al.*, unpubl. res.). However, non-reproductive branches are very likely to reproduce the following year in *S. canaliculata*, and the primordia content of its apical shoot is qualitatively similar to that of the apical shoot of reproductive branches of *S. trifurcata* (R. Milla *et al.*, pers. obs., and see shoots surrounded by a square in Fig. 1).

At the end of the growing season (early November 2006), a second sampling campaign was carried out to document the pre-formation of elements of the following year’s growth just before winter. In *S. canaliculata*, differentiated inflorescence primordia were already detected in this sampling, which was not the case in *S. trifurcata*. Therefore, an additional sampling was carried out in the populations of *S. trifurcata* in early March 2007 to detect if inflorescence pre-formation occurs in this species before the onset of the growing season.

On each date, the following metrics in the selected shoots were obtained. First, shoot compactness ($C_{\text{shoot}}$, g mm$^{-3}$) was calculated as follows. The diameters and height of the shoots were measured to the nearest 0.1 mm, and the volume of shoot covered by foliage ($V_{\text{shoot}}$, mm$^3$) was calculated analogously to $V_{\text{canopy}}$ above. Then, regression equations were fit, separately for each population, between leaf lengths and leaf dry weights of those leaves harvested to measure leaf density ($r^2 > 0.90$ for all regressions). Regression coefficients were used to estimate leaf dry weight of the shoots dissected in August 2006. By adding dry weight of all leaves in a shoot, dry mass of green leaves in a shoot ($M_{\text{shoot}}$) was obtained. Then, analogously to canopy and leaf compactness, an index of shoot compactness ($C_{\text{shoot}}$, g mm$^{-3}$) was calculated as:

$$C_{\text{shoot}} = \frac{M_{\text{shoot}}}{V_{\text{shoot}}}$$

Afterwards, selected shoots were dissected under a stereo-microscope fitted with an ocular micrometer (MS5 Leica Microsystems, Heerbrugg, Switzerland) at ×10 and ×40. Shoot leaves and primordia were dissected acropetally from the more proximal dry leaves to the upper distinguishable primordia >0.025 mm (resolution limit of the stereo-microscope). The length and relative location of each element (senescent leaves, green leaves, leaf primordia with differentiated lobes, undifferentiated leaf primordia <1 mm in length and inflorescence primordia) were recorded from the base to the tip of the shoot. Also, the presence/absence of shoot primordia (>0.025 mm) in each leaf axil was recorded. The number of shoot primordia at leaf axils was expressed on a node basis, by dividing by the number of nodes of each shoot. Also, 3–8 axillary shoot primordia per population were dissected, and their length and the number of pre-formed organs within the primordia were recorded to quantify the developmental stage of axillary shoot primordia.

To describe the internode elongation pattern typical of each species, an additional set of 25 shoots per species, belonging to eight different plants, was harvested at mid-altitude locations (*S. trifurcata* at 540 m, and *S. canaliculata* at 1430 m) at the end of the growing season. Shoots sprouting from a branching point were selected in order to characterize the elongation pattern of shoots exploring canopy areas adjacent to the parent axis. Internode length was measured acropetally at all nodes. Leaves at the most distal end of the shoot were too tightly packed together to allow measurement of internode lengths, thus only internodes ≥0.2 mm were considered. Node order was further categorized as proximal, central or distal within the shoot, to allow comparability of internode lengths among shoots.

**Statistics**

**Statistics for the altitude-per-field-record database.** The number of records was unbalanced among species (see Table 2), and data for several species did not comply with normality–homoscedasticity requirements of parametric analyses. Thus, to test whether specimens of ser. *Pentadactylis* inhabit higher altitude sites than those of ser. *Ceratophyllae*, non-parametric Generalized Linear Models (GLMs) were used (McCullagh and Nelder, 1999). A nested model was run, including the possession of axillary/apical inflorescences (architecture), and species, nested within architecture, as fixed-effect factors, and altitude as the response variable. Poisson error and log link function distribution were specified. Type 3 option of PROC GENMOD (SAS v.9) was used to analyse the main effects.

**Statistics for the case study.** The distribution of data of each variable at each population was checked for normality (Kolmogorov–Smirnov test) and homoscedasticity (Levene tests). When significant heteroscedasticity and/or lack of normality were detected, data were log$_{10}$ transformed, or analysed by non-parametric statistics.

$C_{\text{leaf}}$, number of primordia at the shoot apex, number of primordia at leaf axils and internode lengths complied with the assumptions of parametric analysis. Thus, trends in inter- and intraspecific variation in $C_{\text{leaf}}$ were tested with an analysis of variance (ANOVA) nested model, including species, and altitude nested within species, as fixed-effect factors. ANOVAs for number of primordia also included season as an additional fixed-effect factor. ANOVAs for internode length included species and node position (proximal, central, distal) as fixed-effect factors. All other variables showed heteroscedasticity and/or lack of normality, and were thus analysed by non-parametric tests using GLMs. Poisson error distribution was used when the original data lacked normality and/or
homoscedasticity (for canopy height, $B$, length of axillary primordia and number of pre-formed organs within axilar primordia), and normal error distribution when only heteroscedasticity problems were detected (for $C_{\text{shoot}}$ and $C_{\text{canopy}}$). Log link function was considered for all GLM tests. All the GLMs included species and altitude (nested within species) as fixed-effect factors. Models for organogenetic variables also included season as an additional fixed-effect factor. In all the GLMs, type 3 option of PROC GENMOD (SAS v.9) was used to analyse the main effects. For those models including season as a factor, bifactorial interactions were considered. If interactions were not significant, they were removed and analyses were performed again.

Multiple comparisons among populations were carried out whenever species or altitude effects were detected by ANOVAs or GLMs. Depending on sample size, and normality–homoscedasticity of each variable, the following types of multiple comparison tests were performed. Dunnet’s C correction (recommended when sample size is large, and variance among groups is unequal) was used for canopy height, $B$, and length of axillary primordia comparisons. Dunnet’s T3 correction (recommended when sample size is small, and variance among groups is unequal) was used for $C_{\text{canopy}}$, $C_{\text{shoot}}$, and number of pre-formed organs within axillary primordia. Bonferroni correction (recommended for balanced designs with parametric data) was used for comparisons of $C_{\text{leaf}}$, number of primordia at the shoot apex and number of primordia at leaf axils.

An additional model was run to compare between species the degree of organogenesis of axillary vegetative primordia at the date of detection of inflorescence primordia: November 2006 for $S.$ canaliculata and March 2007 for $S.$ trifurcata. Patterns in length of axillary primordia, and the number of pre-formed organs within axillary primordia were analysed through GLMs using Poisson error distribution, log link function was used, and Dunnet’s C and Dunnet’s T3 corrected multiple comparisons were run. Parametric analyses and all multiple comparison tests were carried out with SPSS 14.0. Non-parametric statistics were performed with SAS v.9.

**RESULTS**

Altitudinal segregation of ser. Ceratophyllae and ser. Pentadactylis as a function of shoot architecture

A total of 818 altitude-per-field-record data from 17 species were compiled, 12 from ser. Pentadactylis and five from ser. Ceratophyllae (Table 2). The remaining three species of ser. Pentadactylis were not included because of lack of data which were reliable and abundant enough. In support of the first hypothesis, field records of species of ser. Pentadactylis were obtained at locations of higher altitude, on average, than records of ser. Ceratophyllae ($F = 345; P < 0.001$, outcome of GLM analysis, see Materials and Methods). On mid-altitude lands, however, there is substantial overlap between species of both series (Fig. 2).

**Variation of pheno-morphological traits in a case study: $S.$ trifurcata–$S.$ canaliculata**

**Canopy traits.** The degree of compactness of the green mass in the crown was higher in $S.$ canaliculata, the highland vicariant, than in $S.$ trifurcata. This pattern was congruent at the canopy, shoot and leaf levels (Table 4, Fig. 3). However, populations of each species located at different altitudes did not differ in a directional way for any of the three proxies of compactness, except a trend for higher $C_{\text{shoot}}$ in high altitude populations of each species. The shoot internode pattern also contributed to a laxer habit of the lowland species. Shoots were composed of more nodes, and longer proximal internode lengths, which positions branching shoots further away from the leader axis in $S.$ trifurcata than in $S.$ canaliculata (Fig. 4). The canopy of $S.$ canaliculata plants was shorter in height than that of $S.$ trifurcata. Also, the $B$, indicated a more intensive production of lateral shoots in $S.$ canaliculata than in $S.$ trifurcata (Table 4, Fig. 3).

Similarly to compactness traits, no significant effect of altitude within each species was detected either for canopy height or for branching intensity.

**Timing and extent of primordia formation.** A significant tendency arose for a progressive accumulation and lengthening of primordial elements as the season proceeded (August < November < March). This pattern remained for all primordia metrics considered here (Table 4).

A remarkable effect of seasonality found here was the later detection of inflorescence pre-formation in $S.$ trifurcata, the lowland species. Inflorescence primordia were recorded in $S.$ canaliculata prior to winter (November), whereas in $S.$ trifurcata they were observed in early spring (March; Table 5). The patterns of size (length) and composition of lateral vegetative primordia at the time when inflorescence pre-formation was detected in each species are shown in Fig. 5.

Regarding the primordial composition of apical meristems, $S.$ trifurcata showed fewer primordia at the shoot apex than its highland vicariant, $S.$ canaliculata (Table 4). When only undifferentiated primordia at the shoot apex were considered, the same pattern was detected. The number of apical primordia differed among locations within species, but not consistently with elevation (Table 5). Further evidence for a higher neoformation–pre-formation ratio in $S.$ trifurcata can be inferred from the following patterns. First, the apical shoots of this species had a higher number of nodes, and internode length was significantly shorter compared with $S.$ canaliculata apical shoots ($P < 0.05$ for both patterns). Secondly, as shown in Fig. 4, internodes at the distal end of branching shoots were much shorter than proximal and central internodes in $S.$ trifurcata, probably indicating development late in the season through neoformation.

Overall, species and altitude did not have a significant effect on the number of axillary primordia per shoot, or their individual composition (number of elements within each axillary primordia; Table 4). The length of axillary shoot primordia was significantly shorter at high altitude locations, within each species range (Table 5). However,
no between-species differences were detected in this regard, when averaged across dates and altitudes.

DISCUSSION

Altitude-related adaptive syndromes and their relationship to shoot architecture

It is shown that (a) as a general trend, ser. *Ceratophyllae* species tend to occur at lowland sites, as compared with ser. *Pentadactylis*; and (b) the trait that separates both series by definition (i.e. shoot architecture) has far-reaching consequences over other traits relevant to adaptation to altitude. It is convenient to highlight here that increasing elevation might not imply decreasing temperatures and increasing environmental harshness *per se*, particularly when different latitudinal areas are compared (Körner, 2007). However, the mountain areas inhabited by species of ser. *Ceratophyllae* and ser. *Pentadactylis* are all constrained to West-European and North Macaronesian regions, which roughly share latitudinal belts.

When a case study within this general pattern (*S. trifurcata* vs. *S. canaliculata*) was explored in detail, it was found that most canopy and organogenetic traits validate the initial idea that subtle modifications in shoot architecture may generate morphs that perform differently under contrasting climates. Therefore, the cushion-like dwarf habit typical of alpine and similar cold environments may be the outcome not only of reductions in growth rate or vegetative growth period, but also of selection pressures favouring architectural models that facilitate prostrate, branched and packed habits, with little apical dominance of vegetative shoots. Körner (1999) argues that, provided with little evidence of metabolic, physiological or cell size constraints accounting for the small size of alpine plants, developmental controls may guide the adaptation to high altitude conditions. This study provides a step towards evaluating the evolutionary significance of subtle differences in the developmental programme of plants from different altitudes. Although correlational and constrained to a single evolutionary event, the value of the evidence found here is strengthened by the facts that: (a) the study system was carefully selected so that putative interfering factors such as phylogeny, growth and rooting habits, microhabitat, etc. were controlled, the most remarkable difference between species being shoot architecture; and (b) the explicit influence of architecture over secondary traits as outlined in Table 1 is straightforward, as it is the adaptive value of the selected traits under contrasting air temperature regimes.

Meristem differentiation to sexual or vegetative development should be a relevant trait from an evolutionary

![Frequency distribution of field records of specimens of ser. Ceratophyllae (234 field records, from five species) and ser. Pentadactylis (584 field records, from 12 species), as a function of altitude. Dashed reference lines are Ceratophyllae and Pentadactylis arithmetic means as indicated. $F_{\text{series}}$ and its $P$-value refer to the results of the GLM described in Materials and Methods.](image-url)
Branch Architecture in Relation to Altitude

A remarkable exception to the above is that length of primordia at leaf axils, compactness of shoots and internode elongation pattern did vary among populations of the same species at different altitudes. Within-species variation in those traits followed the same directional trend as among species (higher compactness and more pre-formation of organs with increasing altitude). This has been referred to as co-gradient variation, whereby evolutionary and plastic responses to an environmental gradient co-vary directly (Conover and Schultz, 1995). This has relevant implications for the adaptive advantage of a given species when it replaces a similar one across a gradient (Lusk et al., 2008). If a functionally relevant trait changes gradually within a species across a gradient, then the appearance of

Further evidence supporting the role of shoot architecture as the leading factor governing variation in the traits studied here is that species, but not altitude, had consistently significant effects: most response variables did not vary among populations of the same species living at different altitudes. Sharper differences in adaptive traits were thus detected in the part of the gradient where the lowland architectural model was replaced by the highland one. At the regional scale of the present study, location of reproductive meristems within the shoot was constant across individuals and locations within each species, which is congruent with the fact that architectural design within species is highly constant in general (Hallé et al., 1978).

Table 4. Effects of species, altitude and season (fixed-effect factors) on the variables studied in this work

<table>
<thead>
<tr>
<th>Specie</th>
<th>Species</th>
<th>Altitude</th>
<th>Season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-statistic</td>
<td>Significance</td>
<td>Direction of effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saxifraga trifurcata</td>
<td>100.6</td>
<td>&lt;0.01</td>
<td>S.t. &lt; S.c.</td>
</tr>
<tr>
<td>Saxifraga canaliculata</td>
<td>52.3</td>
<td>&lt;0.01</td>
<td>S.t. &lt; S.c.</td>
</tr>
<tr>
<td>Saxifraga canaliculata</td>
<td>90.1</td>
<td>&lt;0.01</td>
<td>S.t. &lt; S.c.</td>
</tr>
<tr>
<td>branch architecture</td>
<td>147.7</td>
<td>&lt;0.01</td>
<td>S.t. &gt; S.c.</td>
</tr>
<tr>
<td>branch architecture</td>
<td>49.5</td>
<td>&lt;0.01</td>
<td>S.t. &lt; S.c.</td>
</tr>
<tr>
<td>No. of total leaf primordia</td>
<td>7.7</td>
<td>&lt;0.01</td>
<td>S.t. &lt; S.c.</td>
</tr>
<tr>
<td>No. of undifferentiated leaf primordia</td>
<td>19.3</td>
<td>&lt;0.01</td>
<td>S.t. &lt; S.c.</td>
</tr>
<tr>
<td>Primordia at leaf axils</td>
<td>0.3</td>
<td>NS</td>
<td>None</td>
</tr>
<tr>
<td>No. of axillary primordia &gt;0.025 mm/No. of leaf axes per shoot</td>
<td>2.2</td>
<td>NS</td>
<td>None</td>
</tr>
<tr>
<td>Mean length of axillary shoot primordia &gt;0.025 mm (mm)</td>
<td>2.9</td>
<td>NS</td>
<td>None</td>
</tr>
<tr>
<td>No. of of elements within axillary shoot primordia</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistics performed through nested ANOVA or GLM models, depending on the normality–homo-scedasticity of each variable. S.t.: Saxifraga trifurcata; S.c.: S. canaliculata; NS, not significantly different at P = 0.05; None, either the effect is not significant at P = 0.05, or, for the factor altitude, differences among sites at different elevations do not follow a directional trend.
an alternative species that advances the clinal variation of the trait more rapidly may imply an abrupt adaptive advantage for the latter species, thus favouring the replacement of species. However, for most traits examined here, and also for a group of leaf and reproductive traits measured in this system (Milla et al., 2008), co-gradient variation is not the rule. This emphasizes the role of abrupt changes in discrete traits such as architecture as more relevant features.

A hypothesis on the arrangement of phenomorphology and organogenesis in two altitude vicariants

In Fig. 6 a scheme is presented illustrating how phenological events are arranged in the study species. Below, this model and its consequences over shoot architecture are described. The onset of phenophases, such as vegetative growth, flowering or fruiting, occurs progressively later in the study species as one moves up the altitude gradient (data not shown). At temperate latitudes, timing of growth and reproduction is highly dependent on temperature seasonality (Dahlgren et al., 2007). After the intense period of spring–early summer growth flush, vegetative shoots keep on producing nodes at a slower pace, accumulating smaller leaves and internodes, which is typical of neo-formed growth occurring outside the optimal period for shoot growth (Borchert, 1991). When temperature and day-length decrease in early autumn, the formation of new leaves is slowed down, progressively being replaced by preformation of next-year organs. At this time of the year (autumn), the most relevant phenological discrepancy between both species occurs. The present data show that the initiation of the inflorescence primordia occurs by early autumn in *S. canaliculata*, whereas it takes place by late autumn to early winter in *S. trifurcata*. Interestingly, organogenesis of reproductive primordia harvested on the same dates did not vary among populations of the same species, suggesting photoperiodic control. Initiation of reproductive organogenesis by photoperiodic clues has been extensively described previously (Yanovsky and Kay, 2003). Although this might seem anecdotal initially, it is advocated, as discussed below, that this subtle pheno-organogenetic modulation may indeed be the developmental trigger of the architectural differences between species.

*Saxifraga canaliculata* inhabits higher, colder sites than its lowland relative, thus the elongation of vegetative leaders slows down early. However, since inflorescence initiation occurs early as well, only apical meristems reach a sufficient degree of development to initiate bracts and flower buds in autumn. Previous work in trees demonstrates that meristems that initiate reproductive organs have to surpass a threshold of number of vegetative scales and leaf primordia before being able to initiate bracts or flower buds (Costes, 2003). Thus, poorly developed axillary meristems of *S. canaliculata* have little chance to become reproductive in autumn. On the other hand, milder conditions in autumn allow extension of apical vegetative leaders at *S. trifurcata* sites for longer. This pattern of protracted formation–extension of elements precludes the
Fig. 4. Shoot internode elongation pattern at typical locations of *S. trifurcata* (550 m a.s.l.), and *S. canaliculata* (1430 m a.s.l.). Different letters within each of the two sub-plots indicate significant differences at $P = 0.05$. Symbols are population means ($\pm$ s.e.m.).

Table 5. Composition of axillary and apical primordia, and number of axillary and inflorescence primordia per shoot, at three contrasting seasons

(A) Late season (August 2006)

<table>
<thead>
<tr>
<th>Primordia at shoot apex</th>
<th><em>S. trifurcata</em></th>
<th><em>S. canaliculata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of total leaf primordia</td>
<td>10.2 (0.9)$^a$</td>
<td>14.0 (0.6)$^{ac}$</td>
</tr>
<tr>
<td>No. of undifferentiated leaf primordia</td>
<td>3.5 (0.4)$^a$</td>
<td>4.0 (0.2)$^{bc}$</td>
</tr>
<tr>
<td>Primordia at leaf axils</td>
<td>0.15 (0.01)$^c$</td>
<td>0.14 (0.01)$^a$</td>
</tr>
<tr>
<td>No. of axillary primordia $&gt;0.25$ mm/No. of leaf axils per shoot</td>
<td>0.74 (0.11)$^a$</td>
<td>0.59 (0.07)$^a$</td>
</tr>
<tr>
<td>Mean length of axillary shoot primordia $&gt;0.025$ mm (mm)</td>
<td>2.8 (0.2)$^a$</td>
<td>2.7 (0.2)$^{ab}$</td>
</tr>
<tr>
<td>No. of elements within axillary shoot primordia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reproductive primordia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of inflorescence primordia per shoot</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

(B) End of season (November 2006)

<table>
<thead>
<tr>
<th>Primordia at shoot apex</th>
<th><em>S. trifurcata</em></th>
<th><em>S. canaliculata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of total leaf primordia</td>
<td>12.4 (0.6)$^a$</td>
<td>14.2 (2.0)$^a$</td>
</tr>
<tr>
<td>No. of undifferentiated leaf primordia</td>
<td>15.8 (0.7)$^a$</td>
<td>15.5 (1.2)$^a$</td>
</tr>
<tr>
<td>Primordia at leaf axils</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of axillary primordia $&gt;0.25$ mm/No. of leaf axils per shoot</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean length of axillary shoot primordia $&gt;0.025$ mm (mm)</td>
<td>2.9 (0.2)$^a$</td>
<td>1.6 (0.2)$^{ab}$</td>
</tr>
<tr>
<td>No. of elements within axillary shoot primordia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reproductive primordia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of inflorescence primordia per shoot</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Continued
accumulation of many pre-formed elements in the apical meristem. This, coupled with the fact that inflorescence initiation occurs later, permits axillary meristems to develop pre-formed organs for a longer period and thus reach a more advanced developmental stage than *S. canaliculata* axillary meristems. In this way, by the period of inflorescence initiation for *S. trifurcata* (winter), axillary meristems can develop reproductive structures (see Fig. 5).

This interpretation leads to the new hypothesis that a phenological trait that may be fixed by photoperiodic cues (i.e. timing of inflorescence pre-formation) defines the architectural model of the branch in our species, and not the reverse. Early initiation of apical inflorescence primordia in *S. canaliculata* might be regarded as an efficient way to lengthen the period available for pre-formation of vegetative and reproductive modules, which is highly favourable under short growth season scenarios (Diggle, 1997). On the other hand, later initiation of lateral inflorescence primordia in *S. trifurcata* allows additional extension of vegetative axes by neoformation, protracting the period of vegetative growth until climate conditions permit. The latter model is certainly better suited to lowland conditions.

**CONCLUSIONS**

Terminal inflorescences have frequently been regarded as developmental constraints, since, for instance, they incur additional costs of reduced flexible flowering timing because of limitation of further vegetative extension of the main axes (see Diggle, 1999). Other examples where meristem differentiation and availability is regarded as a developmental constraint for plant growth are frequent in the literature (Watson, 1984; Geber, 1990). Here, the adaptive value of variation in meristem differentiation for the adjustment to contrasting climate regimes is highlighted. Also, it is advocated that architecture models can be determined by organogenetic events, such as, in the present case study, the timing of inflorescence pre-formation. Early initiation of reproductive primordia constrains the length of the vegetative growth season, and promotes apical occurrence of inflorescences in the present case study. This, in turn, leads to a longer pre-formation season, and to a more compacted and packed plant body at all scales. The latter reasoning suggests that the evolution of contrasting architectures might be relatively easy to achieve under certain selective pressures and differential sensitiveness to photoperiodic cues, at least in the *Saxifraga* clade studied here. This idea deserves further experimental testing.

---

**TABLE 5. Continued**

(B) End of season (November 2006)

<table>
<thead>
<tr>
<th>Primordia at shoot apex</th>
<th><em>S. trifurcata</em></th>
<th><em>S. canaliculata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of total leaf primordia</td>
<td>11.8 (0.6)a</td>
<td>16.2 (1.1)b</td>
</tr>
<tr>
<td>No. of undifferentiated leaf primordia</td>
<td>4.3 (0.2)a</td>
<td>4.8 (0.3)b</td>
</tr>
<tr>
<td>Primordia at leaf axis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of axillary primordia &gt;0.025 mm/No. of leaf axils per shoot</td>
<td>1.07 (0.00)a</td>
<td>1.07 (0.00)a</td>
</tr>
<tr>
<td>Mean length of axillary shoot primordia &gt;0.025 mm (mm)</td>
<td>5.77 (0.66)a</td>
<td>2.84 (0.29)b</td>
</tr>
<tr>
<td>No. of elements within axillary shoot primordia</td>
<td>12.2 (1.4)ab</td>
<td>19.3 (1.0)b</td>
</tr>
<tr>
<td>Reproductive primordia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of inflorescence primordia per shoot</td>
<td>0.33 (0.21)a</td>
<td>1.0 (0.00)b</td>
</tr>
</tbody>
</table>

Data are means (s.e.m.). Altitudes sharing a letter within a row are not statistically different at *P* = 0.05.

---

**TABLE 5. Continued**

(C) Prior to spring growth (March 2007)

<table>
<thead>
<tr>
<th>Primordia at shoot apex</th>
<th><em>S. trifurcata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of total leaf primordia</td>
<td>11.8 (0.6)a</td>
</tr>
<tr>
<td>No. of undifferentiated leaf primordia</td>
<td>4.3 (0.2)a</td>
</tr>
<tr>
<td>Primordia at leaf axis</td>
<td></td>
</tr>
<tr>
<td>No. of axillary primordia &gt;0.025 mm/No. of leaf axils per shoot</td>
<td>1.07 (0.00)a</td>
</tr>
<tr>
<td>Mean length of axillary shoot primordia &gt;0.025 mm (mm)</td>
<td>5.77 (0.66)a</td>
</tr>
<tr>
<td>No. of elements within axillary shoot primordia</td>
<td>12.2 (1.4)ab</td>
</tr>
<tr>
<td>Reproductive primordia</td>
<td></td>
</tr>
<tr>
<td>No. of inflorescence primordia per shoot</td>
<td>0.33 (0.21)a</td>
</tr>
</tbody>
</table>
**Fig. 5.** Composition and length of axillary shoot primordia at the time of the year when pre-formation of inflorescences was detected (November 2006 for *S. canaliculata*, and March 2007 for *S. trifurcata*). Altitudes sharing a letter within each variable are statistically equal at $P = 0.05$. Fixed-effect factors (species, and altitude nested within species) were significant at $P = 0.05$ for both variables, although differences between altitudes did not follow any elevation trend for length of lateral primordia. See Materials and Methods for details on statistics. Symbols are population means ($\pm$ s.e.m.). Data for *S. trifurcata* at 1360 m were missing.

**Fig. 6.** Hypothetical arrangement of phenophases and pre-formation of *S. canaliculata* and *S. trifurcata*, as inferred from the results of this study. See Discussion for explanation.
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LITERATURE CITED


