

Intra- and interspecific performance in growth and reproduction increase with altitude: a case study with two *Saxifraga* species from northern Spain

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Summary

1. Improving our knowledge on plant functions underlying shifts in species ranges along altitudinal and latitudinal gradients is a research priority to understand biotic responses to climate change. Although much is known about biological responses to elevation, coupled observations of ecophysiological and reproductive traits with whole-plant performance metrics are rare, and thus our understanding disjunct rather than holistic.

2. We expected growth and reproductive performance to decrease with elevation, irrespective of adjustments in traits, modifications in allocation, or congeneric shifts in populations. To test this hypothesis we assessed growth, reproductive investments and traits related to resource use efficiency and reproductive success in populations of two vicariant species growing along an elevation gradient: *Saxifraga trifurcata* (lowland species) and *S. canaliculata* (highland species).

3. For most metrics, a relationship with elevation arose, mostly related to the replacement of species, but also to within-species variation in *S. trifurcata*. Leaves of *S. canaliculata*, and of the higher populations of *S. trifurcata*, were denser, had lower specific leaf area (SLA) and lower N concentration but higher N resorption efficiency. Collectively, these trends indicate a more conservative resource use pattern at the highlands as compared to the lowlands. Despite this, vegetative growth per unit canopy area was higher in *S. canaliculata* than in *S. trifurcata*, and in the highland than lowland populations of *S. trifurcata*. Reproductive investments, measured as the reproductive mass per unit canopy area, were lower, and reproductive maturity was reached later, in *S. canaliculata*, but reproductive success (fruit set and seed germination) increased at the high altitude limit of each species' range.

4. Performance did not uniformly decrease with altitude. Growth was higher in the uplands possibly because preferential biomass and meristem allocation to the vegetative function compensated for the decrease in growth season length. Similarly, the decrease in reproductive investments in the highland populations was partially compensated for, since fruit set was higher and more viable seeds (per gram invested in reproduction) were produced in the highland limit of each species.

5. This suggests that populations growing in increasingly limiting environments can partially compensate by modifications in allocation and morpho-functional traits.

Key-words: climate change, elevational gradient, leaf traits, reproductive success, *Saxifraga*

Introduction

Knowledge on how plants vary in their phenotypic expression along climate gradients is of paramount significance to

understand plant capability to respond to climate change in real time. Elevational gradients are convenient scenarios to investigate such responses, since key factors for plant development as atmospheric temperature, CO₂ partial pressure, irradiance or rainfall vary abruptly across short distances (Körner 2007).

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Previous empirical work has revealed a high degree of phenotypic adjustment to changing local environments across altitudes. Key traits for plant functioning vary in a clinal fashion along elevation gradients. For instances, traits such as specific leaf area (SLA), carbon gain on a whole year basis, or leaf mass ratio tend to decrease as altitude increases, whereas root thickness, canopy compactness or organ preformation increase towards high altitudes (e.g., Diggle 1997; Körner 1999; Craine & Lee 2003). Although abundant in number and comprehensive in terms of geographical coverage, most original studies on plant response to altitude have been constrained to narrow aspects of the biology of plants. Growth processes, reproductive events, demographic patterns or research on plant morphology are commonly addressed in separate research programs (e.g., Atkin, Botman & Lambers 1996; Diggle 1997; Oleksyn *et al.* 1998; Bühler & Schmid 2001; Fabbro & Körner 2004; Giménez-Benavides, Escudero & Iriondo 2006). This hinders an integrative understanding of plant response to altitude, because each biological process is examined in a different study system. For example, a suit of 52 papers arising from a prospective search in the Web of Science (version 8.9, searched performed on 3 June 2008, for articles published from 1970 to present day) with altitude and/or elevation and plant in the title, examined plant responses to altitude as varied as leaf ecophysiology, phenology, reproduction, survival, sex ratios, germination or interspecific interactions. However, only one article examined several aspects of plant biology in a single study, showing decrease in vegetative growth and height, lower fecundity and seed production, together with later phenological development at higher altitudes for *Lesquerella fendlerii*, and the opposite pattern for *L. pallida*, a related species better fitted to high altitude environments (Dierig *et al.* 2006). We are not aware of previous studies that evaluate how a single species, or populations of closely related species, modulate several functional traits related to resource economy along with traits related to reproductive success in a sharp altitude gradient. Moreover, as Violle *et al.* (2007) pointed out, the relevance of trait variation on plant response to environmental change can only be assessed if performance of plants is simultaneously measured at some study scale. However, studies on clinal variation in traits usually lack estimates of its effects on plant performance.

Here, we set out from the assumption that, under oceanic climates (i.e. in the absence of periodic droughts), the shorter growing season in highlands should imply a net reduction in resource input for plants on a per-whole-year-basis. In addition, environmental severity usually increases with altitude (stronger daily temperature amplitudes, increasing duration of snow cover, higher UV-B radiation, etc.) (Körner 1999; Körner 2007), which is certainly the case under oceanic-type of climates like the one studied here, where water availability is relatively high along the whole gradient (see Materials and Methods). Thus, our working hypothesis is that plant performance will diminish at high altitudes. In particular, we hypothesize that (i) vegetative growth, investment in reproduction, and reproductive success should decrease with altitude; (ii) plant resource use efficiency (i.e. nutrient recycling

in the canopy and leaf traits related to resource retention times) should increase with altitude; and (iii) the preceding trends should hold when comparing populations of the same species living at different altitudes, and when comparing related species typical to different altitude ranges. To evaluate these hypotheses we measured vegetative growth, reproductive investments, size at first reproduction, leaf traits related to resource use efficiency, and traits related to reproductive success in the field in seven populations of two altitudinal vicariant species growing along an elevation gradient: *Saxifraga trifurcata* Schard., Hort. Gott. (lowland species) and *Saxifraga canaliculata* Boiss. & Reut. Ex Engl. (highland species). The above predictions are relevant in a climate warming context because, if supported, the fact that performance decreases with altitude implies that improvement of local conditions throughout the gradient should entail upwards migration of organisms.

Materials and methods

STUDY SPECIES AND SITES

Saxifraga trifurcata and *S. canaliculata* are pulvinular evergreen chamaephytes, endemic to the north of the Iberian Peninsula, which occupy the same microsite: small fissures and holes on limestone rocky sites. In our study area, *S. trifurcata* lives in lowland rocks from 0 to 1400 m a.s.l., whereas *S. canaliculata* grows from nearly 1300 to 1900 m a.s.l. Thus, both species behave as typical altitudinal vicariants, with a very narrow contact zone at approximately 1300–1400 m (Table 1). Both are very similar from a morphological viewpoint (Vargas 1994), but *S. trifurcata* has larger and more lobated leaves than *S. canaliculata*, and *S. canaliculata* has terminal inflorescences, while *S. trifurcata* lateral inflorescences.

The climate at the lowlands is oceanic humid type, with mild winters and warm summers, which turns colder and more humid as altitude increases (Table 1). However, given its oceanic character, typical Mediterranean summer drought is absent even at the lowland sites. The area has abrupt topography, with altitudes from 0 to 2640 m a.s.l. across only 40 km. We located four populations of the lowland *S. trifurcata* and three of the highland *S. canaliculata* along an altitude gradient spanning at approximately 1800 m. The highest population of *S. trifurcata* and the lowest one of *S. canaliculata* coexist. The altitude gradient mirrors growth-season-length and mean annual temperature gradients, ranging from 12 months and 14.7 °C MAT in the lowest population, to 4 months and 3.9 °C MAT in the highest population (growth-season-length: number of months with mean monthly temperature > 5 °C).

MEASUREMENT OF FRUIT SET, VEGETATIVE GROWTH, AND LEAF TRAITS

We visited each population in August 2006, when fruit maturation and vegetative extension had finished. We measured major (D) and minor (d) diameters of 10 randomly selected average-sized individuals per population. Then we counted and cut all infructescences on each individual. Fruit set per plant was estimated as the ratio of fruit number to flower number. Seeds of all populations, but *S. trifurcata* at 540 m a.s.l. (Collection from this population failed), were stored separately for each mother plant for germination assays.

Table 1. Decimal degree coordinates and climate at the study populations of *S. trifurcata* (lowland species) and *S. canaliculata* (highland species)

Site (dec. degree coord.)	Species	Altitude (m a.s.l.)	MAT (°C)	Rainfall (mm year ⁻¹)	Growing season length (months)
4°48'W 43°24'N	<i>S. trifurcata</i>	100	14.7	1248	12
5°7'W 43°17'N	<i>S. trifurcata</i>	540	11.5	1293	12
5°0'W 43°16'N	<i>S. trifurcata</i>	1064	8.2	1602	8
4°59'W 43°14'N	<i>S. trifurcata</i>	1360	6.9	1629	6
4°59'W 43°14'N	<i>S. canaliculata</i>	1430	6.7	1642	6
4°59'W 43°13'N	<i>S. canaliculata</i>	1872	3.9	1676	4

All sites located along an altitude gradient in north Spain. Altitudes are measured in meters above sea level. MAT stands for mean annual temperature. Rainfall is total yearly rainfall in year mm m⁻². Growing season length is the number of months with mean monthly temperature > 5 °C.

The canopy of each plant was also cut and taken to the laboratory, where two 2-year-old non-reproductive branches per plant were sampled. Two-year-old branches contain the current-year leaves (green), and the 1-year-old litter, which stays attached to the stem. One green leaf and one 1-year-old litter were harvested per branch and SLA (m² g⁻¹) and leaf density (Ld, g m⁻³) were measured (see Appendix S1 in Supporting Information for details).

All other current-year green leaves of each canopy were cut, oven-dried, and weighed to compute current-year vegetative growth as follows. First, size of individual plants was estimated as canopy area (A, cm²), assuming an elipsoid shape:

$$A = \pi \times D/2 \times d/2 \quad \text{eqn 1}$$

Then, current-year vegetative growth (hereafter VG, g cm⁻² of canopy) was computed as dry mass of current-year growth per canopy area unit. Canopy area was chosen as the basis to make VG a relative measure, instead of above-ground vegetative biomass. These are cushion-like caespitose chasmophytes, and area or linear dimensions are frequently used to estimate plant size in this sort of species (e.g. Albert, Escudero & Iriondo 2001). Diameters were taken from the first to the last green leaf of each minor and major axes of the plant. Plants were fairly compact, and, particularly those selected for growth measures did not show gaps. Thus, canopy area reflects exposed leaf area. Also, canopy basis allows presenting data on VG in the same units than data on reproductive investments (see below). Anyhow, canopy area correlated tightly with above-ground biomass at each population ($P < 0.01$ for all correlations, r^2 ranging from 0.66 to 0.93, $n = 9$ –13 plants per population). The contribution of roots to total plant biomass could not be estimated for the sampled individuals, because the root system is strongly integrated in the rocky substrate, and could not be dug for most average-sized plants. Anyhow, we dug several small individuals to get a rough impression of root allocation. Roots of small plants of *S. trifurcata* represent c. 18% of total plant dry weight (ranging from to 5% to 45%, $n = 17$); roots of *S. canaliculata* represent c. 12% of total plant dry weight (ranging from 2% to 47%, $n = 14$).

Afterwards, a subsample of oven-dried green leaves and one-year-old litter was taken per plant, and nitrogen resorption efficiency (N_{ref} ratio) was measured as explained in Appendix S1.

REPRODUCTIVE INVESTMENTS AND SIZE AT FIRST REPRODUCTION

In each population we measured major (D) and minor (d) diameters of canopies, and counted the number of infructescences of 200 individuals. Size of plants was estimated as canopy area as explained above (A, cm²). Reproductive investment of the 200 individuals (RI, g cm⁻²) was estimated as dry mass of infructescences per canopy area, as:

$$RI = (I_N \times I_M)/A \quad \text{eqn 2}$$

Where I_N is number of infructescences per individual, and I_M is the typical dry mass of a single infructescence (measured from a composite sample of 20 infructescences per population, typical I_M of *S. trifurcata* and *S. canaliculata* was, respectively, 19.6 and 28.1 mg).

To model size at sexual maturity, we estimated flowering probability curves by fitting a generalized linear model (GLM) (McCullagh & Nelder 1989) for the 200 individuals of each population, with plant size as the dependent variable. We plotted the curves using the estimated parameter for plant size and the formula:

$$P = 1/(1 + e^{\mu + \alpha x}) \quad \text{eqn 3}$$

where μ is the intercept with the X -axis, related to the threshold size for reproduction, and α is the slope of the curve, related to the percentage of reproductive plants (Wesselingh *et al.* 1993).

SEED GERMINATION ASSAYS

Seeds from all populations, but *S. trifurcata* at 540 m a.s.l., were stored in plastic vials at room temperature and darkness for approximately 6 months. Average weight of one *S. trifurcata* and *S. canaliculata* seed was, respectively, 0.039 and 0.034 mg. Germination responses were obtained under a 16-h light/8-h dark photoperiod and three temperature treatments: 10/5, 15/10 and 20/15 °C. (see Appendix S1 for details).

DATA HANDLING AND STATISTICS

Data were arranged in three data bases. Data base 1 (vegetative growth, fruit set and leaf traits) included destructive leaf and branch-based data from the 10 average-sized individuals from each population.

Data base 2 (reproductive investments and flowering probability) included non-destructive size and reproductive investments data of the 200 individuals per population. Data base 3 (germination) included the germination data. Multivariate outliers in data base 1 were identified and discarded. Remaining data were checked for parametric assumptions and transformed or analysed by non-parametric statistics when necessary (see Appendix S1 for details).

Leaf density, leaf thickness, N concentration, N_{ref} , SLA, and VG complied with the assumptions of parametric analysis. Thus, trends in inter- and intraspecific variation were tested with an ANCOVA nested model, with species and altitude nested within species as fixed factors, and plant size as the covariate. Fruit set was analysed by nonparametric tests, using GLMs with the same model structure, binomial error distribution and log-link function.

Reproductive investments and flowering probability did not comply with parametric assumptions, and were analysed with GLMs. Species and altitude nested within species were treated as fixed factors, with plant size as a covariate. We used Poisson distribution and log link function for reproductive investments, and binomial distribution and logit link function for flowering probability.

Seed germination was also analyzed with GLMs, binomial distribution and logit link function. Temperature treatment, species and altitude nested within species were treated as fixed factors. Analyses were performed with SPSS 14.0 and SAS v.9.

Results

ALLOCATION OF BIOMASS TO VEGETATIVE GROWTH AND REPRODUCTIVE INVESTMENTS, AND SIZE AT FIRST REPRODUCTION

Vegetative growth was greater in *S. canaliculata*, the highland vicariant, than in *S. trifurcata*. Also, highland populations of *S. trifurcata* showed higher vegetative growth than its lowland populations, while no intraspecific trend was detected among the populations of *S. canaliculata* (Fig. 1). However, total vegetative growth is a function of each single branch growth rate, and of the number of branches or meristems per canopy unit, so trade-offs may occur between number and biomass of new shoots. When vegetative growth was decomposed in those two components, *S. canaliculata*, the highland species, had a higher number of branches per canopy area than *S. trifurcata*, but growth on a branch basis were similar between species (data not shown). Thus, *S. canaliculata* higher vegetative performance was due to its higher number of modules per canopy area, as opposed to larger yearly shoots.

Reproductive investment, measured as mass of infructescences per unit canopy area, was lower in *S. canaliculata*, the high altitude vicariant. Also, the two highest populations of *S. trifurcata* showed a decrease in reproductive investment, as compared to the two lowest ones of the same species (Fig. 1). Similarly to vegetative growth, highest reproductive investment in *S. trifurcata* was driven by a higher number of infructescences per canopy area in this species, and not by larger individual reproductive modules of this species (not shown).

The size at which individuals become reproductive was significantly larger in *S. canaliculata*, as compared to *S. trifurcata* ($P < 0.05$, *post hoc*s after binomial GLM) (Fig. 2). No altitudinal trend was observed within species at this regard.

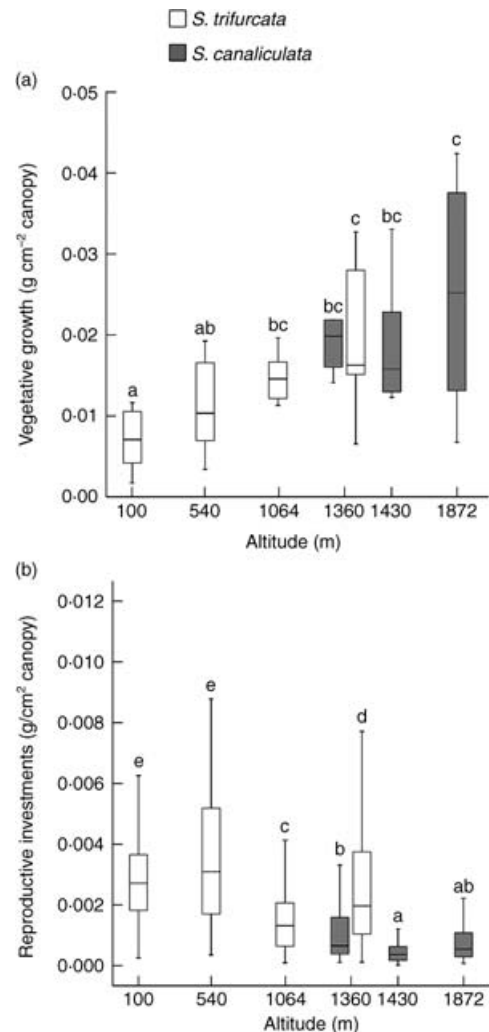


Fig. 1. Vegetative growth and reproductive investments as a function of species and altitudes. Letters in the upper part of the plots mean that locations that share no letter are significantly different at $P = 0.05$ on the basis of multiple comparison tests, after ANCOVA (vegetative growth) or GLM (reproductive investments) analyses. Models included species and altitude nested within species as fixed effects, and plant size as the covariate.

LEAF TRAITS

Leaves of higher altitude plants were significantly denser, and had more dry mass per unit leaf area (i.e. lower SLA). The tendency towards increasing leaf density with altitude was mainly due to the replacement of *S. trifurcata* by *S. canaliculata* at the mid-stretch of the gradient, rather than to differences among populations of the same species (Fig. 3). The SLA gradient was due both to species shift and to a statistically significant decrease with altitude in *S. trifurcata*. Regarding leaf thickness, leaves of *S. trifurcata* highest populations were thicker than lowland ones, but no overall difference between species was detected (Fig. 3).

Both leaf traits indicative of nitrogen conservation showed that this increased with elevation: nitrogen concentration was significantly less in the leaves of highland populations, and resorption of N from senescent leaves was more efficient in the

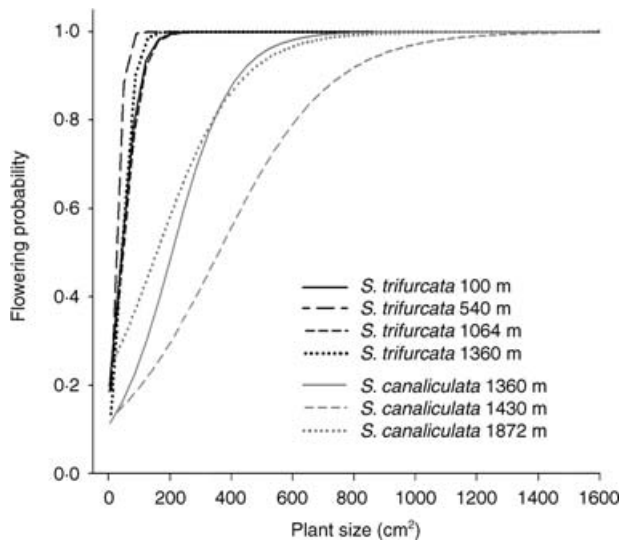


Fig. 2. Flowering probability as a function of plant size. Parametrization of the model was performed taking into account threshold size for reproduction, and percentage of reproductive plants. See Materials and Methods for the formulation of the model. Each curve models data from 200 individuals per population. P -value < 0.01 for each of the seven models.

branches of highland plants. Within-species variation across elevations in *S. trifurcata*, as well as species replacement at c. 1300–1400 m, triggered these trends (Fig. 3).

FRUIT SET AND SEED GERMINATION PERCENTAGES

Fruit set and seed viability (both measures of reproductive efficiency) were higher at the high altitude limit of each species range (Fig. 4). However, no significant differences were found between species when all populations were considered together ($P = 0.08$ for seed viability, and $P = 0.42$ for fruit set for the factor Species in GLMs).

Discussion

Our initial hypothesis was that growth and reproduction would decrease as one moves up an altitude gradient. In accordance with this view, several studies have found decreasing vegetative growth (Oleksyn *et al.* 1998; von Arx, Edwards & Dietz 2006), seed set and seed size (Cruden 1972; Oleksyn *et al.* 1998), or fruit set and reproductive investments (Ladinig & Wagner 2005) with increasing altitude. However, collectively, our results suggest: (i) a lower than expected premium of growing at low altitude for plant performance; and (ii) that adjustment in phenotypic traits, and in biomass allocation to reproductive vs. vegetative functions, may help plants reach higher levels of growth performance at higher altitudes. Other studies have reported non-significant responses or even increases in reproductive and vegetative performance for high altitude plants (e.g. Akhalkatsi & Wagner 1996; Gugerli 1998; Ladinig & Wagner 2007), but functional mechanisms were usually not explored.

VEGETATIVE GROWTH AND RESOURCE RETENTION ABILITY INCREASE WITH ALTITUDE

Vegetative growth was higher in the highland than lowland vicariant and, also, it increased with altitude in the lowland vicariant. This occurred despite the sharp reduction in growth season length along this gradient. Most literature on growth response to elevation has focused on growth potential capabilities of highland vs. lowland plants measured in common garden experiments. Under such circumstances alpine shrubs and herbs do not show, as a general pattern, inferior growth rates than lowland plants (Körner 1999), although this does not hold for trees (Oleksyn *et al.* 1998, Shi, Körner & Hoch 2008). In the field, Sakai, Sasa & Sakai (2006) found that, once plant size is controlled for, as has been done in our study, there is no altitude trend in vegetative growth for *Salix* species. Also, when measured at lower (e.g. leaf) or higher (e.g. community) study scales, carbon uptake potential is not particularly lower at highlands (see refs. in Körner 1999). Körner (1999) concluded at this regard that carbon metabolism does not account for obvious size differences between large lowland and small highland plants, but control on development, morphology and allocation is probably more relevant. An in-depth analysis of mechanisms underlying inherently lower slower growth rates of alpine species also highlighted the irrelevance of metabolic rates, and played up the importance of leaf morphology, in accounting for growth rate variation (Atkin *et al.* 1996).

The leaves of both *Saxifraga* species showed altitudinal differences in morphology and resource re-allocation which indicate an increase in resource retention efficiency up the gradient. SLA and leaf nitrogen decreased, and leaf density and nitrogen resorption efficiency increased at highlands. Previous work in evergreen trees found similar patterns (e.g. Cordell *et al.* 1999; Hultine & Marshall 2000; Li *et al.* 2006). Our results suggest that nutrients and carbon were retained in the plant body for longer in the highlands. Given leaf trait trade-offs that have been found across species (Wright *et al.* 2004), these trends suggest (i) that longevity of leaf tissue, and thus resource retention time, is longer at highlands (consistent with previous results for evergreens, e.g. Cordell *et al.* 1999), and (ii) that leaf tissue is less productive at highlands in terms of instantaneous carbon fixation (also in accordance with reports for evergreens, e.g. Hikosaka *et al.* 2002). However, resource retention ability correlated directly with vegetative growth here, which contrast with most previous literature (e.g., see review in Reich, Walters & Ellsworth 1992). We suggest that it is unlikely that both growth and resource retention trends are linked by a direct physiological mechanism.

An alternative mechanism accounting for the improvement of vegetative performance with altitude is that available resources were preferentially devoted to the vegetative function in *S. canaliculata*, at the expense of cutting investment in reproduction. This was manifest in two patterns: (i) the opposite trends of reproductive investments and vegetative growth upwards the gradient; and (ii) the delay of reproductive adulthood in *S. canaliculata* compared to *S. trifurcata*, as

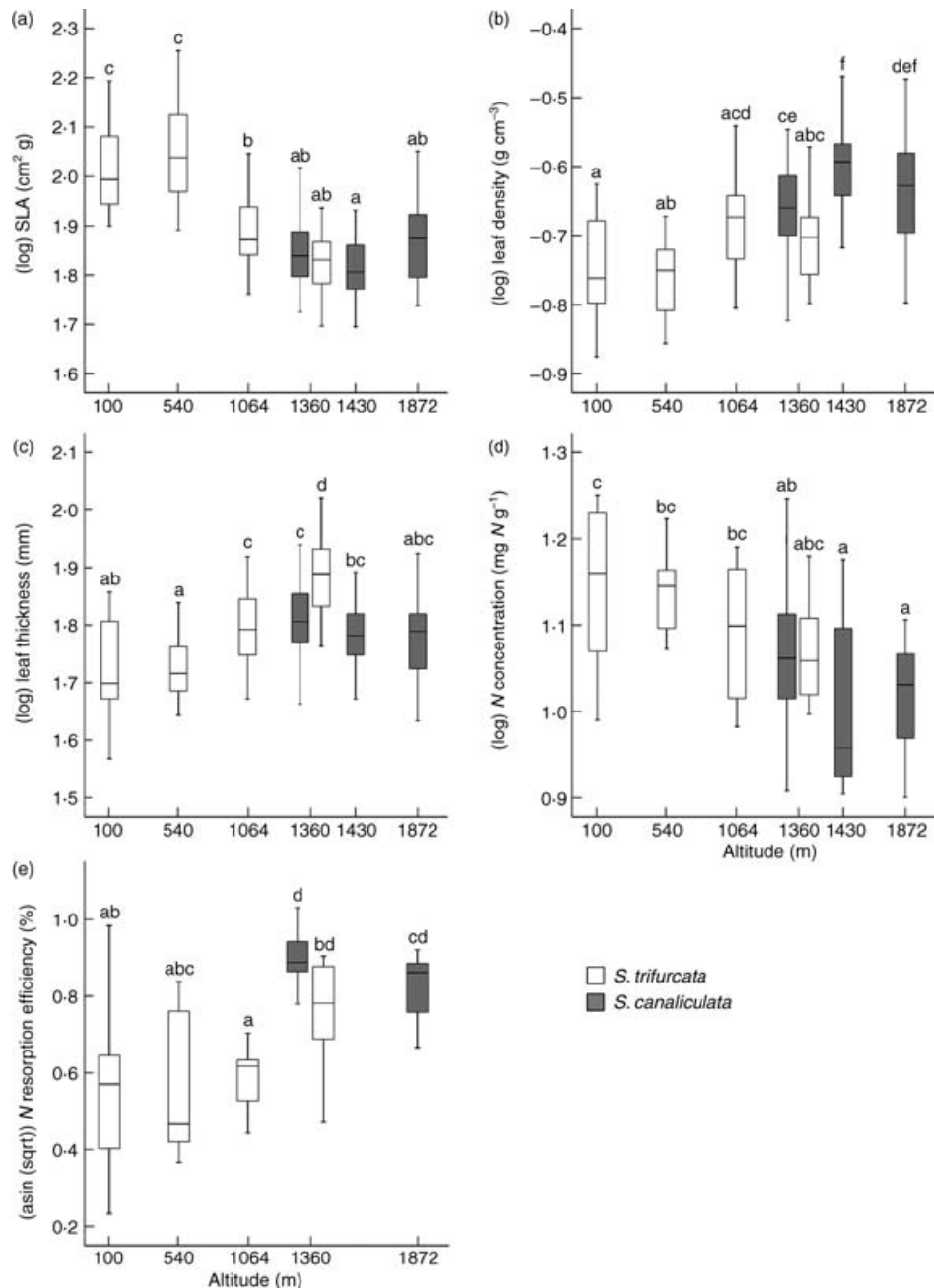


Fig. 3. Leaf trait variations across the altitude gradient. Lettering as in Fig. 1, on the basis of multiple comparison tests after ANCOVA analyses.

shown in the flowering probability curves. A similar decrease in reproductive allocation with altitude, accompanied by sustained vegetative growth, was found in the dwarf shrub *Salix reinii* Fr. & Sav (Sakai *et al.* 2006). Allocation to functions that maximize persistence (e.g. vegetative growth, clonal propagation, etc.) are frequently favoured under harsh environments, at the expense of reducing sexual reproduction (García & Zamora 2003). Persistence through vegetative extension and long life spans (maximum size of plants was larger in *S. canaliculata*, suggesting longer maximum plant longevity in this species, data not shown) is common among alpine plants, at the expense of reproductive effort (Körner 1999; Sakai *et al.* 2006; but see Fabbro & Körner 2004).

An additional developmental mechanism may further support the higher vegetative growth of *S. canaliculata*. As stated in Materials and Methods, *S. canaliculata* possesses apical inflorescences, with vegetative shoots growing in axilar position, while *S. trifurcata* shows the opposite pattern. Apical control of *S. trifurcata*'s apical vegetative leader inhibits branching, and thus few yearly shoots are produced per branch unit in this species (Milla, Giménez-Benavides & Montserrat-Martí in press). In contrast, *S. canaliculata* lacks a vegetative leader, and thus simultaneous growth of multiple lateral vegetative shoots may permit efficient and fast allocation of carbon in the short but benign growing season at highlands. This is in accordance with recent reports suggesting

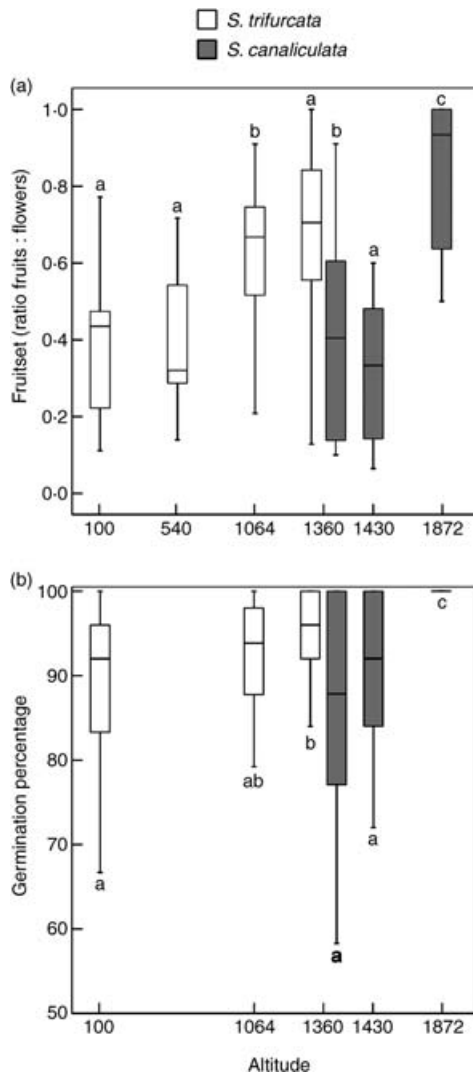


Fig. 4. Variation in fruit set, and germination percentage across the gradient. Lettering as in Fig. 1, on the basis of multiple comparison tests, after GLM analyses. Germination data missing for *S. trifurcata* at 540 m due to incorrect manipulation of seed bearing capsules.

that growth of woody species at high altitudes is limited by temperature constraints on meristem activity rather than by shortage of resources (Shi *et al.* 2008).

Other complementary mechanisms for the maintenance–improvement of vegetative growth with altitude may include increases of facilitative interactions at highlands (Callaway *et al.* 2002), water stress amelioration through reduced evapotranspiration with altitude, which occurs also in oceanic-type gradients like the one studied here (Körner 2007), or increasing photon flux density due to less cloudy conditions during the growing season at highlands.

IMPROVEMENT OF REPRODUCTIVE SUCCESS COUNTERACTS THE REDUCTION IN REPRODUCTIVE INVESTMENTS WITH ALTITUDE

As noted above, the more highland of the two species (*S. canaliculata*), and also the highland populations of *S. trifurcata*,

devoted a smaller fraction of resources to reproduction. However, the monotonic decrease of reproductive investments with altitude contrasted with trends in reproductive success and seed germinability within-species: the highland population of each species was more successful in terms of fruit set and seed viability. The scarce evidence available from literature tells that, although pollinator abundance is lower at high altitudes, fertilization of ovules is not particularly limiting in highland vs. lowland sites (García-Camacho and Totland, unpublished results). Compensatory mechanisms to the scarcity of pollinators, such as long duration of flowers and of stigma receptivity or more efficient pollen transfer are common at high altitudes (Bingham & Orthner 1998; Fabbro & Körner 2004). Long flower life spans, for instances, have been reported for other species of *Saxifraga* from cold habitats (Stenström & Molau 1992). Flowering phenology and synchrony can also affect fruit set significantly. Since flowering season is shorter at highlands (Körner 1999), synchrony at blossom may facilitate attraction of pollinators, abundance of pollen donors and exogamy, improving fertilization ratios. In *Saxifraga oppositifolia* L., for instance, warmer conditions lengthen the flowering period, thus driving inter-individual flowering asynchrony (Stenström, Gugerli & Henry 1997). Moreover, facultative self-compatibility is common among highland *Saxifraga* species, which further assures reproductive process when out-crossing fails (Vargas 1994; Raffl, Marcante & Erschbamer 2007). Regarding viability of seeds, improved germinability of highland seeds may also be the consequence of higher rates of outbreeding due to flowering synchrony or to more efficient pollinator activity.

The above patterns mean that, per biomass unit invested in reproduction, the highland populations of each species produced a higher number of viable seeds. This counteracts the lower total investment in reproduction at highlands, and probably makes the number of viable seeds per unit canopy area similar among populations and altitudes, or even higher at *S. canaliculata*'s upper limit. This is most relevant, and challenges widespread assumptions stating that performance is optimum at the mid-range stretch of species distributions, and decreases towards range margins (Samis & Eckert 2007).

Conclusion

Presumably, a shift towards preferential biomass and bud allocation to vegetative growth across ontogeny, allowed the more highland of the two species, *S. canaliculata*, and the highland populations of *S. trifurcata*, to achieve higher vegetative development than lowland plants. More 'efficient' or successful fruit set and seed germination also compensated for the reduction in reproductive investments at the highland edge of each species range. Overall, then, our results suggest that modulation of traits underlying growth and reproductive performance can counteract the impoverishment of conditions for plant development at high altitudes.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Details on Materials and Methods.

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