Patterns and origin of intraspecific functional variability in a tropical alpine species along an altitudinal gradient

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Background: Intraspecific functional variability (IFV) along altitudinal gradients is a powerful proxy to infer the responses of plants to abrupt environmental changes. We envisage that IFV shows distinctive patterns in tropical and extratropical alpine regions.
Aims: To characterise the patterns and explore the origin of IFV in a tropical alpine species in a context of upward range extension.
Methods: We examined variations in a series of plant functional traits in *Lasiocephalus ovatus*, inside and outside a nurse plant along a 600 m altitudinal gradient in the Ecuadorian Andes, and we studied its genetic variability.
Results: More conservative traits were developed at higher elevation, in contrast to extratropical alpine plants, which commonly develop opportunistic traits in response to late snowmelt close to their upper altitudinal limit. The presence of nurse cushions did not alter this trend. Increasing genetic distance along the gradient suggested that IFV might be partly genetically induced.
Conclusions: Our data combined with existing literature in tropical alpine environments lead the way to a stimulating scientific challenge: determining if patterns of plant altitudinal distribution in tropical alpine areas in response to climate change are predictable from patterns described in extratropical alpine areas.

Keywords: Andes; *Azorella aretioides*; competition; facilitation; genetic distance; *Lasiocephalus ovatus*; páramo; plant functional traits; SLA; upward range extension

Introduction

Upward range extension is a crucial ecological process by which alpine plants can escape the warming effects of past and current climate change when upslope areas are sufficiently large to harbour them (van der Hammen 1974; Simpson 1975; Colwell et al. 2008; La Sorte and Yetz 2010; Dirnböck et al. 2011). Once propagules have reached upslope areas, a large part of the success in upward range extension is expected to depend on the intraspecific functional variability (IFV) in plants along altitudinal gradients to overcome the new pool of environmental constraints. Worldwide, these constraints may include increased aridity and nutrient shortage because of the absence of well-developed soil (e.g. Pérez 2003) and cold temperature due (1) to katabatic winds flowing down from glaciers, when present (Kazanskii 2010) and (2) to the absence of soil structure. However, examining such variability remains mostly a challenge for the future (Albert et al. 2010; Jung et al. 2010).

Along with pattern description, identifying whether IFV is induced genetically or environmentally (plasticity) along altitudinal gradients may contribute to a better understanding of IFV, for at least two reasons: (1) because genetically induced traits adapt less well to rapid environmental changes than environmentally induced phenotypes

(Sultan 2000; Scheepens et al. 2010); and (2) because adaptive traits might have developed for purposes other than to overcome the constraints associated with the gradient (Eckhart et al. 2004; Gonzalo-Turpin and Hazard 2009). Alpine plants are a highly relevant model to test the origin of variation in functional traits, owing to their high morphological variability along steep environmental gradients (Körner 2003).

Biotic parameters are a potential source of intraspecific variability along altitudinal gradients (Hillyer and Silman 2010). This includes positive plant–plant interactions, which are expected to become central drivers of plant range extension to higher altitudes as soon as nurse plants are available in these sites ('stress-gradient hypothesis', Bertness and Callaway 1994). However, it has been shown that upward range extension in plants is often triggered by increased interspecific competition with plants that have migrated from lower vegetation belts (Lenoir et al. 2010; Dirnböck et al. 2011).

Among alpine environments, tropical alpine systems warrant particularly close investigation for various reasons. First, despite a large body of literature on the adaptation of plants to the highly specific environmental constraints inherent to tropical alpine regions (see Simpson 1975; Rundel et al. 1994; Körner 2003; Marquez et al. 2006; and

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references therein), comparatively few studies have been made with extratropical alpine environments in terms of plant population/community (re-)organisation in the face of abrupt environmental changes (Felton et al. 2009; Malhi et al. 2010; Anthelme et al. 2012). Second, tropical alpine areas exhibit unique environmental characteristics among alpine environments. This includes (1) a growing season for plants that lasts all year long (Smith and Young 1987); (2) the absence of prolonged snow cover because of weak seasonal temperature variation with concomitant higher frequency of freeze–thaw cycles, needle-ice activity and solifluction events (Pérez 1987; Francou et al. 2001); and (3) reduced precipitation at higher altitude, which, combined with strong seasonal precipitation variation close to the tropics, results in increased water stress for plants and the absence of developed soils (Rundel et al. 1994; Leuschner 2000; Körner 2003). Third, they are confronted by the rapid intensification of human activities, with a strong impact on biodiversity and ecosystem function (Luteyn 1999; Poulenard et al. 2003). Accordingly, tropical alpine plants are expected to respond and interact distinctively along altitude in comparison with their extratropical counterparts from which half of them originated (Sklenář et al. 2011; Anthelme and Dangles 2012). Specific responses have been shown across a series of architectural/functional characters (Hedberg and Hedberg 1979; Ramsay and Oxley 1997; Anthelme and Dangles 2012), including 'supercooling' aptitude to escape frequent low temperatures at night (Goldstein et al. 1985; Rada et al. 1987; Lipp et al. 1994), higher longevity coupled with higher maximal height and a high allocation of biomass to central aerial stems protected by leaves, as substantial investments of energy and resources to overcome stress related to temperature, water, and nutrients (Smith 1980; Smith and Young 1987; Monasterio and Sarmiento, 1991), and the development of thick, microphyllous leaves to cope with both increased aridity (Leuschner 2000) and xylem embolism (Sklenář et al. 2010). Whereas some of these studies examined IFV, to the best of our knowledge none also explored its patterns, its origin (plasticity vs. genetic selection) and the impact of plant–plant interactions on the outcome of trait variability.

We investigated IFV in the tropical alpine species *Lasiocephalus ovatus* Schtdl. along an altitudinal gradient in the Ecuadorian Andes. Specifically, we explored the following three research questions:

- Do the patterns of IFV in *L. ovatus* correlate with other patterns described in other tropical and extratropical alpine regions?
- Are there any correlations between genetic patterns and the variability of such traits?
- To what extent are IFV patterns in *L. ovatus* influenced by the presence of nurse plants?

By comparing our data with existing literature, we then explore the relevance of using extratropical patterns of plant upward colonisation to predict colonisation in tropical alpine regions under the effects of climate change.

Materials and methods

Target and nurse species

Lasiocephalus ovatus Schltdl. (Asteraceae) is a perennial, evergreen, semi-erect plant that can develop several branches with inflorescences at the top of the stems and a high density of pubescent leaves on branches (Figure 1; Dušková et al. 2010). In our sampling, it was found to reach an average height of 25 cm, with a maximum height of 66 cm. It is distributed in tropical alpine environments in Ecuador from 2700–4850 m (Sklenář and Jorgensen 1999) and Colombia (Luteyn 1999), but was found between 4150 and 4870 m within our study area. *L. ovatus* displays a series of morphological traits such as subcoriaceous leaves and white pubescence, which have been cited to improve nutrient and water uptake, and to ameliorate resistance to cold temperature and solar radiation in tropical alpine environments (Monasterio and Sarmiento 1991). *L. ovatus* was found to be the most tolerant to cold temperature among four tropical alpine species, but it did not rely on supercooling to avoid freezing, making it the 'most extreme life form' sampled by Sklenář et al. (2010). The local 700 m-long gradient occupied by *L. ovatus* in the study region indicates that this species is likely able to adapt to a large array of climatic and edaphic parameters.

Azorella aretioides DC. (Apiaceae), which acts as a nurse plant for *L. ovatus*, is an uncommonly large, compact, evergreen cushion. As other species of the same genus, it is composed of 'many tightly packed branched stems that form a smooth semi-woody exterior surface, and converge below to a single broad basal taproot' and it develops a series of physiological traits related to stress tolerance, including a slow lateral spread (see Kleier and Rundel (2009) for a more detailed description). Its altitudinal distribution in Ecuador and Colombia is between 3500 and 4750 m (Luteyn 1999) and in our study area it is found between 4370 and 4750 m, where it covers 5–11% of the soil surface (Anthelme et al. 2012). Various species of the genus *Azorella* are known for their important positive effects on vegetation, through soil nutrient enrichment and soil water enrichment (Badano et al. 2006; Kleier and Rundel 2009; Anthelme et al. 2012), protection from wind exposure, and improved soil stability (le Roux and McGeoch 2008). As a result, cushion-forming *Azorella* are important ecosystem engineers, with positive effects on the organisation of alpine plant communities.

Study area and sites

The study area was the western alpine slopes of the Antisana (alternatively: Antizana) Ecological Reserve, within the Eastern Cordillera of the Ecuadorian Andes in the vicinity of the Equator (00° 28′ S, 78° 09′ W; Figure 1).
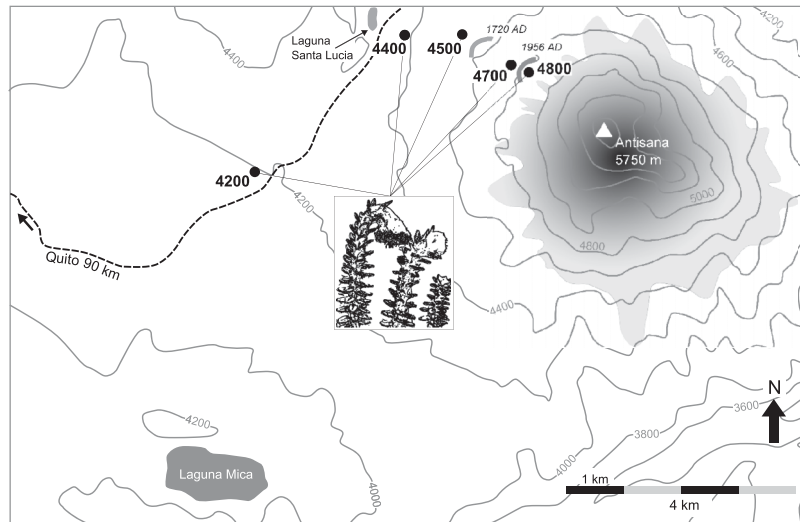
Figure 1. Map of the study area in the Antisana Ecological Reserve, Ecuador Study sites (black circles), access road (dashed line), former limits of glacier 15-alpha (dark grey arcs to the north - north-west of Antisana peak) and Antisana's ice cape (black-grey degraded area). Contour lines are at 200 m altitude. Centre image: *Lasiocephalus ovatus* (Asteraceae).

The local climate in this area is representative of the humid tropical alpine environments of the Andes in Venezuela, Colombia, Ecuador, and North Peru (see Sarmiento 1986 for a review on tropical alpine climates). At the weather station located at 4900 m, just above the highest of our sampling sites, precipitation falls all year round with a slight decrease in November–January. During 2006 and 2007 the yearly total precipitation was 1000 mm and the mean relative humidity was 80%, whereas mean temperature had no seasonal trend and averaged 1.15 °C, yearly (Station ORE IRD-INAMHI, measurements at 1.80 m above soil surface; Maisincho and Carceres 2007). At plant height, temperature can vary by 9 °C within 24 h, which is far higher than the monthly variation of 1 °C in a year (Anthelme et al. 2012) as found commonly in tropical alpine environments (Körner 2003). Under current climate, permafrost is absent and snow cover is only present for a short time at all sites (B. Francou, pers. com.).

The peak of the Antisana Mountain is covered by an ice cap between 5000 and 5750 m a.s.l. (Figure 1); the ice cap has lost more than 30% of its total area in recent decades (Vuille et al. 2008). Above our study sites lies glacier 15-alpha, which lost 280 m of its total length of 1.8 km between 1994 and 2006 (Vuille et al. 2008). Vegetation between 4000 and 4500 m is typical of 'grass páramo' (Luteyn 1999). It is dominated by tussock grasses such as *Calamagrostis intermedia* (J. Presl) Steud. and *Festuca* spp., the evergreen shrub *Chuquiraga jussieui* J.F. Gmel. (Asteraceae-Mutisiae), with *Lasiocephalus ovatus* being one of the most frequent species encountered at all elevations. Above 4500 m, the vegetation becomes fragmented and several growth forms, including cushions, basal rosettes, and tussock grasses, coexist without dominating locally. Herbivory by large mammalian grazers is limited because of the protection given to this area. Nevertheless, grazing by wild llamas has impacted all sites, and sites up to 4700 m were additionally impacted by a few wild horses, rabbits and cattle.

The study sites were located along an altitudinal gradient at five elevations from 4200–4800 m. With the exception of site 4200 (4 km from the nearest site 4400), the maximum distance between sites did not exceed 1 km (Table 1; Figure 1). The highest site was located on the recently deglaciated lateral moraine of glacier 15-alpha. Glaciers descended to 4600 m or less at the end of the Little Ice-Age (1720 AD) and then receded regularly; sites 4500, 4700 and 4800 thus form an approximately 250-year postglacial chronosequence (Jomelli et al. 2009; Figure 1). The last eruption of volcano Antisana occurred in 1802 through lava flows and did not affect our sites. However, the eruption of neighbouring volcano Cotopaxi in 1740 resulted in substantial accumulation of volcanic ash (B. Francou, pers. com.), which might have influenced positively plant colonisation in our sites through soil nutrient enrichment (Wilcox 1959). Deglaciation of sites 4200 and 4400 occurred much earlier in the Holocene period, making the current presence of *L. ovatus* probably driven by a multitude of environmental parameters in addition to the length of ice-free period, including large-scale fires, grazing and interactions with other species. Apart from altitudinal variation, two local environmental specificities were observed: site 4400 has been disturbed regularly by temporary water courses and site 4800 had large boulders up to 3 m tall, in the shelter of which *L. ovatus* was generally found.

Sampling and measurements

At each site, 15 randomly chosen individuals of *Lasiocephalus ovatus* were selected, registered spatially with GPS, and marked with stainless steel rings. Six plant traits were selected in accordance with: (1) their relevance as indicators of shifts in general plant strategies along an

Table 1.   Variation in climate and vegetation cover in the vicinity of sampled plants along an altitudinal gradient between 4200 m and 4700 m (Antisana, Ecuador). Climate variable values are daily mean $\pm$ SE (25 days in March 2009, measurements each 15 minute). Different letters indicate significant differences in values among sites ($P < 0.05$, paired t-tests for microclimate; $P < 0.05$, post-hoc Tukey tests for vegetation cover). Values in brackets represent the frequency of daily freezing events. Vegetation cover estimated at soil surface. n.d., no data.

| Site | Latitude | Longitude | Min. temperature (°C) | | Average wind speed (m s^{-1}) | Vegetation cover (%) |
			Leaf zone	Root zone		
4200	0°29′37.40″ S	78°12′17.72″ W	n.d.	n.d.	n.d.	92 ± 4[a]
4400	0°28′02.26″ S	78°10′33.43″ W	−0.54 ± 0.35[a](0.64)	3.49 ± 0.19[a](0.00)	1.49 ± 0.03[a]	29 ± 6[b]
4500	0°28′02.87″ S	78°10′00.31″ W	−1.01 ± 0.36[b](0.68)	4.03 ± 0.19[b](0.00)	1.00 ± 0.03[b]	91 ± 4[a]
4700	0°28′20.17″ S	78° 09′30.98″ W	−1.12 ± 0.30[b](0.68)	2.13 ± 0.15[c](0.00)	1.37 ± 0.04[c]	27 ± 5[b]
4800	0°28′22.35″ S	78° 09′22.74″ W	−0.93 ± 0.24[b](0.72)	2.07 ± 0.15[c](0.00)	0.42 ± 0.01[d]	00 ± 0[c]

altitudinal gradient; and (2) practicalities in their assessment and measurement. Maximum vegetative plant height (hereafter 'plant height') was measured in the field in February 2010. Apical stems were collected and sealed in hermetic plastic bags. Within 24 h, the number of leaves on a relatively undamaged 10 cm stem section was counted (relative number of leaves: RNL) and the areas of 10 randomly selected green leaves were determined with ImageJ software (http://rsb.info.nih.gov/ij). Mean leaf area for each individual was measured as the mean of these 10 values. Leaves were then oven-dried (96 h, 60 °C) and weighed to obtain leaf dry mass. Specific leaf area (SLA) was calculated as the ratio between leaf area and leaf dry mass. Leaves from 10 randomly chosen individuals at each site were sent to the Instituto Nacional Autónomo de Investigaciones Agropecuarias (INIAP, Quito, Ecuador) for laboratory analysis of total macronutrients using the Kjeldahl method (Ntotal$_{foliar}$: hereafter N$_{foliar}$) and atomic absorption spectroscopy (P$_{foliar}$, K$_{foliar}$).

Soil samples (0–15 cm depth) were extracted from the locations of 10 of the collected individuals at each site. The soil samples were sealed in plastic bags and sent to the INIAP for laboratory analysis of available soil macronutrients (?N$_{soil}$ (NH4+), P$_{soil}$, K$_{soil}$, Ca$_{soil}$ and Mg$_{soil}$) and soil organic matter (SOM), using modified Olsen methodology, principally. Note that given the relative instability of NH$_4$, its relevance as representative of total available N in soil must be taken cautiously.

Microclimatic data were collected at all sites every 15 min over 25 days in March 2009, except at site 4200 where our climatic microstation became damaged. Measurements included the following: wind speed (S-WSA-M003, Onset Computer Corporation, Pocasset, MA), temperature at leaf zone, that is, 10 cm above the soil surface (Onset HOBO-Pro RH/Temp data loggers covered with plastic roofs), and temperature at the root zone, that is, 5 cm below the soil surface (means of three and two spatial replicates, respectively; Onset probes connected to HOBO microstations). In addition, vegetation cover (%) was estimated using photographs of areas of 0.46 m^2 around each individual (delimited by a 76 cm-diameter ring).

Sites 4500 and 4700 were the only locations where *L. ovatus* was sufficiently frequent to be found regularly

both inside and outside cushions of the potential nurse plant *Azorella aretioides*. At these sites, we randomly selected 15 additional individuals located inside cushions at each altitude and made similar measurements of plant traits as for the other individuals. A total of 105 samples were taken into account for plant traits (15 × 5 sites plus 2 × 15 samples in cushions).

Genetic analyses were conducted on 10 individuals at each of the five altitudes, and also inside cushions at sites 4500 and 4700. This subsample ($n = 70$) was chosen randomly within the initial ecological sample ($N = 105$). Material was collected from leaves and stems at least 5 cm long. Nuclear DNA was isolated using the Invitrogen Easy-DNA^{TM} kit (Invitrogen Corp, Carlsbad, CA, USA). In total, 35 pairs of primers for nuclear microsatellite markers (simple sequence repeats; SSRs) from closely related species *Senecio vulgaris L.* and *Senecio squalidus L.* (Liu et al. 2004) were tested and six loci were found to be polymorphic: AY652658, AY652660, AY652670, AY652673, AY652674, and AY652676. These markers were analysed to estimate genetic diversity and genetic distance along the altitudinal gradient, and in the presence/absence of *A. aretioides*. SSRs provide data on neutral diversity, and as such are not directly associated to selection or adaptation. We used these data as a proxy of the adaptive diversity (Holderegger et al. 2006).

Data analysis

Trait variation among sites along the altitudinal gradient was assessed with general linear models (GLMs). We used fixed-effects models with the presence of *Azorella aretioides* (cushion variable) being nested within altitudes 4500 m and 4700 m (altitude variable) to assess the effects of both variables on traits of *Lasiocephalus ovatus*. Microclimatic data were presented in a manner to identify stressful conditions for plants, i.e. minimal temperature and average wind speed. They were compared between sites with a series of paired t-tests, pairs being values for two sites at the same time on the same day (e.g. day two, 11:15 a.m., sites 4500 and 4700), with the assumption that the inherent temporal autocorrelation is compensated by the large number of temporal repetitions (2400) and spatial

repetitions (for temperature at the root zone). Relationships between traits and soil properties, and among traits, were analysed with (multiple) regressions.

Genetic diversity was estimated with expected heterozygosity (He) (Weir 1996). Assessment of genetic distance by standard methods was considered unreliable because the ploidy of *L. ovatus* varies along altitudinal gradients (Dušková et al. 2010). To address this potential problem, we performed principal component analyses (PCA) on matrices based on Bruvo distance, which take distances between microsatellite alleles into account without knowledge of allele copy number or the requirement that individuals have the same ploidy (Bruvo et al. 2004; Clark and Jasienuk 2011). Matrices had the following form: labels of individuals in column one, type of microsatellite marker in column two, and molecular weight of alleles in the next four columns. PCA was performed with the R package Polysat (Clark and Jasienuk 2011).

Results

Microenvironment along the altitudinal gradient

Minimum temperature in the leaf zone was overall negative and remained relatively stable along the gradient, increasing only at 4400 m (-0.54 °C instead of ca. -1 °C at the other sites; Table 1). Temperature in the root zone decreased more sharply along the gradient, from 4.0 °C at 4500 m to 2.1 °C at 4800 m, with the exception at 4400 m where its value (3.5 °C) was lower than that at 4500 m. Freezing events were not observed in the root zone, but their frequency during the observation period was high and increased regularly along the elevation gradient at leaf level, from 0.64 (4400 m) to 0.72 (4800 m). SOM and soil macronutrient concentration were negatively correlated with altitude, (linear regressions in Table 2), but they displayed unusually low values at 4400 m (e.g. N_{soil}: 24.10 ± 2.01 mg kg^{-1} at 4400 m; between 80 and 105 mg kg^{-1} at 4200 and 4500 m). Indeed, vegetation cover was a much better predictor of variations in the concentration of soil

nutrients and SOM than altitude, and made the additive effects of altitude insignificant (e.g. SOM: R^2 (ALT + COV) = 0.63 = R^2 (COV); stepwise regressions; Table 2).

Variation in plant traits

All plant traits for *Lasiocephalus ovatus* displayed significant variations between sites (Figure 2). Only the RNL showed a truly linear increase with elevation (R^2 = 0.49, $P < 0.001$). The majority of other metrics decreased more or less monotonously with elevation (K_{foliar}), and sometimes ended with a plateau (plant height, N_{foliar}). Interestingly, SLA peaked in the middle of the altitudinal gradient ($R^2_{quadratic}$ = 0.23, $P < 0.001$; R^2_{linear} = 0.04, $P > 0.05$). Positive, albeit weak, correlations between N_{soil} and N_{foliar} (Pearson correlation: 0.31; Appendix 1) and K_{soil} and K_{foliar} (0.35) indicated that, to some extent, higher nutrient concentration in the soil resulted in higher nutrient concentration in leaves. SLA co-varied negatively with plant height (R^2 = 0.21, $P < 0.001$).

Genetic diversity and genetic distance

Overall, expected heterozygosity (He) ranged between 0.70 and 0.76 along the altitudinal gradient (Figure 3). The distribution of genetic diversity along the altitudinal gradient showed a hump-backed trend, reaching a peak at the intermediate elevation along the gradient (4500 m: He = 0.77). Genetic diversity correlated linearly with mean SLA (R^2 = 0.71, $P < 0.05$; Figure 3), but not with the variability of SLA (R^2 = 0.08).

In the PCA diagram shown in Figure 4A, axes 1 and 2 represent individuals along with elevation; it shows a structured pattern of genetic distance along the altitudinal gradient. Genetic distance increased significantly along the gradient 4400–4700 m, despite the short spatial distances between the sites at these altitudes (axis 1; ANOVA: $P < 0.001$, post-hoc Tukey tests). In contrast, the segregation of site 4200 along axis 2 (ANOVA: $P < 0.001$) might be purely spatial, owing to its greater distance from the

Table 2.   Concentration of soil organic matter (SOM) and soil extractable macronutrients at each site (4200–4800), and respective effects of altitude (ALT), vegetation cover (COV), and the presence of nurse plants at that altitude (NURSE (ALT)) on soil nutrients. The effects of the variables ALT, COV and ALT + COV were assessed with linear regressions (R^2 adjusted); the variable NURSE (ALT) was assessed with nested GLM (R^2). ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

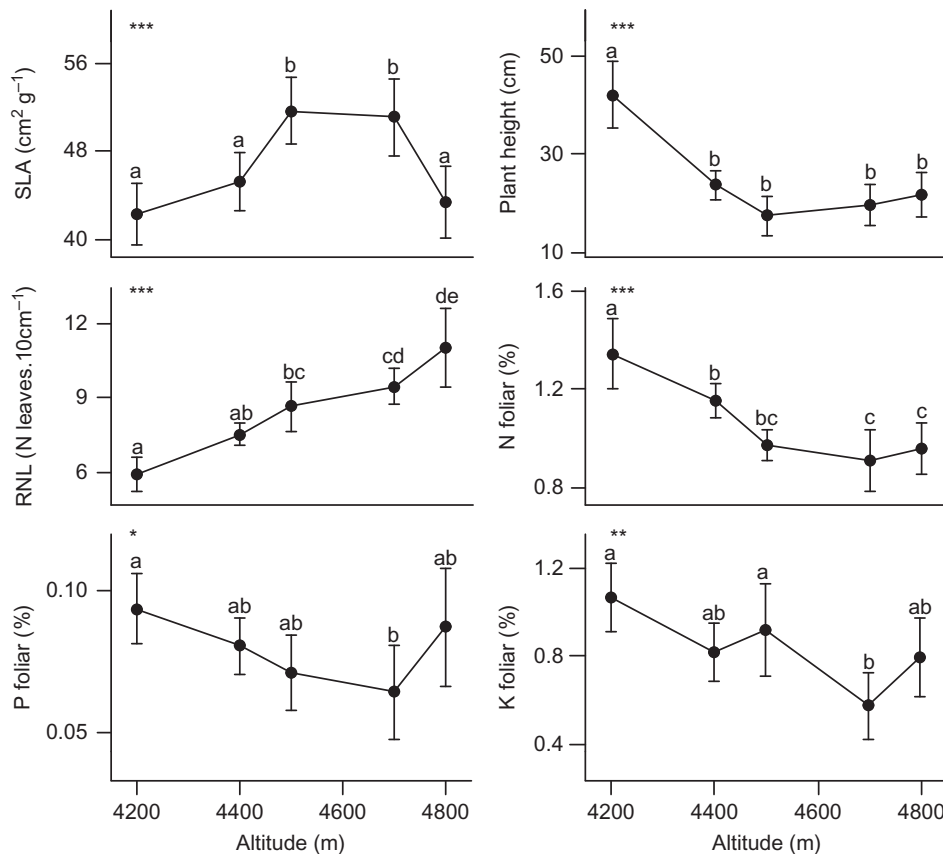|	SOM (%)	N_{soil} (mg kg^{-1})	P_{soil} (mg kg^{-1})	K_{soil} (mmol 100-ml^{-1})	Ca_{soil} (mmol 100-ml^{-1})	Mg_{soil} (mmol 100-ml^{-1})
4200	20.68 ± 3.74	81.10 ± 15.00	13.88 ± 1.78	0.41 ± 0.07	19.04 ± 1.94	2.38 ± 0.13
4400	4.50 ± 0.62	24.10 ± 2.01	5.30 ± 0.55	0.13 ± 0.01	4.76 ± 0.56	1.10 ± 0.05
4500	31.58 ± 3.92	101.30 ± 6.00	14.32 ± 1.27	0.55 ± 0.05	18.32 ± 2.02	4.40 ± 0.19
4500c	58.38 ± 6.51	82.85 ± 6.66	12.67 ± 1.49	0.66 ± 0.06	17.4 ± 1.11	4.36 ± 0.16
4700	2.19 ± 0.41	49.00 ± 5.22	9.33 ± 2.40	0.18 ± 0.02	2.62 ± 0.42	2.26 ± 0.21
4700c	12.75 ± 4.05	50.35 ± 3.09	8.35 ± 1.22	0.36 ± 0.05	6.68 ± 0.56	4.44 ± 0.28
4800	0.07 ± 0.03	14.63 ± 2.59	5.42 ± 0.66	0.12 ± 0.01	1.48 ± 0.09	0.76 ± 0.03
R^2 ALT	0.22***	0.16**	0.10*	0.15**	0.26***	0.03
R^2 COV	0.63***	0.57***	0.26***	0.56***	0.44***	0.48***
R^2 ALT + COV	0.63	0.58	0.25	0.56	0.43	0.59
R^2 NURSE (ALT)	0.86***	0.56**[a]	0.17	0.56***	0.43	0.59***

Figure 2.   Variation in functional traits of *Lasiocephalus ovatus* along the altitudinal gradient. Overall significance of variation is shown top left of each box (one-way ANOVA; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., not significant). Different letters indicate significant differences between sites (post-hoc Tukey tests). Bars are 95% confidence intervals. For abbreviations of plant traits: see methods.
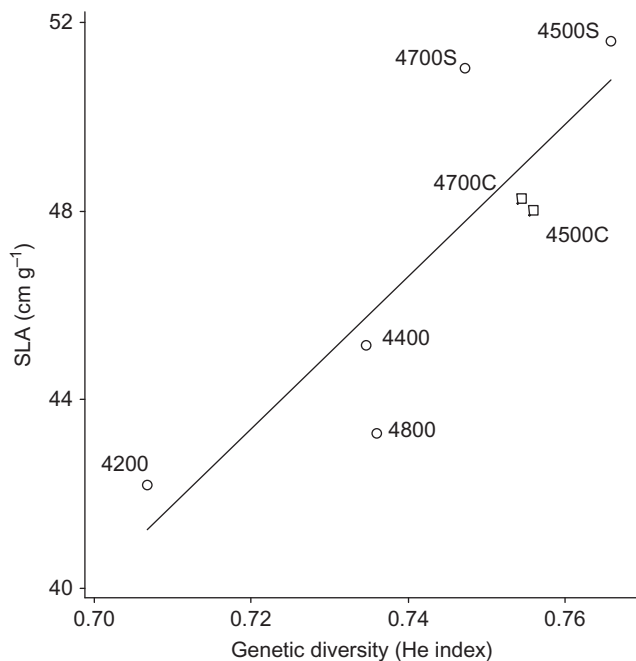

Figure 3.   Relationships between genetic diversity and mean SLA per site along the altitudinal gradient. C, *Lasiocephalus ovatus* inside *Azorella aretioides* nurse plants; S, *L. ovatus* outside nurse plants; line indicates linear regression.

other sites (Figure 1 and Table 1). Multiple regressions showed that more than 10% of the variance for four functional traits was explained by the combination of the two axes: N_{foliar} ($R^2_{adjusted} = 0.30$; $P < 0.01$), RNL ($R^2_{adjusted} = 0.28$), plant height ($R^2_{adjusted} = 0.16$), and K_{foliar} ($R^2_{adjusted} = 0.28$). In contrast, genetic distance did not explain much of the variance in SLA ($R^2_{adjusted} = 0.06$). The greatest intrapopulation variation in genetic distance was found at 4500 m.

Nurse effects on trait variation

Azorella aretioides had an overall positive effect on N_{foliar} concentration in *Lasiocephalus ovatus*, irrespective of altitudinal variation (fixed-effects models at 4500 and 4700 m; Figure 5). This positive effect occurred mostly at the upper site (4700), where K_{foliar} was also enriched significantly in nurse plants (two-sample *t*-tests). SOM, K_{soil} and Mg_{soil} were found to be more abundant inside nurse cushions although linear regressions failed to show correlations between soil and leaf concentration for each of the three macronutrients studied at site 4700. In contrast, available N_{soil} concentration was reduced in cushions at 4500 m. Genetic diversity was almost unaffected by nurse plants: it decreased slightly in the presence of *A. aretioides* at 4500 m
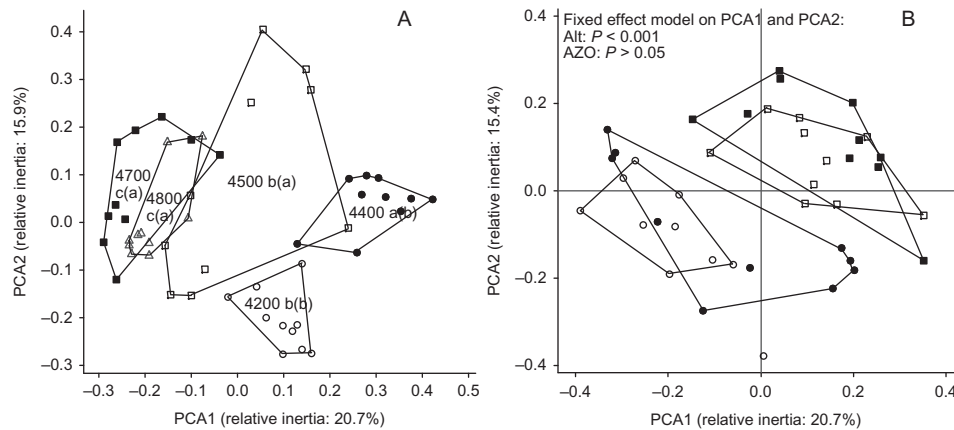
Figure 4. Genetic distances between individuals of *Lasiocephalus ovatus* (PCA on Bruvo distances, biplot 1–2) along the altitudinal gradient, from 4200–4800 m (A). Marks are individuals, with lines representing each altitudinal group. Different letters indicate significant differences between sites along axis 1 with post-hoc Tukey tests (differences along axis 2 are in parentheses). Presence (filled markers) or absence (empty markers) of nurse plant at 4500 m (circles) and 4700 m (squares) (B).
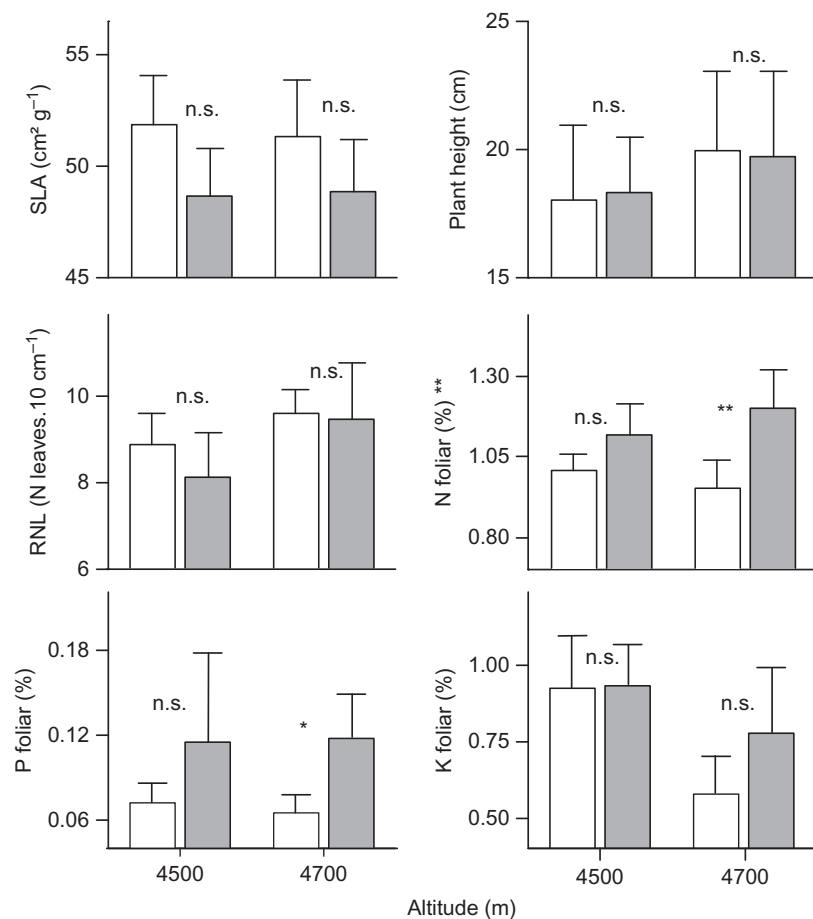

Figure 5. Effects of the nurse plant *Azorella aretioides* on plant functional traits in *Lasiocephalus ovatus*. White bars, without nurse; grey bars, with nurse. Overall significance of nurse effect, where applicable, is indicated to the right of the y-axis legend (fixed-effect model, see Figure 2 for levels of significance). Significance of nurse effect at each elevation indicated within each panel (two-sample *t*-tests). For abbreviations of plant traits: see methods.

(-0.01), whereas it increased at 4700 m when *A. aretioides* was present ($+0.007$; Figure 3). Genetic distance did not vary with the presence of cushions, as observed on PCA biplot 1–2 (Figure 4B).

Discussion

Our altitudinal gradient proved to involve an increasing stress gradient that is primarily driven by soil nutrient and SOM, and secondarily by minimum temperature, whose

decrease along elevation was obvious in the root zone only. This stress pattern highlights the substantial influence of other microenvironmental variations due to local specificities (e.g. boulders at 4800 m weaken winds, vegetation cover negatively affected by temporary water courses that occur at 4400 m). These findings show both the strengths and shortcomings of using altitudinal gradients in ecology (Körner 2003, 2007; Malhi et al. 2010).

Conservative vs. opportunistic strategies at higher elevation

By developing seeds with plumes, *Lasiocephalus ovatus* has a particularly efficient dispersal, which is certainly the primary explanation to its large altitudinal distribution. However, the unimodal and monotonous patterns of IFV observed along the altitudinal gradient indicate that the local wide-ranging altitudinal distribution of this species is also driven by its high capacity to adapt morphologically and physiologically to increasing stress inherent to higher elevations. Combinations of simple functional traits are increasingly used to infer strategies developed by plants to cope with shifts in environmental constraints (e.g. Gross et al. 2007; Maire et al. 2009). Here, by development of a lower stature combined with a higher leaf density, and most of all by reducing CO_2 assimilation (decrease in SLA, Poorter et al. 2009) and by reducing maximal photosynthetic activity, as indicated by a decrease in nitrogen concentration in leaves (Reich et al. 1999), individuals of *L. ovatus* likely adopt a more conservative, stress-tolerant strategy at the upper end of the altitudinal gradient. In accordance with our data, several other studies that examined functional trait variability along altitudinal gradients observed more conservative traits at the upper distribution of tropical alpine plants. At community level, grasses were found to develop high freezing tolerance in Papuasia (Earnshaw et al. 1990) and their photosynthetic nitrogen-use efficiency and SLA decreased along with increasing elevation in the alpine Mont Kinabalu, Malaysia (Hikosaka et al. 2002). At population level, CO_2 assimilation rate decreased along with elevation in two species of *Draba* and various growth forms in the Venezuelan páramo (Rada et al. 1992; Cabrera et al. 1998) whereas the supercooling aptitude of a giant rosette increased along with elevation in the same region (Rada et al. 1987). As an exception, no altitudinal effect of altitude on supercooling capacity in grasses was observed, presumably because grasses did not use this strategy to overcome cold stress (Marquez et al. 2006).

In contrast, in extratropical alpine plants, shifts towards opportunistic traits are observed commonly in the upper part of altitudinal gradients because of seasonal vegetative growth and late snowmelt (Choler 2005; Gonzalo-Turpin and Hazard 2009; Baptist et al. 2010), through the development of higher nutrient concentration in leaves (Morecroft and Woodward 1996; Baptist et al. 2010), larger SLA (Choler 2005; Gonzalo-Turpin and Hazard 2009; Kudo et al. 1999), or decreased life-span (Kudo et al. 1999). None of the studies in tropical alpine environments cited above

evidenced a higher frequency of opportunistic traits in tropical alpine species at the upper limit of their distribution. Given that (1) snowbed duration is one of the crucial factors controlling plant growth in extratropical alpine environments (Rasmus et al. 2009; Wipf et al. 2009) and that this duration is especially low within the tropics, and (2) earlier snowmelt associated with current global warming is expected to have a strong, negative impact on the distribution of alpine plants in seasonal alpine systems (Björk and Molau 2007; Wipf et al. 2009), exploring more thoroughly this distinction between IFV patterns in tropical and extratropical alpine environments pattern constitutes a stimulating line of research.

SLA driven by competition and stress?

The intermediate peak of the distribution of SLA along the altitudinal gradient provides evidence of a second, less expected pattern of traits. Interestingly, a similar IFV pattern was observed for the Himalayan tree *Quercus aquifolioides* Rehd. & Wils., with an initial increase in SLA due to higher water stress at lower elevations (Li et al. 2006). The mechanisms sustaining our pattern are likely different because in humid tropical alpine environments water stress is rather expected to increase with elevation (e.g. Leuschner 2000). Instead, we hypothesise that the much taller growth of individuals at 4200 m (41.9 cm vs. 20.6 cm in other sites, Figure 2) is a response to stronger competition for light with other species at the lowest altitude (Grime 2001), especially with the shrub *Chuquiraga jussieui* and tall tussock grasses, such as *Calamagrostis intermedia*. Reduced SLA at lower elevation may therefore reflect lower plant fitness indebted to increased competition, as shown by the overall negative relationship that was found between SLA and height. This specific pattern of traits, and the similar one observed by Cabrera et al. (1998) for *Draba* spp. in the Venezuelan páramo, corroborate in tropical alpine environments the recent conceptual model proposed by Albert et al. (2011), according to which IFV in plants reach a maximum at the mid distribution range of species.

Structured genetic pattern along the gradient

Despite generating increasing interest, IFV and its causes, namely, genetics or the environment, remain poorly understood, even though this variability might play a central role in the distribution of plant communities and populations (Jung et al. 2010). Across neutral markers, we identified a genetically structured pattern along a tropical altitudinal gradient, which indicates that strong intraspecific variability might in part the result of genetic selection or genetic drift. Two recent studies along alpine altitudinal gradients identified a significant effect of genetic differentiation on SLA: one concluded that genetic selection was involved (Gonzalo-Turpin and Hazard 2009), the other suggested genetic drift (Scheepens et al. 2010). At first glance, the strong correlation between SLA and genetic diversity might

support the hypothesis of genetic selection for this trait in *Lasiocephalus ovatus*. However, the absence of a notable correlation between genetic distance and SLA, as well as between SLA variability and genetic diversity, indicates that this is not the case. Therefore, instead, shifts in SLA might be environmentally induced, or, perhaps, the result of genetic drift. The overall absence of strong correlations between functional traits and soil parameters (Appendix 1) suggests that the origin of IFV may indeed have genetic roots, a hypothesis which needs to be explored more thoroughly. Note that the shift from diploidy to triploidy in *L. ovatus* with increasing elevation, as shown in the same study area (Dušková et al. 2010), was not considered in our study and might also take a substantial part in the origin of IFV along the altitudinal gradient.

Positive but secondary effects of nurse plant

The identification of leaves of *Lasiocephalus ovatus* with higher nitrogen concentration inside than outside of the cushions of *Azorella aretioides* indicates that the presence of nurse plants enables a slightly better accumulation of biomass, as indicated by higher respiration rates (Poorter et al. 2009). This facilitative effect was essentially found at higher altitudes (4700 m), where soil nutrient concentration outside cushions is expected to be very poor because of the increased aridity characterising tropical alpine environments (Leuschner 2000; Pérez 2003) and the more fragmented vegetation cover (see Antonsson et al. 2009 for similar results in a high-latitude alpine area). Accordingly, the fitness of *L. ovatus* likely was ameliorated by soil nutrient enrichment (see increases in K_{soil} and Mg_{soil} in presence of cushions) and possibly increased soil water content, as observed for a large number of species in the same study area (Anthelme et al. 2012).

At the maximum elevation at which mature individuals can develop (4700 m), it is therefore likely that *A. aretioides* contributes to reinforcing populations of *L. ovatus*, and thus reduces the risk of population extinction, by offering a preferential biogenic habitat. Although facilitation does not seem to be a critical factor in explaining IFV patterns in *L. ovatus*, it is conceivable that other species with poorer dispersal capacities will depend more on the presence of nurses to colonise upslope areas in a context of global warming. *Myrosmodes* sp. (Orchidaceae), *Huperzia crassa* (Willd.) Rothm. (Lycopodiaceae), and *Lupinus microphyllus* Desr. (Fabaceae), which are facilitated strongly by *A. aretioides* at higher elevation (Anthelme et al. 2012), might belong to this group.

Conclusions

The observation of correlations between IFV and altitudinal variation is in line with the hypothesis that IFV partly conditions the distribution success of plant populations facing environmental changes (Albert et al. 2010), including their capacity to extend their distribution range upward (Theurillat and Guisan 2001; Cannone et al. 2008).

In the one species studied, IFV might be the result of a combination of plant plasticity and genetic differentiation, whereas facilitative effects by nurse plants may act only as secondary factors for colonisation, by reinforcing the presence of *L. ovatus* at sites where it has already become established. By developing more conservative, as opposed to opportunistic, traits at higher elevations, *L. ovatus* and other tropical alpine species seem to have adapted to a crucial specific characteristic of tropical alpine environments: the absence of pronounced seasonal temperature variations with concomitant absence of persistent snowbeds. This result, combined with available literature on this topic, leads the way to a stimulating challenge for future studies in tropical environments: determining whether the distribution patterns of alpine plants from temperate and (sub)polar alpine environments are, or are not, an adequate model for the distribution of tropical alpine plants, with the objective to evidence a general trend of variation of traits along gradients at both intra- and interspecific levels.

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Appendix 1. Pearson correlations between six plant functional traits and soil properties

	SLA	Plant height	RNL	N_{foliar}	P_{foliar}	K_{foliar}
N_{soil}	0.21	0.12	−0.27	0.31*	0.13	0.40**
P_{soil}	−0.05	0.22	−0.23	0.27	0.08	0.16
K_{soil}	0.10	0.18	−0.20	0.14	0.12	0.35*
Ca_{soil}	−0.06	0.40**	−0.30*	0.16	0.07	0.20
Mg_{soil}	0.46**	−0.11	−0.07	0.04	−0.09	0.32*
SOM	0.11	0.14	−0.17	0.13	0.09	0.37**

SLA, specific leaf area; RNL, relative number of leaves; SOM, soil organic matter. ***, $P > 0.001$; **, $P < 0.01$; *, $P < 0.05$.