

## RESEARCH PAPER

# Influence of altitude on local adaptation in upland tree species from central Argentina

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## Keywords

Altitudinal gradient; *Escallonia cordobensis*; functional traits; germination success; intraspecific variation; *Maytenus boaria*; *Polylepis australis*.

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## ABSTRACT

- Steep climatic gradients boost morphological and physiological adjustments in plants, with consequences on performance. The three principal woody species of the Sierras Grandes Mountains of central Argentina have marked differences in sapling performance along their altitudinal distribution. We hypothesize that the steep gradient of climatic conditions across the species' altitudinal distribution promotes trait differences between populations of different altitudes that are inherited by the following generation.
- Seeds from different altitudes were exposed to three temperature regimes to assess differential germination responses. Saplings were then transplanted to a greenhouse to assess possible variations in attributes and performance after 18 months.
- The three species showed differences in germination responses to temperature among altitudes and/or in sapling attributes and performance. In *Maytenus boaria* and *Escallonia cordobensis*, germination success was higher under high temperatures for the highest-altitude, whereas lower temperatures boosted germination of the lowest altitudes. *Polylepis australis* showed no differences in germination among temperature treatments. In the greenhouse, saplings of the three species from intermediate altitudes showed high performance, whereas the upper and lower populations seemed to be adjusted to tolerating more stressful conditions (i.e., lower temperatures at the upper end and water stress at the lower end), showing lower performance toward both altitudinal limits.
- These patterns agree with those described for saplings growing under field conditions, suggesting adjustments in response to environmental changes undergone by populations along the altitudinal range. The marked adjustments of populations to the local environment suggest a potentially high impact of climatic change on species distribution.

## INTRODUCTION

Mountain environments are characterised by marked climate gradients, defined by changes in temperature, atmospheric pressure, UV radiation, precipitation and length of growing season (Körner 1999). Such climate factors determine gradients of environmental stress, which are important drivers of morphological and physiological variability in plants (Schöb *et al.* 2013; Frei *et al.* 2014; Grassein *et al.* 2014). For instance, leaves of mountain plants generally decrease in length, width and area with increasing altitude, whereas leaf nutrient content per area and leaf thickness increase (Bresson *et al.* 2011; Körner 2012; Vitassé *et al.* 2013). Regenerative traits also vary among populations in response to altitudinal gradients (reviewed in Baskin & Baskin 1998), with selection being expected to favour the local synchronisation of germination with optimum climate conditions for seedling survival (Bischoff *et al.* 2006; Galloway & Etterson 2007; Bischoff & Müller-Schärer 2010; Donohue *et al.* 2010; García-Fernández *et al.* 2013; Giménez-Benavides & Milla 2013).

This tight relationship between gradients of environmental stress and plant strategies and distribution that characterise mountains make this system highly vulnerable to environmental changes associated with global warming (Beniston 2003; Gottfried *et al.* 2012; Elsen & Tingley 2015). Locally differentiated shifts in climate might result in populations either becoming locally extinct, persisting through spatially migrating across ecological niches, or adapting to new conditions in current locations (Aitken *et al.* 2008; Nicotra *et al.* 2010). Many species are expected to be unable to track climate change, since the currently observed migration rates of many species are several orders of magnitude lower than the anticipated isotherm shift under the future climate change scenarios (Davis & Shaw 2001; Giam *et al.* 2010; Pimm *et al.* 2014). Consequently, a decline in cold-adapted species has been predicted in mountain systems under increasing temperatures (Gottfried *et al.* 2012), especially in mountains with restricted areas (Elsen & Tingley 2015).

Local trait variation can predict a plant species' potential to adapt to variable environmental conditions within a given area (Byars *et al.* 2007; Nicotra *et al.* 2010). Plants with broad

altitudinal distribution ranges are exposed to different environmental conditions across the gradient; this situation may lead to variation among populations, ultimately producing local adaptations (Körner 2012; Valladares *et al.* 2014). While irreversible physiological and morphological adjustments or local adaptation might benefit species in the maternal environment, they might also reduce the capacity to respond to climate shifts (Thuiller *et al.* 2008; Nicotra *et al.* 2010; Körner 2012). Accordingly, it is necessary to understand intraspecific variation between different altitudinal populations to improve predictions on the responses of mountain species to changing climate conditions (Thuiller *et al.* 2008; Körner 2012).

Woodlands of the upper vegetation belt of the Sierras Grandes Mountains of Córdoba (central Argentina) are dominated by *Polylepis australis*, with a lower abundance of *Maytenus boaria* and *Escallonia cordobensis*. These woodlands are particularly restricted in terms of altitudinal retreat, because the mountains do not extend above the alpine zone and there is intensive browsing by livestock affecting sapling performance of the three species (Marcora *et al.* 2013). Previous studies revealed that several traits and components of performance of these three species change along their altitudinal distribution (Marcora *et al.* 2008, 2013). Whether such differences are inherited in the following generations, contributing to population performance and persistence, remains to be tested. Among evidence that might provide clues about the ability of species to persist in particular areas after the occurrence of climate changes (Herman & Sultan 2011), persistence ability has been insufficiently explored in species of subtropical latitudes (Körner 2012).

Here we assessed whether the three principal woody species from this mountain system show differences among populations from different altitudes, which would be linked to their success in the early stages of their life cycle (*i.e.* seed germination as well as sapling attributes and performance). We hypothesised that the steep gradient in climate conditions across the altitudinal distribution of the three species promotes trait differences between populations of different altitudes that are inherited by the following generation. We used an experimental approach to test this hypothesis, which consisted in exposing seeds from different altitudes to three temperature regimes (temperatures from home and two other altitudinal belts) with the aim of assessing possible differences in germination responses (*e.g.* high germination rate at the home temperature). Thereafter, saplings were transplanted to a greenhouse to assess variations in their attributes or performance among altitudes.

## MATERIAL AND METHODS

### Study area and species

Seeds were collected from the Sierras Grandes de Córdoba, central Argentina. Seeder trees were selected along an altitudinal gradient from 900 m to 2700 m a.s.l., the highest altitude of this mountain range (Linderos road, 32°5'S, 64°9'W). Along the gradient, several climate variables are highly correlated with altitude, such as mean annual temperature, which decreases from 15.7 °C at 900 m a.s.l. to 7.4 °C at 2700 m a.s.l. ( $r^2 = -0.99$ ; Marcora *et al.* 2008), and annual precipitation, which increases from 600–1000 mm between 600 and 2200 m

a.s.l. ( $r^2 = 0.67$ ; Colladon 2014). These changing conditions lead to consistent environmental shifts along the gradient, with a significant decrease in soil temperature ( $r^2 = -0.97$ ) and increasing soil moisture ( $r^2 = 0.95$ ) towards the upper portions of the gradient (Pais-Bosch *et al.* 2012; Tecco *et al.* 2016), suggesting that climate stress along the altitudinal gradient results from a combination of opposing gradients of temperature and moisture (Tecco *et al.* 2016).

*Polylepis australis* Bitter (Rosaceae) is a tree endemic to the mountains of central and northwest Argentina (Kessler & Schmidt-Lebuhn 2006). It grows up to 16 m in height (Renison *et al.* 2013) and is self-compatible (Seltmann *et al.* 2009), with its pollen and one-seeded nutlets (hereafter referred to as seeds) being wind-dispersed. *Maytenus boaria* Molina (Celastraceae) is distributed across temperate regions of Chile and Argentina, including the central Argentine mountains, as well as Peru and Brazil (Donoso & Wendler 1985). This tree species reaches 5–8 m in height and produces insect-pollinated flowers with abundant pollen and bird-dispersed fruits (one- to two-seeded capsules; Cabello & Camelio 1985). The shrub *Escallonia cordobensis* (Kuntze) Hosseus (Escalloniaceae) is endemic to the Sierras de Córdoba and reaches 4 m in height. To the best of our knowledge, neither pollen nor seed dispersal of this species has been studied, but the white flower type suggests insect pollination.

### Seed collection

Mature fruits of the three species were collected from populations at different altitudes between February and April 2012. The altitudinal range covered for each species was constrained by the availability of accessible adult trees. Fruits of *P. australis* were collected from seven altitudes (900, 1200, 1500, 1800, 2100, 2400 and 2700 m a.s.l.), and those of *E. cordobensis* and *M. boaria* were sampled at four altitudes (900, 1200, 1500 and 1800 m a.s.l.). At each altitude, fruits were collected from a minimum of 20 seeder trees separated from each other by at least 100 m. Seeds of each species and each altitude were pooled. Although this methodology does not allow us to evaluate differences among individuals from the same altitude, seed sampling from several widely-spaced parents allows the inclusion of a much more representative sample of genotypes from each population, and hence should increase ecological realism of the results (Gianoli & Valladares 2012). Fruits of *P. australis* and *E. cordobensis* were stored in paper bags at room temperature; those of *M. boaria* were stored in wet sand at 4 °C until the start of germination experiments (December 2012).

### Germination tests and sapling traits

For the germination experiments, 40 seeds of *P. australis* and *M. boaria* and 50 seeds of *E. cordobensis* were put on filter paper in standard Petri dishes of 9-cm diameter and kept permanently moist with deionised water. Filter paper was replaced weekly to reduce infection by fungi. In the case of *M. boaria*, fruit coat delays germination (Cabello & Camelio 1985); hence, seeds were first scarified with 98% sulphuric acid for 15 min.

Germination experiments were conducted in germination chambers under an alternating photoperiod of 12 h warm white light and 12 h darkness. Three temperature treatments were programmed to simulate natural site conditions from the

highest to the lowest altitudinal belts of Sierras Grandes: 8/4 °C, 20/10 °C and 32/20 °C. These three thermoperiods mimic the day/night temperatures in spring at the higher, intermediate and lowest ends of the gradient, respectively (Marcora *et al.* 2008; Tecco *et al.* 2016). This experiment follows the reciprocal transplants approach, in which populations are exposed to the temperature of the altitudinal belt of origin and temperatures from other altitudinal belts (Kawecki & Ebert 2004). For each temperature treatment, six Petri dishes were used per altitude and per species ( $40 \times 6 \times 3 = 720$  seeds for *P. australis* and *M. boaria*;  $50 \times 6 \times 3 = 900$  seeds for *E. cordobensis*). Germinated seeds (with at least 2 mm radicle length) were removed every 2–3 days and kept in Petri dishes at 20/10 °C for the greenhouse experiments. Germination experiments ended after 60 days, following regular protocols (Baskin & Baskin 1998). At that time, germination under the cold temperature regime had not plateaued for *E. cordobensis* and *M. boaria*.

To characterise populations, 50 seeds per altitude and per species were subjected to a TTC test (1% triphenyltetrazolium chloride) to assess initial seed viability. Additionally, seed mass of *P. australis* and *M. boaria* was calculated by weighing 30 seeds per species and per altitude. Seed mass of *E. cordobensis* was measured in three groups of 200 seeds per altitude because the seeds are very small and light (<0.05 mg).

Between 3 and 4 weeks after germination, seedlings were transferred to plastic pots containing a compost–sand mixture (2:3) and cultivated at 22 °C and 60% relative humidity. Sapling traits and performance measurements (plant height, leaf chlorophyll concentration, leaf area and specific leaf area) were recorded on three dates (6, 12 and 18 months after planting). After sapling harvest (at 10 cm from ground level), leaf and aerial biomass were measured. Specifically, growth height (cm) was measured on the longest sapling shoots. Total leaf chlorophyll concentration was estimated using the SPAD502 chlorophyll meter; SPAD measurements were converted into chlorophyll concentrations ( $\mu\text{g}\cdot\text{cm}^{-2}$ ) using the general relationship provided in Coste *et al.* (2010). This trait was determined as an average of SPAD values from three leaves per individual. Leaf area (LA;  $\text{cm}^2$ ) and specific leaf area (SLA;  $\text{cm}^2\cdot\text{g}^{-1}$ ) were determined as the average of ten whole leaves from the middle region of the sapling, following standard protocols (Pérez-Harguindeguy *et al.* 2013), and leaf area was calculated using the Win FOLIA Pro S program (Regent Instruments, Canada). Sapling mortality of the three species was recorded between 6 and 18 months after planting in the greenhouse. The number of plants of each altitude and species used for trait measurements is indicated in the figures (see Results).

### Data analysis

Differences in seed mass between provenances were tested using univariate general linear models. Differences in germination success (%) and seed viability between altitudes and temperature treatments were analysed using generalised linear models (GLMs) with quasi-binomial error structure of data and logit link function. Altitude and temperature treatments were included as fixed factors, and interaction between factors was included to evaluate if altitudes show different responses to temperatures, *i.e.* if seeds from higher populations show higher germination success at a different temperature than

seeds from lower altitudes. Whenever appropriate, a DGC *a posteriori* test (Di Rienzo *et al.* 2016) was applied to evaluate significant differences between altitude and temperature treatments.

Variations in sapling mortality among altitudes were analysed with GLMs using a binary distribution and logit link function. We used live and dead saplings of each population as independent samples, so the response variable was binary (live/dead) and altitude was included as fixed factor.

Differences in sapling traits (height, LA, SLA, chlorophyll concentration, sapling and leaf dry biomass) among altitudes were analysed using GLM, with altitude as fixed factor. Some response variables were  $\log_{10}$  or square root-transformed to obtain normal distribution of residuals. In addition, we assessed the relationship of each sapling trait to altitude using linear regression and binary logistic regression for mortality. After visual inspection of the graphs, we added the term ‘altitude squared’ where necessary to determine whether variables had an optimum at intermediate altitudes. All statistical analyses were performed using Infostat (Di Rienzo *et al.* 2016) in R, and graphs were made in R 3.2.4 (R Development Core Team 2016).

## RESULTS

### Effects of altitude on germination response to temperature

Germination success of the three species differed among altitudes, with some showing different germination responses to temperature treatments (Table 1). For *P. australis*, germination success increased with increasing altitude (Fig. 1a), whereas for *M. boaria* and *E. cordobensis*, the highest germination percentages were recorded at intermediate altitude (1500 m a.s.l.; Fig. 1b, c).

Germination success of *P. australis* differed among altitudes, without statistically significant differences between temperature treatments or their interaction (Fig. 1a; Deviance<sub>A</sub> = 227.54, df = 6,  $P_A < 0.0001$ ; Deviance<sub>T</sub> = 8.14, df = 2,  $P_T = 0.056$ ; Deviance<sub>A+T</sub> = 23.12, df = 12,  $P_{A+T} = 0.17$ ). When germination success was analysed including interaction between altitude and temperature treatments alone, without the principal effects of both factors, the population from 2100 m a.s.l. showed significant differences in temperature treatments, *i.e.* germination success was lower at low temperatures than at high temperatures.

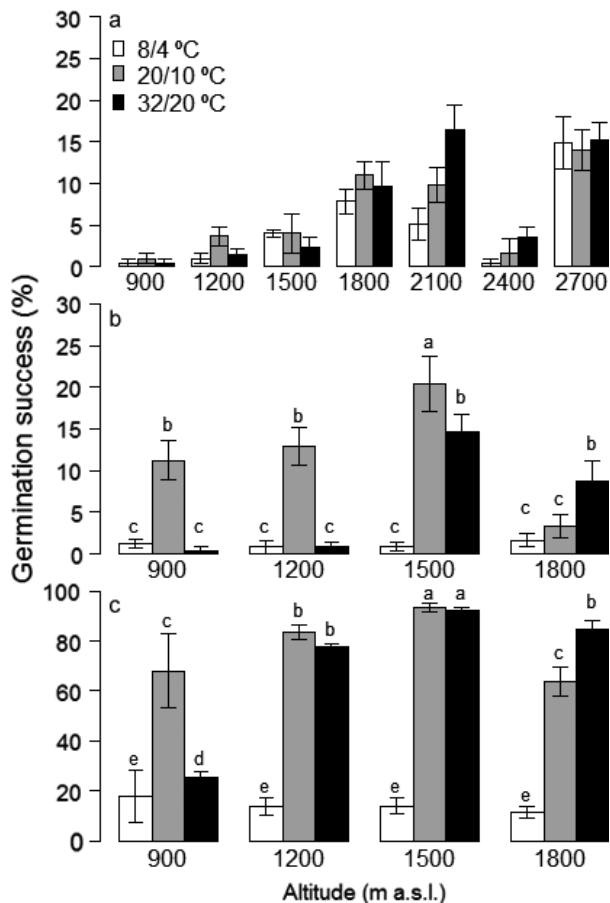
In *M. boaria* there were significant differences in germination success between temperature treatments for all altitudes (Fig. 1b; Deviance<sub>A</sub> = 46.47, df = 3,  $P < 0.0001$ ; Deviance<sub>T</sub> = 124.08, df = 2,  $P_T < 0.0001$ ; Deviance<sub>A+T</sub> = 58.35, df = 6,  $P_{A+T} < 0.0001$ ). Seeds from the lowest to intermediate altitude (*i.e.* 900 to 1500 m a.s.l.) showed higher germination success at intermediate temperatures (20/10 °C), whereas seeds from the highest altitude (1800 m a.s.l.) had higher germination success under warm conditions (32/20 °C; Fig. 1b). Under cold conditions, seeds from all four altitudes showed lower germination success than at higher temperatures.

In *E. cordobensis* significant differences in germination success were observed between temperature treatments at all altitudes (Fig. 1c; Deviance<sub>A</sub> = 167.19, df = 3,  $P_A < 0.0001$ ; Deviance<sub>T</sub> = 1319.09, df = 2,  $P_T < 0.0001$ ; Deviance<sub>A+T</sub> = 240.54, df = 6,  $P_{A+T} < 0.0001$ ). Populations from all altitudes showed lowest germination success under the coldest

**Table 1.** Variation in seed and sapling traits of the three study species among populations from different altitudes on 18-month-old saplings cultivated in the greenhouse. Test value shows F-values corresponding to ANOVA using GLM; analysed deviance values corresponding to GLM for mortality are shown. Degrees of freedom of general and GLM was 6 for *Polylepis australis* and 3 for *Maytenus boaria* and *Escallonia cordobensis*. The other columns summarise the explained proportion of variance (adjusted  $R^2$ ) in intraspecific variation of functional traits in linear regressions or binary logistic regression and slope for altitude and their squared term (–, negative; +, positive;  $\Omega$ , unimodal; U, u pattern). For mortality, Nagelkerke  $R^2$  is reported.

trait	<i>polylepis australis</i>				<i>maytenus boaria</i>				<i>escallonia cordobensis</i>			
	linear model	regression			linear model	regression			linear model	regression		
seed trait	test value	$R^2$	altitude		test value	$R^2$	altitude		test value	$R^2$	altitude	
seed mass	15.24***	0.31	+***	n***	49.81***	0.56	–***	ns	6.27*	0.70	–*	
sapling traits												
plant biomass	6.11***	0.062	+*	n**	4.57**	0.045	ns	ns	2.06 ns	0.014	+*	n*
height	8.08***	0.115	+**	n***	6.23***	0.03	+**	n**	0.14 ns	0	ns	ns
leaf area	4.38***	0.044	+*	n*	4.46**	0.052	ns	ns	2.07 ns	0	ns	ns
SLA	3.60**	0.047	–**	U**	0.60 ns	0	ns	ns	0.85 ns	0.001	ns	ns
leaf biomass	12.09***	0.118	+***	n***	4.44**	0.051	ns	ns	2.56 ns	0.002	ns	ns
chlorophyll	2.60*	0.021	+**	n*	1.01 ns	0.004	ns	ns	2.27 ns	0.024	ns	ns
mortality	27.31***	0.082	–*	U**	1.31 ns	0.01	ns		3.76 ns	0.31	ns	

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; ns,  $P > 0.05$ .



**Fig. 1.** Seed germination success (%; Mean  $\pm$  SE) in response to three different temperature treatments for (a) *Polylepis australis*, (b) *Maytenus boaria* and (c) *Escallonia cordobensis*. Letters indicate mean differences between altitudes  $\times$  temperature regimes (DGC,  $P < 0.05$ ).

temperature regime. Seeds from the highest altitude (1800 m a.s.l.) showed higher germination success at warm (32/20 °C) than at intermediate temperatures, whereas the opposite

pattern was observed for the lowest populations (900 m a.s.l.; Fig. 1c). Seeds from the two intermediate altitudes (1200 and 1500 m a.s.l.) did not differ in germination success between intermediate and warm temperature treatments (Fig. 1c).

Initial seed viability (assessed with TTC test) of *P. australis* and *M. boaria* was different among altitudes, whereas there were no differences for *E. cordobensis*. Seed viability was low in *P. australis* for populations from 900, 1200, 1500 and 2400 m a.s.l. (Deviance = 23.99,  $df = 6$ ,  $P < 0.001$ ), whereas in *M. boaria* the lowest seed viability was in the population from 1500 m a.s.l. (Deviance = 10.12,  $df = 3$ ,  $P = 0.018$ ). Seed mass of the three species varied along the altitudinal gradient (Table 1). *P. australis* exhibited a hump-shaped pattern in seed mass along the gradient (Fig. 2a), and seed mass of *M. boaria* and *E. cordobensis* decreased with increasing altitude (Figs 3a and 4a).

#### Effects of altitude on sapling traits and performance

We found significant differences in several sapling traits and performance components among altitudes for the three species grown in the greenhouse. These differences were consistent on the three sampling dates (*i.e.* 6-, 12- and 18-month-old saplings). Thus, for simplicity, we only report results for the 18-month-old saplings.

For *P. australis*, there were significant differences among altitudes in mortality, sapling dry biomass, height and chlorophyll concentration, as well as in leaf dry biomass, LA and SLA. In most cases, trait variations showed unimodal responses to altitude (Table 1, Fig. 2). The offspring from the lowest and highest sites of the altitudinal gradient had the highest mortality and attributes indicative of overall lowest growth performance (lowest sapling biomass, height and LA) despite their higher SLA (Fig. 2). In addition, significantly lower chlorophyll concentrations were found in saplings from lower altitudes (900 and 1200 m a.s.l.) than in those from higher altitudes.

In *M. boaria*, sapling dry biomass, height, leaf biomass and LA varied significantly among altitudes (Table 1). Attributes related to increased growth performance (*i.e.* high values for



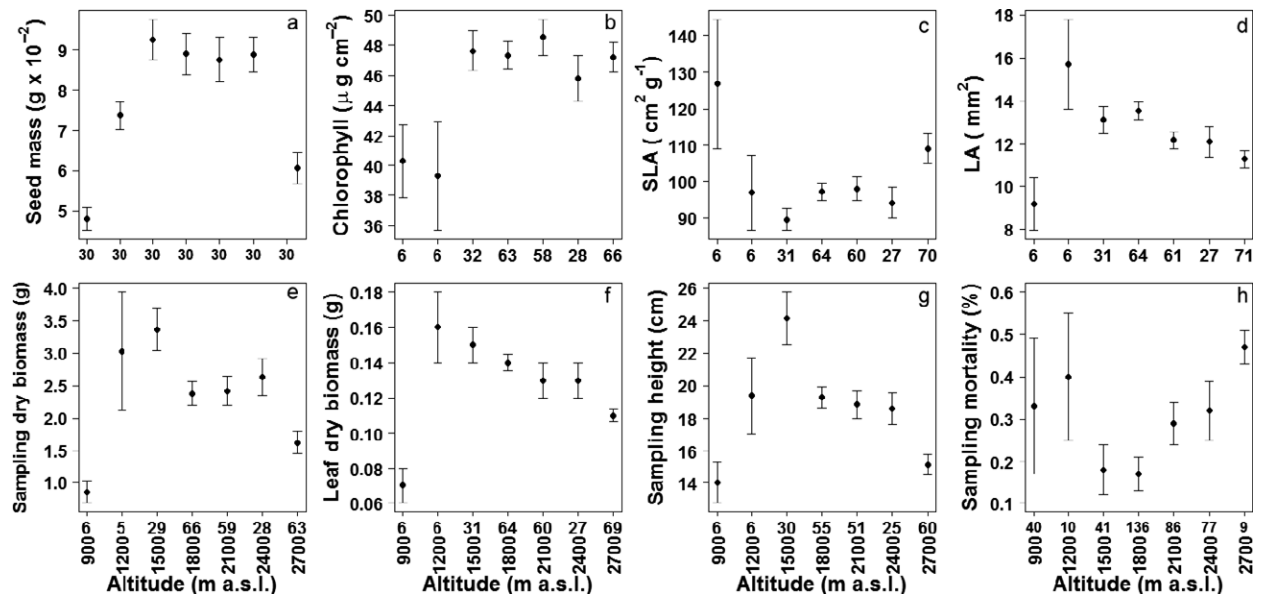


Fig. 2. Attribute variation in seeds from different altitudes and saplings grown in the greenhouse for 18 months measured for *Polyloplepis australis*. Means  $\pm$  SE. Number of replications (N) per elevation is indicated in each figure.

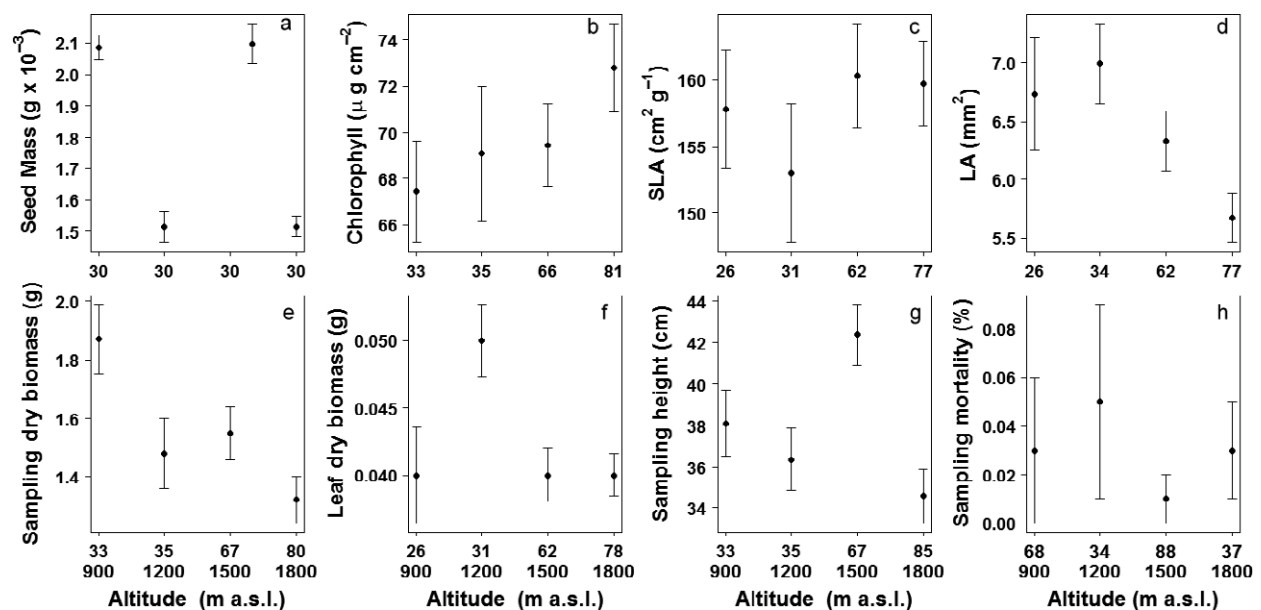


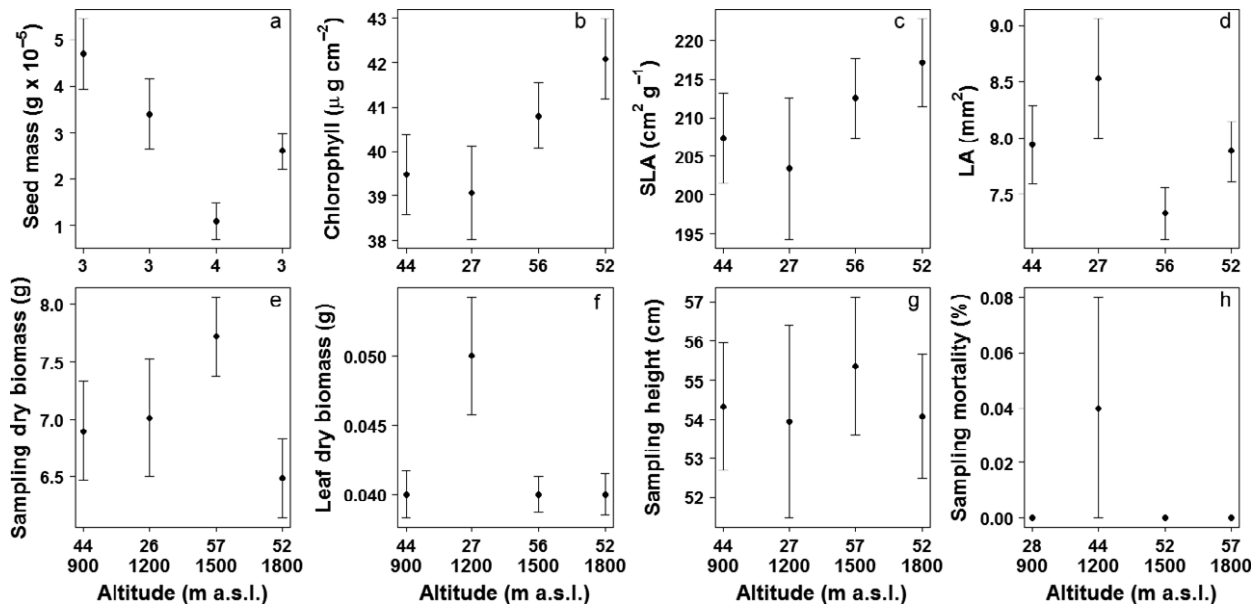
Fig. 3. Attribute variation in seeds from different altitudes and saplings grown in the greenhouse for 18 months measured for *Maytenus boaria*. Means  $\pm$  SE. Number of replications (N) per elevation is indicated in each figure.

sapling height and biomass, large leaves) were mostly observed in saplings from lower or intermediate altitudes, whereas the opposite was observed for saplings from higher altitudes (Fig. 3).

Saplings of *E. cordobensis* did not differ significantly in traits among altitudes (Table 1). However, sapling dry biomass showed a unimodal pattern, with the highest biomass in saplings from 1500 m a.s.l. and the lowest in saplings from 1800 m a.s.l. (Fig. 4).

## DISCUSSION

Our experimental approach shows that the main three woody species from the upper vegetation belt of the Sierras Grandes in central Argentina exhibit differences in germination, as well as in sapling attributes and performance among elevational provenances when grown under standard conditions in the greenhouse. These differences were in line with our hypothesis and suggest adjustments of populations in response to



**Fig. 4.** Attribute variation in seeds from different altitudes and saplings grown in the greenhouse for 18 months measured for *Escallonia cordobensis*. Means  $\pm$  SE. Seed mass of *E. cordobensis* shows three values per elevation; each value corresponds to 200 seeds (see Methods for details). Number of replications (N) per elevation is indicated.

environmental changes along an altitudinal gradient (Körner 2012; Grassein *et al.* 2014). Such adjustments might be adaptive when the offspring environment is predictable and similar to that of the maternal environment (Galloway & Etterson 2007; Bischoff & Müller-Schärer 2010; Donohue *et al.* 2010); however, those adjustments might hinder rapid population response to climate shifts (see below). In the Sierras Grandes, low temperatures and a short growing season would be the main constraints to seedling establishment at high altitudes, whereas decreasing soil moisture, increasing temperature and water stress would limit sapling establishment toward lower altitudes (Pais-Bosch *et al.* 2012; Marcora *et al.* 2013; Tecco *et al.* 2016).

#### Differences in germination success in response to temperature among seeds from different altitudes

For *M. boaria* and *E. cordobensis* highest germination success occurred at different temperatures among altitudes. For *P. australis* only the populations at 2100 m a.s.l. showed differences in germination response to temperature. Exposure of mother plants to certain environmental factors, *e.g.* temperature and photoperiod, can promote changes in their seeds, and those determinants (either genetic or non-genetic) in turn affect germination response and seedling establishment (reviewed in Baskin & Baskin 1998; Bischoff *et al.* 2006; Giménez-Benavides *et al.* 2007). Accordingly, the observed differences in germination response to temperature among populations of these species might show adaptations to local environmental conditions (Dunbabin & Cocks 1999; Galloway 2005; Donohue *et al.* 2010) that would favour recruitment under analogous conditions of the mother plants (see below).

Our results for *M. boaria* and *E. cordobensis* show that germination success in populations from the upper extreme of the species' distribution tends to improve with increasing

temperature, whereas germination of populations from lower altitudes seems to be boosted by cooler temperatures. Although these results may at first seem counterintuitive, they suggest an adaptive response to local environmental conditions for successful seedling establishment, through avoiding the constraints that characterise each extreme in the altitudinal gradient, *i.e.* germination will be stimulated by the most benign conditions. On the one hand, the patterns observed in upper populations are similar to those found for other species in cold environments; indeed, some populations avoid germination during or immediately after severe winters (*e.g.* Giménez-Benavides & Milla 2013) and germinate only at relatively high temperatures. For example, species from Northern Europe need higher relative temperatures for germination than Mediterranean species (Fenner & Thompson 2005), consistent with findings reported for Arctic species (Baskin & Baskin 1998). On the other hand, towards the warmer extreme of the species distribution range, drought is expected to be the main hazard for seedlings (Fenner & Thompson 2005; Engelbrecht *et al.* 2007; Giménez-Benavides *et al.* 2007), and reduced germination in response to high temperatures may preclude successful seedling establishment in populations during unfavourably dry conditions (*e.g.* Levine *et al.* 2008), thereby ensuring local persistence of peripheral populations (Giménez-Benavides *et al.* 2007).

In contrast, *P. australis* populations from different altitudes showed no differences in germination among temperature treatments, suggesting no local adaptation in germination response to temperature. Consequently, germination success of *P. australis* would be less affected by changes in temperature than that of the other two species. For *M. boaria* and *E. cordobensis*, germination success of populations might be favoured only under maternal environmental conditions, and might not be able to acclimate to rapid climatic shifts. As mentioned

above, the adaptive significance of such responses can be related to specific habitat requirements along the altitudinal distribution (Bradshaw 1965).

Germination success among populations of *P. australis* and *E. cordobensis* reached values similar to those assessed for their initial viability, but was lower for *M. boaria*. Possible changes in the requirement for cold stratification to break dormancy among populations should be assessed in *M. boaria* (Giménez-Benavides & Milla 2013). Finally, germination response of all three species does not seem to be affected by seed mass, as suggested by the different altitudinal patterns of each variable.

#### Differences in sapling attributes and performance among offspring of different altitudes

Populations from different altitudes grown in the greenhouse showed differences in sapling attributes and performance components. Such differences were likely due to irreversible physiological and morphological adjustments or local adaptation (Pigliucci *et al.* 2006; Herman & Sultan 2011; Körner 2012). In any case, the differences in sapling traits and performance components among altitudinal populations observed in this study are related to environmental conditions of mother plants, as suggested by the similar responses of plants under greenhouse conditions to those of their counterparts cultivated in the field across the altitudinal gradient (Marcora *et al.* 2013).

Most *P. australis* sapling traits that showed differences among altitudes followed a unimodal pattern, with attributes indicative of increased performance in saplings from intermediate altitudes (*i.e.* largest saplings with highest chlorophyll content and lowest mortality). Similarly, the tallest sapling of *M. boaria* and the largest sapling biomass of *E. cordobensis* correspond to populations from an intermediate altitude (1500 m a.s.l.), also describing a unimodal pattern in response to elevation. Accordingly, a unimodal response to altitude was found for different components of performance of adult trees of *P. australis* growing along the altitudinal gradient (Marcora *et al.* 2008) and for saplings of *P. australis* and *E. cordobensis* grown under field conditions (Marcora *et al.* 2013).

Furthermore, saplings of all three species from both extremes of the species altitudinal distribution seem to be adjusted to tolerate more stressful conditions that prevail at the two ends of this gradient due to the opposing gradients of temperature and moisture (*i.e.* lower temperatures at the upper end and water stress at the lower end; Marcora *et al.* 2013; Tecco *et al.* 2016). This adjustment would have negative consequences on performance due to trade-offs in strategies for resource acquisition and use (Wright *et al.* 2005; Reich 2014; Stahl *et al.* 2014). Accordingly, traits from the two extremes of the altitudinal gradient appear to be governed by security rather than vigour (Körner 2012). Reduced sapling growth in *M. boaria* and *E. cordobensis* and reduced leaf size and biomass in *P. australis* from high altitudes might be induced either by a shorter growing season or by the direct influence of low temperatures and high radiation stress endured by seeder trees (Cornelissen *et al.* 2003; Körner 2012; Stahl *et al.* 2014). These intraspecific variations in response to altitude were assumed to be an adaptation, consisting of enhanced photosynthesis at lower temperatures and increased resistance to freezing temperatures (Goldstein *et al.* 1985). Likewise, the decreased performance of populations from

lower altitudes would be related to water stress, which is a strong selective pressure at the lower limit of the species distribution (Cornelissen *et al.* 2003; Engelbrecht *et al.* 2007; Jump *et al.* 2009; Normand *et al.* 2009; Alberto *et al.* 2013). A similar influence of water stress on tree performance at the lower limit was found for other mountain species, such as *Polylepis sericea* (Colmenares-Arteaga *et al.* 2005), *Fagus sylvatica*, *Populus tremuloides*, *Pinus ponderosa* and *Picea rubens* (cited in Körner 2012).

While both SLA and chlorophyll concentration are usually used as performance indicators (*e.g.* Cornelissen *et al.* 2003), in our study neither trait matched variations in sapling performance. The direction of SLA variation recorded for *P. australis* saplings in our study was unexpected, in that the unimodal pattern of sapling performance was not coupled to a common SLA trend but to an opposite pattern, with the lowest SLA being recorded for saplings from intermediate altitudes (*i.e.* those with highest sapling and leaf biomass, LA and height). This SLA trend could be related to the unimodal reduction in LA toward the altitudinal limit of the species distribution. A reduction in LA without an increase in leaf thickness (not measured here) results in a reduction in leaf mass, which in turn can result in a surprisingly high SLA (Witkowski & Lamont 1991; Wilson *et al.* 1999). Another explanation for the observed pattern is that high SLA values under stressful conditions might facilitate rapid growth responses under short favourable climate periods (Gotsch *et al.* 2010), such as wet periods at lower altitudes or warm temperatures at higher altitudes. However, no such rapid response was recorded in our study under the favourable greenhouse conditions during the 18 months of our experiment. Although SLA is a commonly used trait to explain interspecific differences in seedling performance (Cornelissen *et al.* 2003), our results show the need to be cautious in drawing conclusions, at least in this mountain system, since variation in SLA among populations of *P. australis* would not correctly reflect variations in sapling performance, as discussed by Sánchez-Gómez *et al.* (2008) and Stahl *et al.* (2014).

Sapling biomass is usually related to chlorophyll concentration because this is an integrative measure of overall leaf functional status associated with structure, N and pigment concentrations (Kitajima & Hogan 2003; Díaz-Espejo *et al.* 2007; Martin *et al.* 2007). However, in our study, while *P. australis* saplings from intermediate and higher altitudes showed no differences in chlorophyll concentration, they did show differences in height and biomass. Similarly, sapling biomass in *M. boaria* and *E. cordobensis* populations varied with altitude, with no differences in chlorophyll concentration. At higher altitudes, increased allocation to underground structures (Hertel & Wesche 2008) or a trade-off between relative growth rate and frost resistance (Körner 2012) might explain the lack of relationship between the two traits. Additionally, variation in leaf density and thickness can affect photosynthesis rates (Sack *et al.* 2013). Dense leaves can reduce photosynthesis by decreasing assimilative leaf compounds and limiting diffusion of CO<sub>2</sub> and penetration of light into the leaf, thereby modifying the relationship between photosynthesis and SLA (Niinemets 1999).

Remarkably, seed mass might also affect early seedling development (Fenner & Thompson 2005). The initial differences in seedlings among seeds of different size can achieve parity after different periods, such as 105 or 160 days after germination (Fenner & Thompson 2005). In this study, the 18-month

period under greenhouse conditions seemed long enough to overcome this potential effect of seed mass, at least for *M. boaria* and *E. cordobensis*. However, this effect cannot be discarded for *P. australis*, since seed mass and some sapling traits followed similar altitudinal patterns. Future studies should include the influence of seed mass on sapling traits.

Finally, genetic differences between traits described for many woody species occurring at high altitudes are a result of directional selection, which may promote populations from such high altitudinal limits to exhibit lower phenotypic plasticity in growth and morphological traits in response to temperature changes than populations from low altitudes (Debat & David 2001; Vitassé *et al.* 2013). In our study, saplings of all three species from altitudinal extremes tended to exhibit the overall poorest performance (at least in terms of biomass). Indeed, saplings from lower and normally drier altitudes, and those from upper and normally cooler altitudes were not able to take advantage of the wetter and milder environment of the greenhouse. Further studies should be conducted to determine whether contrasting environmental stress factors at the altitudinal distribution extremes in such systems might reduce phenotypic plasticity of these woody species, which need to be assessed including intra-population variability.

## CONCLUSION

Populations in rapidly changing environments can persist by either adapting to conditions in their usual habitat or migrating to other areas; otherwise they might become locally extinct

(Aitken *et al.* 2008; Nicotra *et al.* 2010). Our findings in relation to the very early phases of the plant life cycle suggest that both germination success and sapling performance of the main woody species of the Sierras Grandes Mountains appear to be well adjusted to their current maternal environments, which might become detrimental under climate warming scenarios (Herрман & Sultan 2011), especially for *P. australis*, which seems to have a broader number of adjusted sapling traits. Additional studies are necessary to explore the influence of differences among individuals in the patterns observed and the adaptive value of the observed intraspecific variability in our study species, which could be evaluated through reciprocal transplants and specific genetic studies (González-Martínez *et al.* 2006).

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## REFERENCES

- Aitken S.N., Yeaman S., Holliday J.A., Wang T., Curtis-McLane S. (2008) Adaptation, migration or extinction: climate change outcomes for tree populations. *Evolutionary Applications*, **1**, 95–111.
- Alberto F.J., Aitken S.N., Alía R., González-Martínez S.C., Hänninen H., Kremer A., Lefevre F., Lenormand T., Yeaman S., Whetten R., Savolainen O. (2013) Potential for evolutionary responses to climate change—evidence from tree populations. *Global Change Biology*, **19**, 1645–1661.
- Baskin C.C., Baskin J.M. (1998) *Seeds: ecology, biogeography, and evolution of dormancy and germination*. Academic Press, San Diego, CA, USA, 665 pp.
- Beniston M. (2003) Climatic change in mountain regions: a review of possible impacts. *Climatic Change*, **59**, 5–31.
- Bischoff A., Müller-Schärer H. (2010) Testing population differentiation in plant species – how important are environmental maternal effects. *Oikos*, **119**, 445–454.
- Bischoff A., Vonlanthen B., Steinger T., Müller-Schärer H. (2006) Seed provenance matters — Effects on germination of four plant species used for ecological restoration. *Basic and Applied Ecology*, **7**, 347–359.
- Bradshaw A.D. (1965) Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, **13**, 115–155.
- Bresson C.C., Vitassé Y., Kremer A., Delzon S. (2011) To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech? *Tree Physiology*, **31**, 1164–1174.
- Byars S.G., Papst W., Hoffmann A.A. (2007) Local adaptation and cgradient selection in the alpine plant, *Poa hiemata*, along a narrow altitudinal gradient. *Evolution*, **61**, 2925–2941.
- Cabello A., Camelio M.E. (1985) Germinación de semillas de maiten (*Maytenus boaria*) y producción de plantas en vivero. *Ciencias Forestales*, **11**, 3–17.
- Colladon L. (2014) *Anuario Pluviométrico 1992–2012. Cuenca del Río San Antonio, Sistema del Río Suquia, Provincia de Córdoba*. Instituto Nacional del Agua y del Ambiente (INAA) y Centro de Investigaciones de la Región Semiárida (CIRSA), Córdoba, Argentina, 86 pp.
- Colmenares-Arteaga M., Rada F., Luque R. (2005) Anatomía foliar de *Polylepis sericea* Wedd. (Rosaceae) a dos altitudes en los Altos Andes venezolanos. *Plantula*, **3**, 141–148.
- Cornelissen J.H.C., Lavorel S., Garnier E., Diaz S., Buchmann N., Gurvich D., Reich S.P.B., ter Steege H., Morgan H.D., van der Heijden M.G.A., Pausas J.G., Poorter H. (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, **51**, 335–380.
- Coste S., Baraloto C., Leroy C., Marcon É., Renaud A., Richardson A.D., Roggy J.-C., Schimann H., Uddling J., Hérault B. (2010) Assessing foliar chlorophyll contents with the SPAD-502 chlorophyll meter: a calibration test with thirteen tree species of tropical rainforest in French Guiana. *Annals of Forest Science*, **67**, 607.
- Davis M.B., Shaw R.G. (2001) Range shifts and adaptive responses to Quaternary climate change. *Science*, **292**, 673–679.
- Debat V., David P. (2001) Mapping phenotypes: canalization, plasticity and developmental stability. *Trends in Ecology & Evolution*, **16**, 555–561.
- Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. (2016) Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba, Argentina. Available from <http://www.infostat.com.ar>
- Díaz-Espejo A., Nicolás E., Fernández J.E. (2007) Seasonal evolution of diffusional limitations and photosynthetic capacity in olive under drought. *Plant, Cell and Environment*, **30**, 922–933.
- Donohue K., Rubio de Casas R., Burghardt L., Kovach K., Willis C.G. (2010) Germination, postgermination adaptation, and species ecological ranges. *Annual Review of Ecology, Evolution and Systematics*, **41**, 293–319.
- Donoso C., Wender J. (1985) Antecedentes fenológicos y gen ecológicos de *Maytenus boaria*. *Bosque*, **6**, 93–99.
- Dunbabin M.T., Cocks P.S. (1999) Ecotypic variation for seed dormancy contributes to the success of capeweed (*Arctotheca calendula*) in Western Australia. *Crop and Pasture Science*, **50**, 1451–1458.
- Elsen P.R., Tingley M.W. (2015) Global mountain topography and the fate of montane species under climate change. *Nature Climate Change*, **5**, 772–776.
- Engelbrecht B.M., Comita L.S., Condit R., Kursar T.A., Tyree M.T., Turner B.L., Hubbell S.P. (2007) Drought sensitivity shapes species distribution patterns in tropical forests. *Nature*, **447**, 80–82.
- Fenner M., Thompson K. (2005) *The ecology of seeds*. Cambridge University Press, Cambridge, UK, 263 pp.
- Frei E.R., Ghazoul J., Matter P., Heggli M., Pluess A.R. (2014) Plant population differentiation and climate change: responses of grassland species along an elevational gradient. *Global Change Biology*, **20**, 441–455.



- Galloway L.F. (2005) Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytologist*, **166**, 93–100.
- Galloway L.F., Etterson J.R. (2007) Transgenerational plasticity is adaptive in the wild. *Science*, **318**, 1134–1136.
- García-Fernández A., Iriondo J.M., Bartels D., Escudero A. (2013) Response to artificial drying until drought-induced death in different elevation populations of a high-mountain plant. *Plant Biology*, **15** (s1), 93–100.
- Giam X., Bradshaw C.J., Tan H.T., Sodhi N.S. (2010) Future habitat loss and the conservation of plant biodiversity. *Biological Conservation*, **143**, 1594–1602.
- Gianoli E., Valladares F. (2012) Studying phenotypic plasticity: the advantages of a broad approach. *Biological Journal of the Linnean Society*, **105**, 1–7.
- Giménez-Benavides L., Milla R. (2013) Comparative germination ecology of two altitudinal vicariant *Saxifraga* species endemic to the north of Spain. *Plant Biology*, **15**, 593–600.
- Giménez-Benavides L., Escudero A., Iriondo J. (2007) Local adaptation enhances seedling recruitment along an altitudinal gradient in a high mountain Mediterranean plant. *Annals of Botany*, **99**, 723–734.
- Goldstein G., Rada F., Azócar A. (1985) Cold hardiness and supercooling along an altitudinal gradient in Andean giant rosette species. *Oecologia*, **68**, 147–152.
- González-Martínez S.C., Krutovsky K.V., Neale D.B. (2006) Forest-tree population genomics and adaptive evolution. *New Phytologist*, **170**, 227–238.
- Gotsch S.G., Powers J.S., Lerdau M.T. (2010) Leaf traits and water relations of 12 evergreen species in Costa Rican wet and dry forests: patterns of intra-specific variation across forests and seasons. *Plant Ecology*, **211**, 133–146.
- Gottfried M., Pauli H., Futschik A., Akhalkatsi M., Barančok P., Alonso J.L.B., Coldea G., Dick J., Erschbamer B., Fernández Calzado M.R., Kazakis G., Krajčič J., Larsson P., Mallaun M., Michelsen O., Moiseev D., Moiseev P., Molau U., Merzouki A., Nagy L., Nakhutsrishvili G., Pedersen B., Pelino G., Puscas M., Rossi G., Stanisci A., Theurillat J.-P., Tomaselli M., Villar L., Vittoz P., Vogiatzakis I., Grabherr G. Continent-wide response of mountain vegetation to climate change. *Nature Climate Change*, **2**, 111–115.
- Grassein F., Lavorel S., Till-Bottraud I. (2014) The importance of biotic interactions and local adaptation for plant response to environmental changes: field evidence along an elevational gradient. *Global Change Biology*, **20**, 1452–1460.
- Herman J.J., Sultan S.E. (2011) Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. *Frontiers in Plant Science*, **2**, 1–10.
- Hertel D., Wesche K. (2008) Tropical moist *Polylepis* stands at the treeline in East Bolivia: the effect of altitude on stand microclimate, above- and below-ground structure, and regeneration. *Trees*, **22**, 303–315.
- Jump A.S., Mátyás C., Peñuelas J. (2009) The altitude-for-latitude disparity in the range retractions of woody species. *Trends in Ecology & Evolution*, **24**, 694–701.
- Kawecki T.J., Ebert D. (2004) Conceptual issues in local adaptation. *Ecology Letters*, **7**, 1225–1241.
- Kessler M., Schmidt-Lebuhn A.N. (2006) Taxonomical and distributional notes on *Polylepis* (Rosaceae). *Organisms, Diversity & Evolution*, **6**, 67–69.
- Kitajima K., Hogan K.P. (2003) Increases of chlorophyll a/b ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. *Plant, Cell and Environment*, **26**, 857–865.
- Körner C. (1999) *Alpine plant life: functional plant ecology of high mountain ecosystems*. Springer, New York, NY, USA, 345 pp.
- Körner C. (2012) *Alpine treelines: functional ecology of the global high altitude tree limits*. Springer, Basel, Switzerland, 220 pp.
- Levine J.M., McEachern A.K., Cowan C. (2008) Rainfall effects on rare annual plants. *Journal of Ecology*, **96**, 795–806.
- Marcora P.I., Hensen I., Renison D., Seltmann P., Wesche K. (2008) The performance of *Polylepis australis* trees along their entire altitudinal range: implications of climate change for their conservation. *Diversity and Distributions*, **14**, 630–636.
- Marcora P.I., Renison D., País-Bosch A.I., Cabido M.R., Tecco P.A. (2013) The effect of altitude and grazing on seedling establishment of woody species in central Argentina. *Forest Ecology and Management*, **291**, 300–307.
- Martin R.E., Asner G.P., Sack L. (2007) Genetic variation in leaf pigment, optical and photosynthetic function among diverse phenotypes of *Metrosideros polymorpha* grown in a common garden. *Oecologia*, **151**, 387–400.
- Nicotra A.B., Atkin O.K., Bonser S.P., Davidson A.M., Finnegan E.J., Mathesius U., Poot P., Purugganan M.D., Richards C.L., Valladares F., van Kleunen M. (2010) Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, **15**, 684–692.
- Niinemetts Ü. (1999) Components of leaf dry mass per area – thickness and density – alter leaf photosynthetic capacity in reverse directions in woody plants. *New Phytologist*, **144**, 35–47.
- Normand S., Treier U.A., Randin C., Vittoz P., Guisan A., Svenning J.C. (2009) Importance of abiotic stress as a range-limit determinant for European plants: insights from species responses to climatic gradients. *Global Ecology and Biogeography*, **18**, 437–449.
- País-Bosch A.I., Tecco P.A., Funes G., Cabido M. (2012) Efecto de la temperatura en la regeneración de especies leñosas del Chaco Serrano e implicancias en la distribución actual y potencial de bosques. *Boletín de la Sociedad Argentina de Botánica*, **47**, 401–410.
- Pérez-Harguindeguy N., Díaz S., Garnier E., Lavorel S., Poorter H., Jaureguierry P., Bret-Harte M.S., Cornwell W.K., Craine J.M., Gurvich D.E., Urcelay C., Veneklaas E.J., Reich P.B., Poorter L., Wright I.J., Ray P., Enrico L., Pausas J.G., de Vos A.C., Buchmann N., Funes G., Quétier F., Hodgson J.G., Thompson K., Morgan H.D., ter Steege H., van der Heijden M.G.A., Sack L., Blonder B., Poschlod P., Vaieretti M.V., Conti G., Staver A.C., Aquino S., Cornelissen J.H.C. (2013) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, **61**, 167–234.
- Pigliucci M., Murren C.J., Schlichting C.D. (2006) Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology*, **209**, 2362–2367.
- Pimm S.L., Jenkins C.N., Abell R., Brooks T.M., Gittleman J.L., Joppa L.N., Raven P.H., Roberts C.M., Sexton J.O. (2014) The biodiversity of species and their rates of extinction, distribution, and protection. *Science*, **344**, 1246752.
- R Development Core Team. (2016) *R: A Language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from URL: <http://www.R-project.org>.
- Reich P.B. (2014) The world-wide ‘fast–slow’ plant economics spectrum: a traits manifesto. *Journal of Ecology*, **102**, 275–301.
- Renison D., Cuyckens G.A.E., Pacheco S., Guzmán G., Grau H.R., Marcora P., Robledo G., Cingolani A.M., Domínguez J., Landi M., Bellis L., Hensen I. (2013) Distribución y estado de conservación de las poblaciones de árboles y arbustos del género *Polylepis* (Rosaceae) en las montañas de Argentina. *Ecología Austral*, **23**, 27–36.
- Sack L., Scoffoni C., John G.P., Poorter H., Mason C.M., Mendez-Alonso R., Donovan L.A. (2013) How do leaf veins influence the worldwide leaf economic spectrum? Review and synthesis. *Journal of Experimental Botany*, **64**, 4053–4080.
- Sánchez-Gómez D., Zavala M.A., Valladares F. (2008) Functional traits and plasticity linked to seedlings’ performance under shade and drought in Mediterranean woody species. *Annals of Forest Science*, **65**, 311.
- Schöb C., Armas C., Guler M., Prieto I., Pugnaire F.I. (2013) Variability in functional traits mediates plant interactions along stress gradients. *Journal of Ecology*, **101**, 753–762.
- Seltmann P., Cocucci A., Renison D., Cierjacks A., Hensen I. (2009) Mating system, outcrossing distance effects and pollen availability in the wind-pollinated treeline species *Polylepis australis* BITT. (Rosaceae). *Basic and Applied Ecology*, **10**, 52–60.
- Stahl U., Reu B., Wirth C. (2014) Predicting species’ range limits from functional traits for the tree flora of North America. *Proceedings of the National Academy of Sciences USA*, **111**, 13739–13744.
- Tecco P.A., País-Bosch A.I., Funes G., Marcora P., Zeballos S.R., Cabido M., Urcelay C. (2016) Mountain invasions on the way: are there climatic constraints for the expansion of alien woody species along an elevation gradient in Argentina? *Journal of Plant Ecology*, **9**, 380–392.
- Thuiller W., Albert C., Araujo M.B., Berry P.M., Cabeza M., Guisan A., Hickler T., Midgley G.F., Paterson J., Schurr F., Sykes M.T., Zimmermann N.E. (2008) Predicting global change impacts on plant species’ distributions: Future challenges. *Perspectives in Plant Ecology, Evolution and Systematics*, **9**, 137–152.
- Valladares F., Matesanz S., Guilhaumon F., Araujo M.B., Balaguer L., Benito-Garzón M., Cornwell W., Gianoli E., van Kleunen M., Naya D.E., Nicotra A.B., Poorter H., Zavala M.A. (2014) The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters*, **17**, 1351–1364.
- Vitassé Y., Hoch G., Randin C.F., Lenz A., Kollas C., Scheepens J.F., Körner C. (2013) Altitudinal adaptation and plasticity in seedling phenology of temperate deciduous tree species. *Oecologia*, **171**, 663–678.
- Wilson P., Thompson K., Hodgson J.G. (1999) Specific leaf area and dry leaf matter content as alternative predictors of plant strategies. *New Phytologist*, **143**, 155–162.
- Witkowski E.T.F., Lamont B.B. (1991) Leaf specific mass confounds leaf density and thickness. *Oecologia*, **88**, 486–493.
- Wright I.J., Reich P.B., Cornelissen J.H., Falster D.S., Garnier E., Hikosaka K., Lamont B.B., Lee W., Oleksyn J., Osada N., Poorter H., Villar R., Warton D.I., Westoby M. (2005) Assessing the generality of global leaf trait relationships. *New Phytologist*, **166**, 485–496.