Morphological and phenological differences in *Nothofagus pumilio* from contrasting elevations: Evidence from a common garden

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Abstract   We tested the hypothesis that contrasting elevations select distinct growth patterns and vegetative phenology in *Nothofagus pumilio*, a winter deciduous tree that dominates mountain forests of Patagonia. Analysis of saplings maintained under common-garden conditions for 4 years showed a significant decrease in shoot annual growth, leaf size, and a delay in bud-break, and leaf expansion with increased elevation of their site of origin. Rapid gain in height seems to be advantageous at low elevation in such light-demanding species. Lower stature high-elevation plants have wider branching angles and greater branching ratios (number of branches/number of internodes) than low-elevation plants. Compact growth at high elevation may be related to strong winds and irradiance. Plants from different elevations had distinct growth patterns during the common-garden experiment. This could be of importance in Mediterranean-climate areas characterized by highly unpredictable precipitation regimes. Also, liberation of growth-suppressed seedlings may follow different environmental signals in low- and high-habitats, which might explain such time-dependent responses to optimal conditions under cultivation. While these greenhouse-grown *N. pumilio* saplings showed heritable differences in plant architectural traits and leafing phenology, it was not clear how the genotypes characteristic of particular elevations would respond to longer growing seasons such as those predicted under global warming.

Key words: environmental cue, genetic basis, Nothofagaceae, South America.

INTRODUCTION

Because of the sessile nature of land plants, their features reflect adaptation to the local environment. This is particularly relevant in species with limited seed dispersal growing in habitats under strong selective pressures. Changing physical conditions of the environment, such as those along steep elevational gradients, can exert diversifying selection on plants a few hundred meters apart. Also phenological differences may arise that impose severe restrictions on gene flow and reinforce genetic differentiation among local populations of widespread plants. Several greenhouse experiments have documented genetically based variation in plant traits with elevation, although only a few studies have analysed the effects of elevation on phenological rhythms (Housman *et al.* 2002; Stinson 2004; references therein). Phenological rhythms of the vegetative stages and architectural features of the dominant tree species inhabiting high-elevation environments are intimately related to plant growth. Thus understanding these rhythms is important in predicting potential responses to climate change.

In southern South America, *Nothofagus* (southern beech) trees are characteristic elements of temperate forests. The plant architecture of the *Nothofagus* species has been studied exclusively on plants growing under natural conditions (Raffaele *et al.* 1998; Puntieri *et al.* 1999, 2003; Passo *et al.* 2002). These studies have shown that growth parameters are species-specific and time-dependent (Raffaele *et al.* 1998). However, the distinction between adaptive variation and plasticity on morphological features of *Nothofagus* is still a matter of speculation. Moreover, variation in phenological patterns along an elevation gradient has only been measured in the field (Rusch 1993; Barrera *et al.* 2000; Díaz 2004) but whether the variation observed was genetically or environmentally based was unknown.

The aim of this study was to analyse size and shape components of plant architecture, leaf traits, and phenological stages during early leaf expansion in populations of *Nothofagus pumilio* (Poepp. et Endl.) Krasser from contrasting elevations under common garden conditions. We tested the hypothesis that distinct physical settings at contrasting elevations exert diversifying selection pressures that may operate at each stage of the life cycle, including seedlings, generating heritable variation in those traits. This extends a set of

studies on genetic differentiation on various characteristics of *N. pumilio* along altitudinal gradients in northern Patagonia, Argentina (Premoli 2003, 2004).

Study species

Nothofagus pumilio, common name 'lenga', is a winter deciduous tree that dominates high-elevation forests of southern Argentina and Chile. It is distributed from approximately 35° to 55°S on the island of Tierra del Fuego. At the northern end of its range, *N. pumilio* occurs from 1000 to 1600 m a.s.l and shows marked structural and functional variation with increasing elevation, such as a consistent decrease in leaf size and growth period (Rusch 1993). As the altitude of *N. pumilio* habitats increases, physical conditions change, such as a decrease in soil temperature and the period with frost-free soils, and an increase in total annual precipitation and percentage of snowfall (Barrera *et al.* 2000). These result in strong phenological differences, such as a delay of bud swelling and leaf initiation of about 5–9 days with each increase of 100 m in elevation (Barrera *et al.* 2000). Isozyme data show that low elevation populations maintain higher levels of polymorphism, and there are significant restrictions of gene flow between populations occupying the upper- and lower-most distributional belts within a given mountain range (Premoli 2003). Furthermore, in high elevation *N. pumilio* show a greater photosynthetic rate as a heritable response to nitrogen-poor volcanic soils, whereas the use of water appears to be plastic (Premoli & Brewer 2007).

METHODS

Source populations and seedling collection

The study site was at 41°12′S and 71°20′W, 10 km from Bariloche city, within Nahuel Huapi National Park. Source populations were located on the western slopes of Cerro Blanco, in the ecotone between humid temperate forest and the Patagonian steppe. Annual precipitation is approximately 1000 mm, concentrated in autumn–winter as rain or snow, and the mean temperature is 5–6°C (Kitzberger *et al.* 2005). Temperature decreases at an approximate rate of 0.5°C/100 m resulting in mean temperatures of 3–4°C at approximately 1500 m (Rusch 1993). Collecting sites were single-species, old-growth erect forest of *N. pumilio* undergoing gap-phase regeneration. Dominant *N. pumilio* trees at the low elevation site were up to 26–28 m tall, they can attain 80–120 cm in diameter at breast height (d.b.h.), and trees can live for at least 280 years (Heinemann *et al.* 2000). Field data indicate that tree height and d.b.h. of mature erect *N. pumilio* decrease with elevation almost three-fold (Barrera *et al.* 2000).

Nothofagus pumilio grows on Andisols, which are mainly derived from volcanic ash (Singer & Morello 1960; Veblen & Ashton 1979). These soils have a high capacity to retain P and to stabilise organic matter, which often leads to N and P deficiencies (Mazzarino *et al.* 1998). Unpublished data from the study site indicate the lack of available organogenic elements in the soil, which is particularly critical at higher elevations where organic C and total N can be as low as 2.8% and 0.11%, respectively (A.C. Premoli, Universidad del Comahue) compared to 17% and 0.80% at lower elevations, respectively (M.J. Mazzarino, Universidad del Comahue). At these high elevations *N. pumilio* individuals, particularly those growing near the timberline are dwarf plants with small sclerophyllous leaves, an organization expected in plants living under mineral-poor conditions (Larcher 1995).

In November during the austral growing season 1999–2000, we collected 200 randomly selected seedlings that had germinated under natural conditions from each of two elevations. Field collection of seedlings avoids limitations of reduced germination rates of *N. pumilio* under experimental conditions (Premoli 2004). Seedlings were harvested from 1100 and 1540 m a.s.l. at Chall Huaco valley. The high elevation site is located below the timberline. Low-elevation plants were, on average, 3 years younger than high-elevation ones at the time of sampling (mean ± SD; 5.1 ± 1.0 and 8.3 ± 2.1 years for low- and high-elevation plants, respectively; $t_{118} = -10.6$, $P < 0.01$). Nevertheless, sampled plants from different elevations were of similar size (4.4 ± 3.1 and 4.7 ± 2.2 cm for low- and high-elevation plants, respectively; $t_{118} = -0.68$, $P = 0.5$).

Common garden

Seedlings were collected in trays containing their own soil to avoid transplantation stress. Once in the greenhouse, plants were separated from soil, their roots were washed, and seedlings were individually planted in pots.

Plants were cultivated for 4 years under homogeneous conditions in a naturally-lighted greenhouse at Laboratorio Ecotono, Universidad Nacional del Comahue, Bariloche, at 876 m a.s.l. Seedlings were tagged and planted in individual pots of increasing size from 1 to 3 L throughout the 4-year experiment using a commercial mixture of 80% soil/20% humus. They were homogenously watered and fertilized regularly. Plants were randomized within the greenhouse and periodically rotated to minimize differences due to microenvironmental effects.

Architectural measurements

The geometry of tree architecture was described by three branch characteristics: angle relative to the main axis A1, type as determined by bud outgrowth and fate (hereafter branching probability), and length (Honda *et al.* 1997 and references therein). We took these measures on leafless individuals during late winter from August to September 2004 before the growing period of that year started.

Morphological features of *N. pumilio* were quantified on 60 randomly selected plants from each provenance. At the time of measurement, a total of four growing periods had elapsed under common garden conditions, e.g. austral summers 2000, 2001, 2002, and 2003. Previous studies on *N. pumilio* (Raffaele *et al.* 1998; Puntieri *et al.* 1999) indicated that each axis develops only one Growth Unit (GU) per year. Moreover, GU boundaries can be identified by several markers: (i) short internodes; (ii) cataphylls; and (iii) change in stem colour and texture between the older and the younger GU (Puntieri *et al.* 1999). We determined the age of each individual by identifying and counting of GUs on the main axis, which is defined as the longitudinal growth produced by the apical meristem bearing lateral axes (hereafter A1 and A2, respectively).

We recorded size variables for each GU on the main and >1 cm-long secondary axes that were produced during the 4-year common-garden experiment. Variation over time in GU morphology was measured within branching orders A1 and A2 to the nearest mm. Measurements on each axis were grouped according to the growing period when the corresponding axis was produced. In the case of A1, axis age was recorded from germination, whereas in the case of A2, axis age was counted from the inception from A1. Size variables at the axis level were: total length of A1 and A2, and number of internodes and branches of A1. The latter two variables were only measured on A1 because many plants had only produced short A2 at the time of measurement.

We quantified shape variables by branch angle and branching probability. Branch angle was calculated from measurements of the angle between the mother shoot A1 and the respective A2 shoots following Honda *et al.* (1997). Branching probability on A1 was obtained as the ratio of total number of branches/total number of internodes. At the time of measurement most plants had produced A2 branches. We restricted measurements of branch angle to those A2 branches >1 cm long while all A2 branches were considered in measures of branching probability.

Leaf traits

Leaf morphology was evaluated by measures of leaf size and shape of 10 expanded leaves that were col-lected from each of 50 plants per elevation during early October 2004. At the time of sampling, each plant carried at most 30–40 leaves. Also, given *a priori* striking architectural differences between low- and high-elevation plants, we followed a random non-destructive sampling scheme and collected leaves from different positions within each plant to represent a sample of the entire individual. We computed total leaf area, maximum leaf width and length, and the distance from the insertion of the petiole to the point of maximum leaf width (hereafter distance) with the program SigmaScan Pro 5.0.

Leafing phenology

Leaf emergence of adult *N. pumilio* follows a flushing type (Rusch 1993). This means that leaves expand simultaneously as a flush usually at the onset of the growing season (Kikuzawa 1989) and shoot elongation follows within a short period after bud break (Kozlowski 1971). Previous observations over the duration of the common garden experiment indicated that leaves of seedlings also flush at once as in adults (Andrea Premoli unpubl. data, 2003). Therefore, phenological observations were carried out during the leafing process from the onset of bud burst until leaf expansion from August to early November 2004. We recorded the number of plants at each phenological stage including all plants maintained in the common garden (196 and 166 plants from low and high elevation, respectively). Phenological stages recorded during leafing were: resting buds, swollen buds, outbreaking leaves, and fully expanded leaves as in Rusch (1993). Each plant was recorded as belonging to a particular phenological stage when at least one bud and/or leaf had attained a given phenophase. Observations were carried out every 7–10 days from 1 August until all plants had reached the final stage of fully expanded leaves, 3 months after the first observation. Phenological stages during leaf senescence were not recorded because leaves were retained on plants in the greenhouse.

Data analysis

We analysed architectural features of each individual produced during the last 4 years under greenhouse conditions, i.e. Southern Hemisphere growing seasons 2000, 2001, 2002, and 2003. This allows for the comparison of growth patterns from each elevation through time. Dependent variables for each GU were shoot length on A1 and A2, and number of internodes and branches on A1. The overall combined effects of treatment (low and high elevation) and growing period (time) on these dependent variables were tested using

Table 1. Summary statistics of one way repeated measures ANOVA for the effects of elevation with two levels (low and high) and time with four levels (growing seasons 2000–2003) on shoot length, number of internodes, and number of branches of the principal axis A1 and shoot length of the secondary axis A2

Source of Variation	Shoot length A1			Number of internodes A1			Number of branches A1			Shoot length A2		
	d.f. effect	d.f. error	F	d.f. effect	d.f. error	F	d.f. effect	d.f. error	F	d.f. effect	d.f. error	F
Elevation (E)	1	118	27.2***	1	117	4.4*	1	118	0.03	1	10	0.02
Time (T)	3	354	94.1***	3	351	133.2***	3	354	63.2***	3	30	21.2***
E × T	3	354	5.9***	3	351	3.8**	3	354	5.2**	3	30	1.5

*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. d.f., degrees of freedom.

repeated-measures ANOVA (Gurevitch & Chester 1986). Repeated-measures ANOVA determines if differences in response variables between the low and high elevation change over time. Normality of residuals between observed and predicted values of dependent variables was evaluated by using normal plots (Sokal & Rohlf 1981). To analyse the effect of elevation on the dependent variables we ran independent planned comparisons (Mead 1988) for each of the following contrasts: 2000–2001, 2001–2002, and 2002–2003.

To test for potential effects of the initial size at plant collection ($A1_i$) on total shoot length ($A1_f$) we calculated the relative growth rate as RGR = ln ($A1_f/A1_i$) and regressed this value against ln ($A1_i$) for low- and high-elevation plants, respectively. Initial plant size was calculated as the sum of GU length on A1 previous to the measuring period, i.e. up to 2000. To analyse the differential dependence of RGR to initial shoot length for low- and high-elevation plants during the 4-year greenhouse experiment, we compared regression slopes by using the two slopes comparisons test (Zar 1996). Differences in RGR of plants from different elevations were analysed by testing the vertical position on a graph of the relationship between RGR and ln (initial shoot length), i.e. the intercept (Zar 1996).

Between-elevation differences of branching patterns (probability and angles) and within-plant averages of leaf traits (area, width length^{-1}, and distance) were tested by one-way ANOVA analyses. Phenological differences between low- and high-elevation plants at each observational date were tested by χ^2 tests comparing the frequency of plants at each phenophase. Phenological lags of high-elevation plants were analysed by χ^2 tests between the frequency of individuals in each phenophase at T(i) *versus* those at T(i-1) for high- and low-elevation plants, respectively.

RESULTS

Repeated-measures ANOVA indicated that elevation had a significant effect over time on shoot length of A1 (34.2 ± 8.4 and 26.4 ± 8.5 cm in low- and high-

elevation plants) and the number of internodes of A1 (16.0 ± 3.1 and 14.5 ± 3.9 in low- and high-elevation plants, respectively) (Table 1; Fig. 1a,b). Yet, plants from different elevations produced overall a similar number of A2 units, i.e. number of branches (5.1 ± 2.3 and 5.1 ± 2.6, low- and high-elevation, respectively) and of comparable lengths (8.1 ± 4.3 and 7.9 ± 3.6 cm, low- and high-elevation, respectively) (Fig. 1c,d).

Architectural features differed over the duration of the common garden experiment. During the growing periods 2000–2001 and 2002–2003 low-elevation plants attained larger shoot lengths of A1 and A2 than high-elevation ones (Table 2). Additionally low-elevation plants produced more internodes on A1 during 2002–2003 than high-elevation individuals. In contrast, during 2001–2002, high-elevation plants developed longer A1, A2, and more internodes and branches on A1 than low-elevation individuals (Table 2).

Low-elevation plants attained significantly greater mean RGR (±1SD) 2.2 ± 0.7 than high-elevation ones 1.8 ± 0.7 ($t_{117} = 3.39$; $P < 0.001$). Initial shoot length had a non-significant effect on the difference in RGR between the two groups of plants during the 4-year greenhouse experiment ($t_{116} = 1.51$; $P > 0.05$).

Branching characteristics differed significantly in plants from contrasting elevations. Overall branching probability of high-elevation plants was 0.51 ± 0.19 while it was 0.42 ± 0.17 for low-elevation ones ($F_{1,118} = 6.70, P = 0.01$). In addition, the mean branching angle was 83.96° ± 10.19 for high-elevation individuals and 76.08° ± 9.84 for low-elevation ones (one way ANOVA, $F_{1,118} = 18.10, P < 0.0001$).

Similarly, leaf traits on plants from contrasting elevations differed. High-elevation plants produced significantly smaller leaves than low-elevation plants (Table 3). Moreover, leaves from high elevation were relatively narrower, having smaller width to length ratios and shorter distances from the insertion point of the petiole to the maximum leaf width (Table 3).

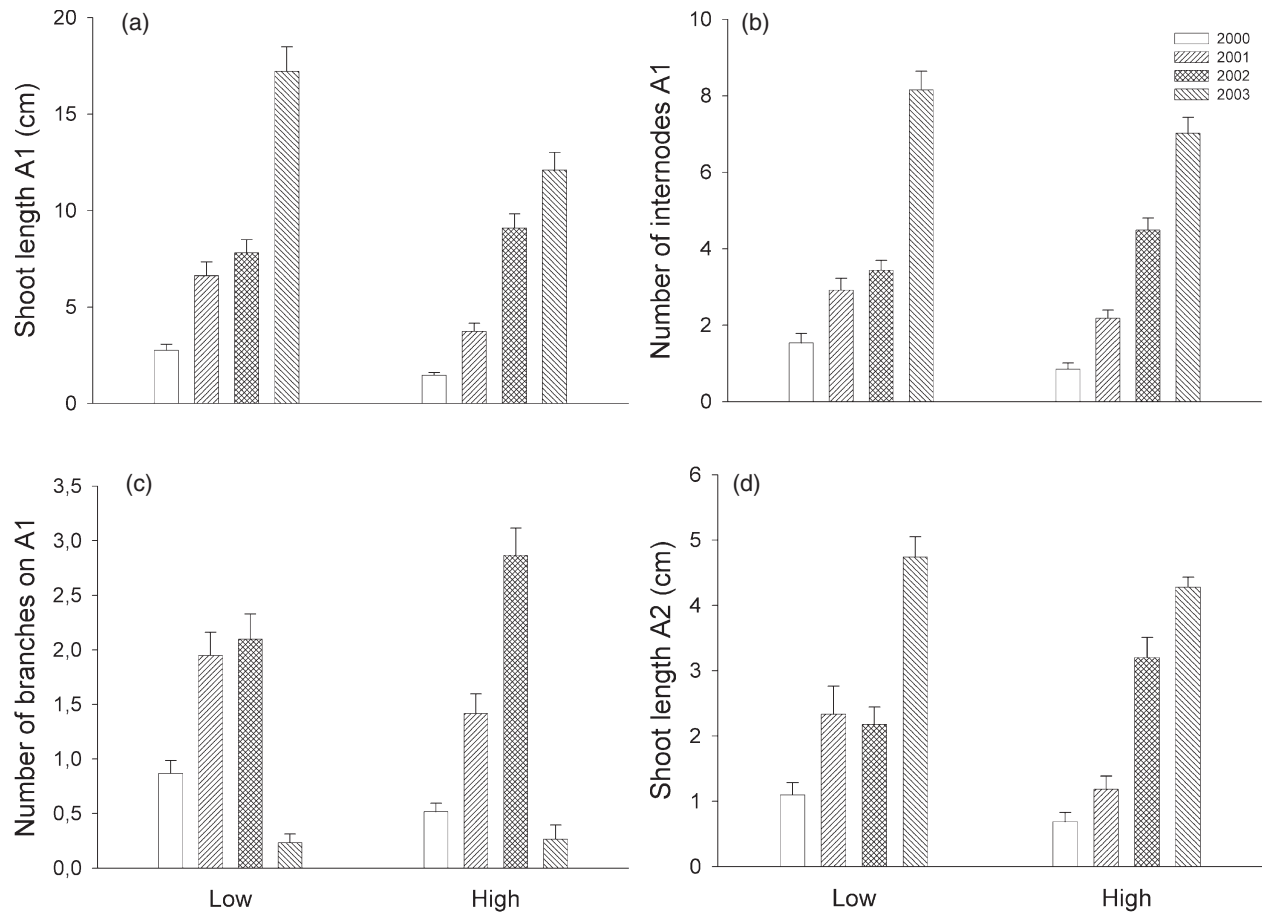Plants from different elevations presented significant phenological differences during the 10-week

Fig. 1. Average architectural features of common garden-grown *Nothofagus pumilio* saplings from low and high elevation (1100 and 1540 m a.s.l.) attained during the growing seasons 2000–2003. These are shoot length (a), number of internodes (b), and number of branches (c) on the principal axis A1, and shoot length on the secondary axis A2 (d).

Table 2. Differences of mean values for architectural traits studied in low and high elevation greenhouse-grown *Nothofagus-pumilio* seedlings during consecutive growing periods from 2000 to 2003

Growing period	Shoot length A1			Number of internodes A1			Number of branches A1			Shoot length A2		
	Low	High	$F_{1,118}$	Low	High	$F_{1,118}$	Low	High	$F_{1,118}$	Low	High	$F_{1,118}$
2000–2001	**3.86**	2.27	3.8*	1.38	1.33	0.01	1.08	0.90	0.4	**1.25**	0.59	3.6*
2001–2002	1.19	**5.36**	9.5**	0.52	**2.30**	9.8**	0.15	**1.45**	8.1**	0.71	**2.19**	9.1**
2002–2003	**9.4**	2.99	10.5**	**4.72**	2.53	7.0**	NA	NA	NA	**2.63**	1.33	5.1*

Contrasts were run between low- and high-elevation plants on shoot length, number of internodes, and number of branches of the principal axis A1 and shoot length of the secondary axis A2. Numbers in bold depict significantly greater values. *$P < 0.05$ and **$P < 0.01$. NA, not applicable.

observation period from August 27 until all the plants reached the fully expanded leaves phenophase. Buds and leaves of low-elevation plants started to burst and expand earlier than those from high elevation. As a result, for the first 4 weeks of the observational period that yielded significant results, high-elevation plants had a greater proportion of plants with resting buds than low-elevation ones (Fig. 2). During the second and third weeks of observations, low-elevation plants reached the maximum proportion of individuals with swollen buds and recently opened leaves, respectively. High-elevation plants did so during the third and fourth weeks. Consequently, phenological peaks of swollen buds and outbreaking leaves had a delay of 2 weeks between elevations. Furthermore, fully expanded leaves were produced earlier by low-elevation plants.

Table 3.   Leaf traits of greenhouse-grown *Nothofagus pumilio* individuals from low and high elevation (1100 and 1540 m a.s.l., respectively)

Elevation	Low	High	*F*	*P*
Area (cm²)	**5.19** (1.32)	3.40 (1.03)	56.90	<0.0001
W/L	**0.72** (0.44)	0.69 (0.59)	5.7	0.02
Distance (cm)	**1.30** (0.20)	1.10 (0.17)	29.2	<0.0001

Area is an indication of leaf size. Width to length ratio (W/L) and distance reflect leaf shape. Distance portrays the length from the insertion of the petiole to the point of maximum leaf width. Results are presented as means (standard deviation). Numbers in bold depict significantly greater values by one-way ANOVA.
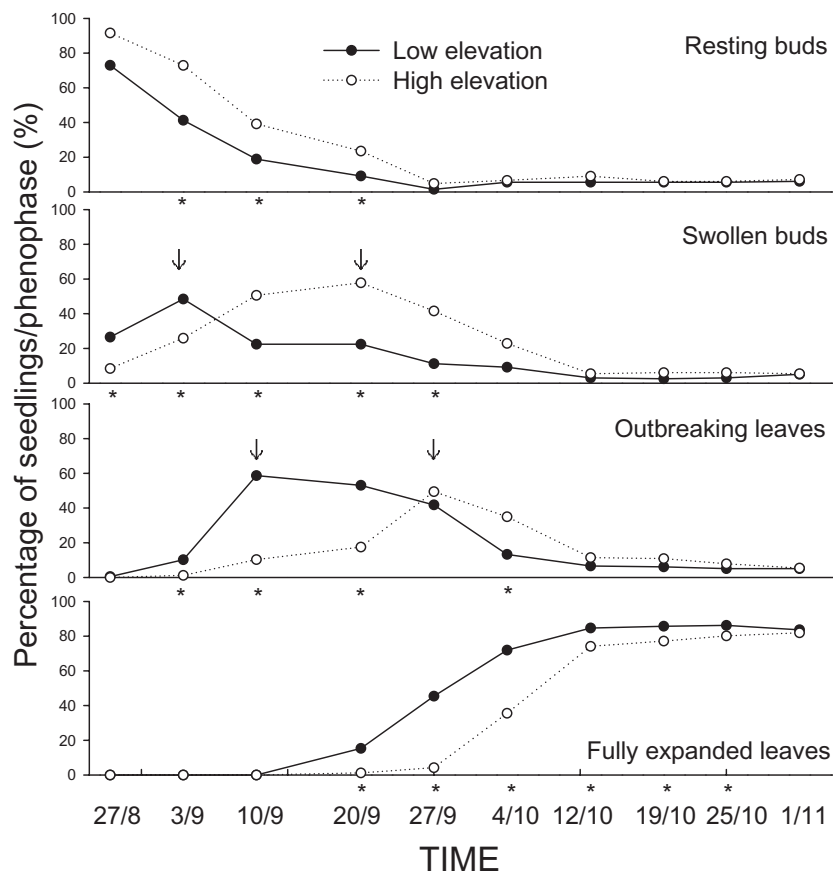

Fig. 2.   Phenological stages during a 10-week observation period that yielded significant differences between plants from low and high elevation (1100 and 1540 m a.s.l.) depicted by closed and open symbols, respectively. Arrows indicate between-elevation significant delay at a given phenophase. Asterisks indicate significant differences ($P < 0.01$) tested by χ^2 tests between low- and high-elevation plants at each phenophase and observation date.

DISCUSSION

Architectural traits followed genetically determined schemes in the deciduous *Nothofagus pumilio* inhabiting contrasting elevations. Repeated-measures ANOVA yielded comparable results to overall RGR and indicated that low-elevation plants significantly outgrow high-elevation ones. Growth patterns of *N. pumilio* plants also differed qualitatively with elevation. High-elevation individuals had higher branching probability

and wider angles than low-elevation ones. This contrasts with the classical view that branching patterns within a given species are independent of the environment (Horn 1975). A comparative study on saplings of a Tasmanian tree species growing under contrasting light environments suggested that light exerts a strong influence on branch orientation (King 1998). Other studies have also documented that different growing environments may produce intraspecific variation in plant geometry. For example, open-grown saplings of

sugar maple (*Acer saccharum*) exhibit fuller crowns and denser branching than those growing in shaded forest understoreys (Steingraeber *et al*. 1979). This was considered a phenotypically plastic response to changes in microenvironment during the life of an individual, which often begins in a shaded environment and finally thrives in the sunlit canopy. Therefore, tree architecture is the result of a trade-off at the sapling stage between (often rapid) vertical growth, and (often less rapid) lateral growth (Ackerly & Donoghe 1998). At low elevation, saplings of *N. pumilio* grow underneath the forest canopy within gaps (Heinemann *et al*. 2000). Therefore, for such light-demanding species (Donoso 1981; Veblen 1985) selection to achieve height rapidly seems to be advantageous at low elevation. In contrast, high-elevation saplings have abundant branches and wide shoot angles from the vertical. This may be related to increased irradiance and linked to other functional traits to avoid self-shading, such as narrow leaves and reduced leaf size (Givnish 1987). In addition, at higher elevations prevailing strong winds may increase bud damage, resulting in more profuse branching. Tree form is a result of a complex interaction between the genetic plan of the species (deterministic architecture) and responses to environmental events (opportunistic architecture) (Hatta *et al*. 1999). Our common garden data strongly suggest that variations in quantitative growth and qualitative architectural traits, i.e. size and shape, are genetically determined in *N. pumilio*. These correlate to other differences that were documented in *N. pumilio* occupying different elevations including isozymes (Premoli 2003), genetically controlled eco-physiological traits (Premoli & Brewer 2007), and structural characteristics (Rusch 1993; Barrera *et al*. 2000). Genetic differences with elevation measured by isozymes were interpreted as a result of diversifying selective pressures across small distances along elevational gradients (Premoli 2003).

On a yearly basis, plants from different elevations seem to follow different growth patterns. It was suggested that variation in shoot length and number of internodes during initial plant growth was time-dependent (Raffaele *et al*. 1998). In particular, annual variation in growth could be of importance in Mediterranean-climate areas characterized by highly unpredictable precipitation regimes (Llorens *et al*. 2004). Such conditions are found at the northern range of *N. pumilio* where the growing and dry seasons overlap. Chall Huaco valley is located towards the eastern-most and therefore driest edge of the range of the species, enhancing this effect. Annual variation in growth patterns may also result from differential investments in aerial *versus* underground structures in plants from different elevations. High-elevation *N. pumilio* have increased leaf C, lignin, and lignin/N probably in response to a lack of available organogenic

elements in the soil (Premoli 2004). On the other hand, an experimental study of fire severity on early regeneration of *N. pumilio* showed that final biomass of seedlings from low elevation sites at Chall Huaco was positively related to direct radiation (Kitzberger *et al*. 2005). Thus, during early growth, high-elevation plants may invest in root development to improve nutrient uptake, while low-elevation individuals growing in the forest understorey may favour early shoot elongation to increase light absorption. If reversal of growth in some years were due to investment in different aerial structures, such as the length of the principal axis and the number and/or longitude of branches produced on an individual plant, one would have predicted a negative relationship among those variables. The lack of such a significant pattern suggests that differential allocation to above- *versus* below-ground structures may account for the observed differences during consecutive growing seasons. Furthermore, a delay in growth responses as indicated by different architectural traits in high-elevation plants compared to low-elevation ones (Table 2) may indicate that the latter respond more rapidly to favourable growing conditions under cultivation in the greenhouse. *N. pumilio* produce seedling banks and small saplings with diameters of only 3 mm can last for almost 25 years on the forest floor (Karin Heinemann, Universidad Nacional del Comahue, unpubl. data 2002). It is possible that liberation of growth-suppressed seedlings may follow distinct environmental signals in low and high elevation habitats, which may explain such time-dependent responses.

Leaf initiation and expansion were delayed 2 weeks in high-elevation common-garden grown individuals. A longer delay of 3–5 weeks was measured under field conditions along a similar 400 m gradient at the southern edge of the species range (Barrera *et al*. 2000). Extreme climatic conditions in the south may strengthen the heritable pattern observed at northern latitudes, and/or phenology is also driven by environmental cues. Asynchronous phenology along elevation gradients is often interpreted as an adaptation for avoiding the potential harm of late frosts (Borchert 1980; Aber *et al*. 1996). However, internal constraints limit phenological and physiological responses to temperature (Starr *et al*. 2000). Nevertheless, complex phenological responses in plants inhabiting mountain regions are expected (Stinson 2004). Field data indicate that leaf formation in *N. pumilio* seems to be temperature-dependent (Rusch 1993). Our results indicate that it is clearly not temperature levels that are controlling leafing phenology, otherwise bud burst would be expected to have occurred earlier in high-elevation seedlings. Also leafing is coupled with flowering in *N. pumilio* (Rusch 1993). While low-elevation populations at Chall Huaco valley start flowering in October, those 100 m further up do so 15 days later

(Díaz 2004). Our results show that altitudinal differences in patterns of vegetative growth and phenology of *N. pumilio* are affected by genetic controls. We could then hypothesize that in addition to the differences in leaf emergence reported here, flowering phenology may also be genetically determined. Date and abundance of flowering reproductively isolate nearby populations and thus, influence gene flow rates, reinforcing diversifying selection pressures at contrasting microhabitats. This explains the significant among-population genetic divergence measured in *N. pumilio* at small spatial scales along elevational gradients (Premoli 2003).

Whether age differences between low- and high-elevation plants affect growth responses under cultivation in the greenhouse is difficult to disentangle. Although high-elevation seedlings were on average 3 years older than low-elevation ones, they were of similar size at collection time. This indicates that extreme physical conditions most probably suppressed growth of high-elevation seedlings before transplanting. As a result, we would have expected liberation of growth in high-elevation plants under the common-garden experiment, which did not occur. We believe this was mainly due to genetically determined effects. Conversely, distinct architectural features and phenological rhythms may also be due to preconditioning effects of the respective environments that governed their growth patterns in the greenhouse, or to distinct developmental pathways that were set up early in the life of seedlings before the transplant. An ongoing reciprocal transplant experiment, consisting of sets of seedlings from different elevations that where planted under their own and contrasting altitudinal provenances, will produce valuable information on the mechanisms and contrasting roles of genetic *versus* plastic effects ruling growth patterns and phenology of *N. pumilio*. However, the question still remains whether genetically determined responses will benefit or constrain plants such as *N. pumilio* inhabiting altitudinal gradients. For example longer growing seasons are predicted under global warming and deterministic phenological responses may put high-elevation individuals at a competitive disadvantage relative to plants that can trigger growth early.

ACKNOWLEDGEMENTS

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