

Environmental v. genetically driven variation in ecophysiological traits of *Nothofagus pumilio* from contrasting elevations

Andrea C. Premoli^{A,C} and Carol A. Brewer^B

^ALaboratorio Ecotono, Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, Quintral 1250, 8400 Bariloche, Argentina.

^BDivision of Biological Sciences, The University of Montana, Missoula, MT 59812, USA.

^CCorresponding author. Email: apremoli@crub.uncoma.edu.ar

Abstract. *Nothofagus pumilio* (Poepp. et Endl.) Krasser is a broadleaved deciduous tree that dominates high-elevation forests in the southern Andes. We evaluated the degree to which differences in stomatal density and physiological traits (net assimilation, conductance and water use efficiency) were related to environmental and genetic influences with elevation by comparing plants growing under field and common garden conditions. Low-elevation leaves under field conditions had fewer stomata, although this pattern was not maintained in the common garden. Assimilation rates were >40% higher for high-elevation plants in the field, and 18% higher in the common garden, than those for low-elevation plants. In addition, under field conditions high-elevation plants tended to have higher stomatal conductance and lower instantaneous water use efficiency than did low-elevation plants; however, these differences were not significant in the common garden. Thus, assimilation seems to be under genetic control whereas ecophysiological traits related to the use of water appear to be more responsive to environmental cues. Our results suggest that plants growing along elevational gradients may show complex ecophysiological patterns. These patterns may be acquired by genetically driven responses to conditions that are fixed throughout the life span of individuals, such as soil nutrients. Also plastic adjustments may favour opportunistic use of available water during the dry season, particularly under Mediterranean-type climate regimes with summer drought.

Introduction

Changing physical factors along elevational gradients, such as temperature, leaf-to-air-vapour density gradient, solar radiation, wind exposure and soil characteristics, induces many visible changes in the composition of plant communities and in the growth habit of individual plants (e.g. Billings and Mooney 1968; Jones 1992; Larcher 1995). These different environmental conditions eventually lead to one of the sharpest shifts in plant dominance at treeline, from forests to shrubland and herbaceous vegetation, often over short distances (Körner 1999; Cuevas 2000).

However, inter- and intraspecific patterns at contrasting elevations can be difficult to predict, in part because of the complexity of the physical conditions and the range of biological responses associated with them. For example, studies comparing photosynthetic responses of low- and high-elevation plants reported lower assimilation rates in high-elevation than in low-elevation plants (e.g. Pisek and Winkler 1958; Slayter and Morrow 1977; Tranquillini and Havranek 1985; Cabrera *et al.* 1998; Kao and Chang 2001). The opposite trend also has been observed where plants from high elevations have photosynthetic rates higher than those of their lowland counterparts (e.g. herbaceous plants of the Austrian Alps; Körner and Diemer 1987). Similar results have been found for *Picea abies* (L.) Karst. seedlings in common glasshouse trials (Oleksyn *et al.* 1998) and for *Nothofagus cunninghamii* (Hook.) Oerst. cuttings from two different elevations (Hovenden and

Brodribb 2000). However, these photosynthetic responses with elevation may have no strong association with morphological traits related to CO₂ uptake, such as stomatal density (Jones 1992). Whereas conductance may increase with elevation, owing to smaller humidity deficits (Körner and Mayr 1981) or to greater carboxylation efficiency because of the reduced CO₂ concentration partial pressure at higher elevations (Zhang *et al.* 1993), reports on stomatal characteristics are inconsistent. For example, some studies report a tendency for higher stomatal density with elevation (Woodward 1987). Others indicate fewer stomata at high elevations (e.g. Schoettle and Rochelle 2000), or no changes in stomatal density (Körner *et al.* 1989). Although the pattern and frequency of stomata on any leaf surface are under tight genetic control, they may be modified by environmental parameters.

The objective of the present study was to analyse genetic (long-term) v. acclimative (plastic short-term) responses of ecophysiological traits on plants from contrasting elevations growing in a common garden and under natural field conditions. This work complements other studies linking plant morphology and function, to better understand patterns of variation resulting from restricted gene flow and divergent selective forces along elevational gradients. An ideal species for exploring environmentally driven (plastic) responses compared with genetically fixed variation in ecophysiological traits at contrasting elevations is the southern beech, *Nothofagus pumilio*

(Poepp. et Endl.) Krasser. *N. pumilio* is a winter-deciduous tree that produces extensive pure forest stands along elevational gradients from ~1000 to 1600 m above sea level in north-western Patagonia. Variable physical conditions characterise *N. pumilio* forests along these gradients, including decreases in soil temperature and the period with frost-free soils, as well as increased total annual precipitation and percentage of snowfall at high elevation (Barrera *et al.* 2000). These physical factors exert differential selection pressures which, in combination with phenological barriers that reproductively isolate nearby populations, result in marked genetic differences (Premoli 2003). Also, on the basis of common garden studies of saplings from different elevations, plant architecture and leaf morphology are tightly linked and differ significantly, suggesting genetic control. Plants from high elevations tend to have lower stature, wider branching angles, greater branching ratios (number of branches/number of internodes) and smaller leaves than low-elevation plants. Compact growth at high elevation may be related to strong winds and irradiance (Premoli *et al.* 2007). Yet, the genetic basis of ecophysiological characteristics with elevation has not yet been investigated.

The present study was conducted in northern Patagonia, an area with a Mediterranean-type climate characterised by summer drought and unpredictable rainfall during the growing season. *N. pumilio* is found predominantly on Andisol soils. These soils are mainly derived from volcanic ash (Singer and Morello 1960; Veblen and Ashton 1979) and have a high capacity to retain P and to stabilise organic matter, which often leads N and P deficiencies (Mazzarino *et al.* 1998). As a result, *N. pumilio* individuals, particularly those growing near timberline, exhibit characteristics expected in plants living under mineral-poor conditions, i.e. dwarf stature, small sclerophyllous leaves (Larcher 1995). Consequently, physiological characteristics of *N. pumilio* along an elevational gradient would be expected to reflect the variability in these soil-nutrient environments.

In the present study, we used field data from adult trees in combination with a common garden experiment on seedlings representing the extremes of the 400-m elevational gradient. We hypothesised that responses for characteristics related to environmental variables were likely to be constant during the life cycle of *N. pumilio* individuals (e.g. soil nutrient conditions) and would be genetically fixed. In contrast, the traits affected by unpredictable physical variables (e.g. water availability) would be driven by plasticity. Moreover, we predicted that any observed differences in ecophysiological traits between plants from contrasting elevations would suggest genetic differences, particularly when measured on common garden plants, whereas similar responses (i.e. lack of significant differences) would be indicative of the influence of environmental conditions. To test this hypothesis, we used data on stomatal density and the physiological attributes of net photosynthesis (A_{\max}), stomatal conductance (g) and instantaneous water use efficiency (WUE, calculated as CO_2 assimilated/ H_2O transpired).

Materials and methods

Field sites and common garden experiment

Populations at elevational extremes of the lower and upper distributional range of *N. pumilio* were chosen. This sampling

design follows results from a genetic analysis of *N. pumilio* populations along elevational gradients that indicated significant isozyme differences between populations located at elevational extremes, which were also genetically alike if located within a similar elevational belt on different mountain ranges (Premoli 2003).

To analyse environmental effects on ecophysiological traits, we compared adult plants under field conditions from different elevations, whereas genetic effects were analysed on seedlings grown in a common garden. Given that the present study was designed to compare low- with high-elevation plants, the unavoidable methodological limitations of this design related to age differences between adult plants in the field and common garden seedlings were minimised by limiting analyses to between-treatment (low and high elevation) comparisons within each set of plants (field and common garden). Moreover, we used only mature, fully expanded leaves of seedlings that were morphologically similar to those observed and measured on adult trees. Likewise, we compensated for potential environmental differences by making all measurements of low- and high-elevation plants within each growing condition (field or common garden) during the same day.

Five mature trees from two populations in Challhuaco Valley (41°15'S, 71°18'W; elevations 1100 and 1540 m) were randomly selected for measurements. Source areas for common garden-grown plants were two populations at each of three locations from three mountain ranges where genetic studies had already been conducted, namely Challhuaco Valley, Cerro Otto (41°08'S, and 71°21'W; elevations 1120 and 1400 m) and Cerro López (41°06'S, 71°33'W; elevations 1250 and 1620 m). For the common garden experiment we harvested seedlings of similar size from each of these locations, most of which had recently germinated under natural conditions during the austral spring (October–December). Collection of *N. pumilio* seedlings instead of seeds avoided the limitation of low germination rates under experimental conditions (Premoli 2004; Premoli *et al.* 2007). Seedlings were collected in trays containing their own soil to avoid transplantation stress. In the greenhouse, plants were separated from soil, their roots were washed and seedlings were individually transplanted into 1-L containers in a substrate consisting of a mixture of peat and their native forest soil. Seedlings were grown in a randomised arrangement in an open space in the vicinity of Universidad Nacional del Comahue, Bariloche, at 876 m above sea level, 30 km from seedling source sites. Plants were watered, fertilised and rotated regularly to minimise confounding environmental effects. Each year plants were repotted into larger containers. Seedlings were maintained under conditions typical of understory *N. pumilio* forests (PAR ~700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at solar noon). At the time of measurement, common garden-grown plants were at least 27 months old and growing in 2-L pots. All measurements were made on five randomly selected seedlings from each elevation and location, for a total of 30 plants.

Stomatal density

Stomatal density was estimated from surface impressions (clear enamel nail polish) made from mid-blade adaxial and abaxial leaf surfaces (Brewer and Smith 1997). Care was taken to avoid

veins. For each of the five trees from each population of adult trees and common garden seedlings, five leaves were evaluated with five replicates per leaf. Stomatal density was estimated by counting the number of stomata per field at $\times 400$ magnification for five fields. These data were corrected by area and reported values are the number of stomata mm^{-2} .

Gas exchange measurements

Net photosynthesis (A_{max}) and leaf conductance to water vapour (g) were measured on adult trees (late January) and common garden seedlings (March) on consecutive days during each measurement period. The number of individual plants selected, as well as the number of repeated-measurements on each plant, was designed to reduce environmental and genetic effects under field and greenhouse conditions. Given the small size of the leaves relative to the size of the leaf chamber of the infrared gas analyser, gas exchange measurements were performed on entire lateral branches containing between four and eight attached leaves, i.e. leaf sets. This methodology was previously used in other physiological studies on closely related *Nothofagus* (Premoli 1994). Field data were gathered from sun-exposed leaves at a height of ~ 1.5 m on five randomly selected adult trees at each elevation and measurements were repeated consecutively five times to reduce the influence of short-term environmental fluctuations. Common garden measurements were performed on five randomly selected seedlings from each elevation and seedling source (i.e. populations from the Challhuaco Valley, Cerro Otto and Cerro López mountain ranges).

Assimilation and conductance were measured under ambient conditions (relatively similar for field and common garden plants) with a portable closed-flow infrared gas analyser system (Li-Cor Inc., Lincoln, NE, Model 6200) that also monitored leaf temperature, chamber humidity and photosynthetic photon flux density (PPFD). To control for possible short-term responses to the environment, measurements of A and g were made on consecutive clear days. Ambient air temperature and relative humidity were monitored with a digital thermohygrometer during each set of gas-exchange measurements on a given individual. Air temperature varied between 22 and 24°C, and relative humidity between 27 and 30%. All measurements were made on cloud-free days under full sun light conditions by noon to avoid influences of changing light levels on photosynthesis. No light response data are available for *N. pumilio*, but the light saturation point of the closely related *Nothofagus dombeyi* (Mirb.) Oerst. (also shade intolerant) is achieved at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Caldiz 1999). Although, light environments differed between the field and the greenhouse (see below), we assumed that our measurements represented a reasonable approximation of A_{max} for both field and common garden plants.

Net gas exchange was measured inside a sealed, 0.25-L cuvette. Leaves of both mature trees and seedlings were positioned at their natural height and orientation to the sun. Measurements were initiated within 15 s of placing the leaves in the cuvette, and CO_2 depletion was measured over the next 15–30 s. On completion of each measurement, the cuvette was flushed with air. Fans in the cuvette maintained leaf temperature within approximately $\pm 2^\circ\text{C}$ of air temperature. The initial

concentration of CO_2 in the cuvette was within $5 \mu\text{L L}^{-1}$ of ambient CO_2 levels ($355 \mu\text{L L}^{-1}$) and did not decrease more than $25 \mu\text{L L}^{-1}$ during the measurement interval. Vapour pressure of air in the chamber was maintained within $\pm 5\%$ of ambient vapour pressure by regulating the amount of flow diverted through the desiccant. After they were measured, leaves were collected and surface area was determined with a scanner and a DOS-based program developed by F. Lema, Atomic Center, Bariloche. Net photosynthesis and conductance were reported on a one-sided leaf area basis.

Soil nutrient concentration

A composite soil sample was collected from the high-elevation population (1540 m) in the Challhuaco Valley. It consisted of five soil subsamples collected at different microsites at a depth of 10–20 cm after removal of organic matter. Samples were air-dried and pooled for analysis. Organic carbon was determined by wet digestion by the Walkley–Black method (Nelson and Sommers 1996) and total nitrogen by the regular semi-micro Kjeldahl method using a block digester (Bremner 1996). These were analysed by the Soil Laboratory at Universidad Nacional del Comahue, Bariloche, which also provided information on soil nutrient content from the low-elevation population at Challhuaco.

Data analysis

Stomatal densities for leaves representing populations from different elevations and growing conditions were compared by one-way ANOVA. Differences with elevation in net photosynthesis (A), stomatal conductance (g) and instantaneous water use efficiency (WUE, calculated as CO_2 assimilated/ H_2O transpired) were analysed according to one-way ANOVA models; each set of field and common garden plants was analysed separately. Possible differences in stomatal density and gas exchange rates among common garden-grown plants from different seedling sources (i.e. mountain ranges) were analysed by factorial two-way ANOVA models, with mountain range and elevation as fixed effects. We also analysed the fixed effects of elevation (low and high) and growing condition (field and common garden) on stomatal density and gas exchange rates by two-way ANOVAs.

Results

Stomata in *N. pumilio* were present on the abaxial surface only. Stomatal density was significantly different for leaves on trees in the field from different elevations. Trees from high elevation under field conditions had 35% more stomata than low-elevation plants (Table 1). In contrast, there was no significant difference in stomatal density for common garden seedlings from different elevations. Although elevation had no effect on common garden plants, differences were significant when samples were pooled for the two-way ANOVA, which yielded significant ($P < 0.001$) results for both of the fixed effects, elevation ($F_{1,76} = 16.4$) and growing conditions ($F_{1,76} = 96.0$), as well as the interaction between them ($F_{1,76} = 26.5$). As a result, the mean adjusted stomatal densities varied from 155.9 to 199.1 stomata mm^{-2} for low- and high-elevation plants, respectively. Additionally, the adjusted mean stomatal densities for plants in different growing

Table 1. Stomatal density, assimilation, stomatal conductance and instantaneous water use efficiency for *Nothofagus pumilio* plants from low- and high-elevation growing in the field (adult) and in a common garden (seedlings)

WUE, water use efficiency. Between-elevation comparisons were tested by one-way ANOVA. Significant ($P < 0.05$) differences between low- and high-elevation plants are indicated with an asterisk. Values are means (\pm s.e.)

Growing conditions	Site	Low	High	Low	High	Low	High	Low	High
		Stomatal density (stomates mm ⁻²)		Assimilation rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		Conductance (mmol H ₂ O m ⁻² s ⁻¹)		WUE ($\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$)	
Field	Challhuaco	180.7* (8.7)	278.8* (12.7)	10.5* (0.6)	18.3* (0.6)	111.0* (8.8)	248.2* (18.1)	97.1* (5.5)	77.0* (3.4)
	Common garden	124.0 (11.9)	116.7 (12.3)	6.6 (0.7)	7.6 (0.8)	133.9 (27.6)	118.6 (18.6)	53.2 (6.9)	67.3 (6.2)
Common garden	Lopez	107.2 (15.7)	123.7 (10.4)	6.7 (0.5)	7.3 (0.6)	119.3 (22.6)	135.7 (28.5)	59.5 (4.9)	60.3 (9.6)
	Otto	127.0 (7.0)	152.7 (9.2)	5.6* (0.6)	8.2* (0.6)	131.6 (21.9)	150.5 (19.0)	45.1 (5.4)	57.5 (6.1)
	Adjusted means ^A	119.4 (6.8)	131.1 (7.1)	6.3* (0.4)	7.7* (0.4)	128.2 (13.0)	134.9 (12.5)	52.6 (3.5)	61.7 (4.1)

^AEffect of elevation on ecophysiological traits recorded on greenhouse-grown plants analysed by two-way ANOVA, with elevation and seedling origin as fixed effects.

conditions were 229.8 and 125.2 stomata mm⁻² for field and common garden plants, respectively.

Assimilation for adult trees growing under field conditions at high elevation was 43% higher than for trees from low elevation. Common garden seedlings showed a similar trend, although the difference of ~18% was not as great as that observed for adult plants growing in the field (Table 1). Stomatal conductance in field plants from high elevations was twice as high as that in lower-elevation plants, resulting in significantly lower instantaneous WUE in the high-elevation plants. No significant differences were found for either conductance or WUE for common garden seedlings from low or high elevations.

The combined two-way ANOVA on the effects of seedling origin and elevation on net assimilation for common garden-grown plants yielded a significant effect for elevation ($F_{1,24} = 6.7$, $P = 0.02$), as well as the interaction between elevation and seedling origin ($F_{1,74} = 1.3$, $P = 0.03$). Seedling origin had no significant effect on any of the ecophysiological variables measured.

PFD was not significantly different (one-way ANOVA, $P > 0.05$) within any set of measurements of field or common garden plants. However, light intensity during measurement was significantly greater in the field than in the common garden ($F_{1,76} = 63.7$, $P < 0.0001$). The average quantum flux density during field measurements was 2006 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (± 1 s.d. 203) and 2152 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (± 151) at low and high elevations, but only 1545 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (± 440) and 1504 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (± 432) during measurements of low- and high-elevation seedlings in the common garden, respectively.

Elevation (low and high) and growing conditions (field v. common garden) had a significant effect on assimilation, stomatal conductance and instantaneous water use efficiency (two-way ANOVA $F_{1,76} = 39.3$, $F_{1,76} = 27.9$ and $F_{1,76} = 14.5$, $P < 0.001$, respectively). Although the interaction between elevation and growing conditions was significant for assimilation and stomatal conductance (two-way ANOVA $P < 0.001$), it was not so for instantaneous water use efficiency (two-way ANOVA $P = 0.15$). Overall, high-elevation plants had greater assimilation and stomatal conductance (12.3 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and 188.2 mmol H₂O m⁻² s⁻¹) than did low-elevation plants (9.1 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and 123.0 mmol H₂O m⁻² s⁻¹). This resulted in less significant instantaneous WUE in high- than

low-elevation plants (64.8 and 79.4 $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$, respectively). Moreover, adult plants growing in the field had greater mean adjusted assimilation (14.4 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (179.6 mmol H₂O m⁻² s⁻¹) and WUE (87.0 $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$) than did common garden-grown seedlings (7.0 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, 131.6 mmol H₂O m⁻² s⁻¹, 57.1 $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$). One possible explanation for the difference in such values between the field and common garden plants may be that field measurements were performed at higher light intensities, whereas common garden plants were acclimated to and measured in a relatively low-light environment. Also, field plants attained higher leaf temperatures (27.0°C \pm 0.4) during measurement than did common garden seedlings (24.9 \pm 0.2°C) ($F_{1,76} = 13.8$, $P < 0.001$). Furthermore, measurements on field plants were made during midsummer (late January), whereas those on seedlings were made in the late summer (March).

We found marked reductions in soil nutrients at high elevation. Organic carbon and total nitrogen were as low as 2.8% C and 0.1% N at the high-elevation sites, compared with 12.2% C and 0.7% N from the low-elevation sites (M. J. Mazzarino, unpubl. data).

Discussion

Our results suggest that individuals of *N. pumilio* growing at elevational extremes differ substantially in ecophysiological traits. Plant age, growth and measurement conditions, as well as the interaction between genotype and environmental influences explain the variation in the ecophysiological traits we measured. We found significant differences for all ecophysiological variables we measured between low- and high-elevation plants in field conditions. However, the lack of equivalent differences for traits related to the use of water in the common garden plants suggests environmental (i.e. plasticity) rather than genetic control. In contrast, we found higher assimilation rates for both field and common garden plants from high elevations, suggesting the existence of genetically driven effects on net photosynthesis. Thus, it is possible that developmental differences between adult plants growing in the field and seedlings grown in common garden conditions may be a confounding factor in the interpretation of our results. However, our analyses were conservative because we compared individuals from high and

low elevations within each set of plants separately. This approach allowed us to explore combined environmental and genetic effects on field plants and the mostly genetic influences on the common garden-grown seedlings. Also, assimilation rates of common garden plants were affected significantly only by elevation and not by seedling origin (i.e. mountain range). This reinforces data from other studies that indicated that genetic differences are maintained by a steep phenological gradient that results in reproductive isolation and limited gene exchange between nearby populations of *N. pumilio* along the elevational gradient (Premoli 2003). Phenological differences, such as a 2-week delay in bud initiation and leaf expansion in high-elevation saplings of *N. pumilio*, were shown to have a genetic basis as demonstrated in common garden trials (Premoli *et al.* 2007). As a result, greater gene flow is maintained among populations at similar elevations rather than among close populations inhabiting different elevational strips within a given location. This marked between-population genetic divergence in *N. pumilio* is enhanced by distinct selective forces that change with elevation.

Although the number of stomata on leaves of trees in high- and low-elevation field conditions was significantly different, this pattern was not conserved in the common garden. Overall, stomatal density was nearly 40% higher at high elevation, corresponding to generally smaller leaves. Although the data presented here suggest environmental control of stomatal density, leaf size seems to be genetically controlled as suggested by a 4-year common garden experiment on *N. pumilio*, which indicated that high-elevation plants bear smaller leaves (Premoli *et al.* 2007). Decreasing leaf size and increasing stomatal density with elevation were found also in a glasshouse experiment on the evergreen rainforest tree, *Nothofagus cunninghamii* (Hovenden and Vander Schoor 2006). That study showed that these traits were environmentally controlled in response to irradiance. Other reports in the literature suggest that stomatal density varies highly with elevation (e.g. Woodward 1986; Köerner *et al.* 1989; Schoettle and Rochelle 2000). One possible explanation is that stomatal density is sensitive to CO₂ concentration and, for some species, the number of stomata per unit area increases with increasing elevation, as CO₂ partial pressure decreases (Woodward 1987). Another possible explanation for elevational differences in stomatal density is that rates of leaf expansion may differ between high and low elevations, but no data on the number of stomatal pores/epidermal cells are available for *N. pumilio*.

Further, stomatal conductance was more than twice as great in the high- v. low-elevation field populations, but differences were not significant in the common garden. This pattern has been reported for other species as well (Köerner and Mayr 1981; Jones 1992). Several explanations have been offered in the literature. Although vapour pressure tends to decrease with increasing elevation, relative humidity tends to increase because of adiabatic cooling (Nobel 1991; Jones 1992). Consequently, the vapour pressure deficit tends to decrease with increasing elevation. Further enhancing this influence is that leaves at high elevation tend to be smaller, resulting in leaf temperatures that are typically closer to air temperature via convective cooling influences (Foster and Smith 1986). In conjunction with greater stomatal density, higher stomatal opening may lead to observed

patterns of stomatal conductance at high and low elevations (Nobel 1991; Jones 1992).

Ecophysiological responses of *N. pumilio* related to the use of water, such as stomatal conductance, water use efficiency and stomatal density, seem to be plastic given that potential differences measured in the field were eliminated in the common garden. Plasticity is one of the mechanisms by which a species can maximise fitness (Bradshaw 1965). In particular, phenotypic plasticity should be high for characteristics that are likely to respond to unpredictable changing conditions of the physical environment, especially in plants inhabiting stressful marginal habitats. We expected this to be the case for characteristics of *N. pumilio* related to the use of water, such as *g*, WUE, and stomatal density. In our field sites in Andean Patagonia, the climate is a Mediterranean-type where most of the annual precipitation falls in winter as snow and summers are dry because of the presence of a subtropical high-pressure cell in the south-eastern Pacific (Veblen *et al.* 1996). Especially, at the Challhuaco field site where we made field measurements and collected seedlings, *N. pumilio* is at the easternmost and driest part of its range. Given that low moisture availability can lead to premature leaf abscission in *N. pumilio* (Veblen *et al.* 1996), we would predict that phenotypic plasticity in ecophysiological characteristics is advantageous if it allows reduced water loss during critical times. Thus, when water is readily available, variations in stomatal control or leaf conductance via changes in stomatal density would not likely lead to maximising water uptake within the same growing season.

Genetic differentiation is predicted for characteristics adapted to environmental variables that are likely to remain constant during the life cycle of an organism (Cordell *et al.* 1998). In the case of *N. pumilio*, we observed similar trends of increased assimilation rates in field- and common garden-grown plants, suggesting heritable variation that may be related to nitrogen-poor volcanic soils found at high elevations. Corresponding to the critical lack of available organogenic elements in the soil at higher elevation, leaves of *N. pumilio* at high-elevation sites had higher lignin (26%) than leaves at low-elevation sites (only 15%) (Premoli 2004). Thus, when insufficient nitrogen is available, greater amounts of carbohydrates may be converted into storable forms (starch and fat) or utilised in secondary metabolism as increased lignin and foliar N (Larcher 1995). This pattern may be positively related to assimilation rates owing to increased levels of Rubisco and other N-containing constituents of the photosynthetic apparatus (e.g. Wong 1979; Field and Mooney 1986; Evans 1989) at higher elevations. Slow-growing species may accumulate considerable amounts of secondary compounds such as lignin and phenolics (Mooney and Gulmon 1982) and this also may be the case for individuals of *N. pumilio* growing at high elevation.

Other studies have reported higher rates of net photosynthesis in glasshouse-grown saplings from high elevations. For example, in the evergreen *N. cunninghamii*, higher assimilation rates were positively correlated with stomatal density and conductance (Hovenden and Brodribb 2000). This is a cool temperate rainforest species from Tasmania that can keep its stomata open under relative cell water content deficits of up to 85% (Read 1985). Therefore, drought tolerance in this species may be attributed to a tight link between leaf biochemistry and

leaf morphology. A report by Oleksyn *et al.* (1998) showed that increased assimilation rates and higher nitrogen content in needles of *Picea abies* from high elevation, grown in a common garden, was correlated with proportionally higher dry-mass partitioning to roots. As in these other studies, there is a strong association between genetically based morphological and functional traits in *N. pumilio* at contrasting elevations. In a companion paper, Premoli *et al.* (2007) reported a significant decrease in annual shoot growth and leaf size, but an increase in branching probability along with wide shoot angles (from the vertical) for high-elevation 4-year-old greenhouse-grown saplings. Increasing the availability of carbon by increasing assimilation rates at high elevation does not necessarily translate into bigger plants. Instead, *N. pumilio* may differ in whole-plant architecture and leaf structure, such as smaller leaf size with high lignin content. As such, these leaves also can be considered as sun leaves (Givnish 1987), a growth form that is also associated with thick leaves and increased photosynthesis rates per unit area (Boardman 1977). Therefore, the observed morphology of *N. pumilio* could be the result of divergent selective forces (e.g. temperature, light environment) along elevational gradients, and not to any particular physiological limitations related to carbon acquisition. This interpretation may support the hypothesis that treeline formation results from sink limitations in treeline species (Körner 1998; Hoch *et al.* 2002), which, in *N. pumilio*, may be related to nitrogen deficiencies in the soil and the influence of low temperatures and a shortened growing season on cell and tissue formation.

In summary, ecophysiological traits in plants inhabiting elevational gradients may show complex adaptive responses, combining genetic differences with important influences resulting from phenotypic plasticity. This enhances the potential of widespread species to adapt to changing environmental conditions along such variable gradients.

Acknowledgements

We thank F. Kitzberger and P. Soares for valuable field and laboratory assistance. G. Goldstein, Ch. Körner, A. Schoettle and three anonymous reviewers made insightful comments on earlier versions of this manuscript. This research was funded by the International Foundation for Science (D/2544-1) and Universidad Nacional del Comahue to A. Premoli, and by Montana EPSCoR, Turner Foundation, University of Montana Creative Research Grants, and a Fulbright Senior Research Fellowship to C. Brewer. We are grateful to Administración de Parques Nacionales in Argentina for permitting us to work in protected areas. A. Premoli is a member of CONICET.

References

- Barrera MD, Frangi JL, Richter LL, Perdomo MH, Pinedo LB (2000) Structural and functional changes in *Nothofagus pumilio* forests along an altitudinal gradient in Tierra del Fuego, Argentina. *Journal of Vegetation Science* **11**, 179–188. doi: 10.2307/3236797
- Billings WD, Mooney HA (1968) The ecology of arctic and alpine plants. *Biological Review* **4**, 481–530.
- Boardman NK (1977) Comparative photosynthesis of sun and shade plants. *Annual Review of Plant Physiology* **28**, 355–377. doi: 10.1146/annurev.pp.28.060177.002035
- Bradshaw AD (1965) Evolutionary significance of phenotype plasticity in plants. *Advances in Genetics* **13**, 115–155.
- Bremner JM (1996) Nitrogen-total. In 'Methods of soil analysis. Part 3. Chemical methods. Soil Science Society of America Book Series no. 5'. (Ed. DL Sparks) pp. 1085–1121. (American Society of Agronomy: Madison, WI)
- Brewer CA, Smith WK (1997) Patterns of leaf surface wetness for montane and subalpine plants. *Plant, Cell & Environment* **20**, 1–11. doi: 10.1046/j.1365-3040.1997.d01-15.x
- Cabrera HM, Rada F, Cavieres L (1998) Effects of temperature and elevation on photosynthesis of two morphologically contrasting plant species along an altitudinal gradient in the tropical high Andes. *Oecologia* **114**, 145–152. doi: 10.1007/s004420050430
- Caldiz MS (1999) Estructura genética del arrayán, *Luma apiculata* (DC.) Burret (Myrtaceae), una especie endémica del noroeste patagónico: su relación con el sistema reproductivo y modo de regeneración. Licenciatura in Biology Thesis. Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, Bariloche, Argentina.
- Cordell S, Goldstein G, Mueller-Dombois D, Webb D, Vitousek PM (1998) Physiological and morphological variation in *Metrosideros polymorpha*, a dominant Hawaiian tree species, along an altitudinal gradient: the role of phenotypic plasticity. *Oecologia* **113**, 188–196. doi: 10.1007/s004420050367
- Cuevas JG (2000) Tree recruitment at the *Nothofagus pumilio* alpine timberline in Tierra del Fuego, Chile. *Journal of Ecology* **88**, 840–855. doi: 10.1046/j.1365-2745.2000.00497.x
- Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C₃ plants. *Oecologia* **78**, 9–19. doi: 10.1007/BF00377192
- Field C, Mooney HA (1986) The photosynthesis-nitrogen relationship in wild plants. In 'On the economy of plant form and function'. (Ed. T Givnish) pp. 25–55. (Cambridge University Press: Cambridge)
- Foster JR, Smith WK (1986) Influence of stomatal distribution on transpiration in low-wind environments. *Plant, Cell & Environment* **9**, 751–759. doi: 10.1111/j.1365-3040.1986.tb02108.x
- Givnish TJ (1987) Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytologist* **106**, 131–160.
- Hoch G, Popp M, Körner Ch (2002) Altitudinal increase of mobile carbon pools in *Pinus cembra* suggests sink limitation of growth at the Swiss treeline. *Oikos* **98**, 361–374. doi: 10.1034/j.1600-0706.2002.980301.x
- Hovenden MJ, Brodribb T (2000) Altitude of origin influences stomatal conductance and therefore maximum assimilation rate in Southern Beech, *Nothofagus cunninghamii*. *Australian Journal of Plant Physiology* **27**, 451–456.
- Hovenden MJ, Vander Schoor JK (2006) The response of leaf morphology to irradiance depends on altitude of origin in *Nothofagus cunninghamii*. *New Phytologist* **169**, 291–297. doi: 10.1111/j.1469-8137.2005.01585.x
- Jones HG (1992) 'Plants and microclimate: a quantitative approach to environmental plant physiology.' 2nd edn. (Cambridge University Press: New York)
- Kao W, Chang K (2001) Altitudinal trends in photosynthetic rate and leaf characteristics of *Miscanthus* populations from central Taiwan. *Australian Journal of Botany* **49**, 509–514. doi: 10.1071/BT00028
- Körner Ch (1998) A re-assessment of high elevation treeline positions and their explanation. *Oecologia* **115**, 445–459. doi: 10.1007/s004420050540
- Körner Ch (1999) 'Alpine plant life: functional ecology of high mountain ecosystems.' (Springer: New York)
- Körner Ch, Diemer M (1987) In situ photosynthetic responses to light, temperature and CO₂ in herbaceous plants from low and high elevations. *Functional Ecology* **1**, 179–194. doi: 10.2307/2389420
- Körner Ch, Mayr R (1981) Stomatal behavior in alpine plant communities between 600 and 2600 meters above sea level. In 'Plants and their atmospheric environment'. (Eds J Grace, ED Ford, PG Jarvis) pp. 205–218. (Blackwell: Oxford)
- Körner Ch, Neumayer M, Menendez-Reidl SP, Smeets-Scheel A (1989) Functional morphology of mountain plants. *Flora* **182**, 353–383.

- Larcher W (1995) 'Physiological plant ecology.' 3rd edn. (Springer: New York)
- Mazzarino MJ, Bertiller M, Schlichter T, Gobbi M (1998) Nutrient cycling in Patagonian ecosystems. *Ecologia Austral* **8**, 167–181.
- Mooney HA, Gulmon SL (1982) Constraints on leaf structure and function in reference to herbivory. *Bioscience* **32**, 198–206. doi: 10.2307/1308943
- Nelson DW, Sommers LE (1996) Total carbon, organic carbon and organic matter. In 'Methods of soil analysis. Part 3. Chemical methods. Soil Science Society of America Book Series no. 5'. (Ed. DL Sparks) pp. 961–1010. (American Society of Agronomy: Madison, WI)
- Nobel PS (1991) 'Physiochemical and environmental plant physiology.' (Academic Press: New York)
- Oleksyn J, Modrzynski J, Tjoelker MG, Zytkowski R, Reich PB, Karolewski P (1998) Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. *Functional Ecology* **12**, 573–590. doi: 10.1046/j.1365-2435.1998.00236.x
- Pisek A, Winkler E (1958) Assimilationsvermögen und Respiration der Fichte (*Picea excelsa* Link.) in verschiedener Höhenlage und der Zirbe (*Pinus cembra* L.) an der alpinen Waldgrenze. *Planta* **51**, 518–543. doi: 10.1007/BF01883340
- Premoli AC (1994) Genetic, morphological, and ecophysiological variation in geographically restricted and widespread species of *Nothofagus* from southern South America. PhD Thesis, University of Colorado, Boulder, CO.
- Premoli AC (2003) Isozyme polymorphisms provide evidence of clinal variation along altitudinal gradients in *Nothofagus pumilio* (Poepp. et Endl.) Krasser. *Journal of Heredity* **94**, 218–226. doi: 10.1093/jhered/esg052
- Premoli AC (2004) Variación en *Nothofagus pumilio* (Poepp. et Endl.) Krasser. In 'Variación intraespecífica en las especies arbóreas de los bosques templados de Chile y Argentina'. (Eds ZC Donoso, AC Premoli, L Gallo, R Ipinza) pp. 145–172. (Editorial Universitaria: Santiago, Chile)
- Premoli AC, Raffaele E, Mathiasen P (2007) Contrasting elevations maintain heritable morphological and phenological differences in *Nothofagus pumilio*. *Austral Ecology* **32**, 515–523.
- Read J (1985) The dynamics of *Nothofagus cunninghamii* rainforest associations in Tasmania—an ecophysiological approach. PhD Thesis, University of Tasmania, Hobart, Tas.
- Schoettle AW, Rochelle SG (2000) Morphological variation of *Pinus flexilis* (Pinaceae), a bird-dispersed pine, across a range of elevations. *American Journal of Botany* **87**, 1797–1906. doi: 10.2307/2656832
- Singer R, Morello JH (1960) Ectotrophic forest tree mycorrhiza and forest communities. *Ecology* **41**, 549–551. doi: 10.2307/1933331
- Slyter RO, Morrow PA (1977) Altitudinal variation in the photosynthetic characteristics of snow gum, *Eucalyptus pauciflora* Sieb. ex Spreng. I. Seasonal changes under field conditions in the Snowy Mountains area of south-eastern Australia. *Australian Journal of Botany* **25**, 1–20. doi: 10.1071/BT9770001
- Tranquillini W, Havranek WM (1985) Influence of temperature on photosynthesis in spruce provenances from different altitudes. In 'Establishment and tending of subalpine forest: research and management'. (Eds H Turner, W Tranquillini) pp. 41–51. (Berichte der Eidgenössischen Anstalt für das forstliche Versuchswesen)
- Veblen TT, Ashton DH (1979) Successional pattern above timber-line in south-central Chile. *Vegetatio* **40**, 39–47. doi: 10.1007/BF00052013
- Veblen TT, Donoso C, Kitzberger T, Rebertus AJ (1996) Ecology of southern Chilean and Argentinean *Nothofagus* species. In 'The ecology and biogeography of *Nothofagus* forests'. (Eds TT Veblen, RS Hill, J Read) pp. 293–354. (Yale University Press: New Haven, CT)
- Wong SC (1979) Elevated atmospheric partial pressure of CO₂ and plant growth. I. Interactions of nitrogen nutrition and photosynthetic capacity in C₃ and C₄ plants. *Oecologia* **44**, 68–74. doi: 10.1007/BF00346400
- Woodward FI (1986) Ecophysiological studies on the shrub *Vaccinium myrtillus* L. taken from a wide altitudinal range. *Oecologia* **70**, 580–586. doi: 10.1007/BF00379908
- Woodward FI (1987) Stomatal numbers are sensitive to increases in CO₂ from pre-industrial levels. *Nature* **327**, 617–618. doi: 10.1038/327617a0
- Zhang J, Marshall JD, Jaquish BC (1993) Genetic differentiation in carbon isotope discrimination and gas exchange in *Pseudotsuga menziesii*. *Oecologia* **93**, 80–87. doi: 10.1007/BF00317668

Manuscript received 10 February 2006, accepted 15 May 2007