

Nature vs nurture in the leaf morphology of Southern beech, *Nothofagus cunninghamii* (Nothofagaceae)

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Summary

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- Leaf morphology varies predictably with altitude, and leaf morphological features have been used to estimate average temperatures from fossil leaves. The altitude–leaf morphology relationship is confounded by the two processes of acclimation and adaptation, which reflect environmental and genetic influences, respectively.
- Here we describe the relationship between altitude and leaf morphology for Southern beech, *Nothofagus cunninghamii* (Hook.) Oerst.. Cuttings from several trees from each of four altitudes were grown in a common glasshouse experiment, and leaf morphology related to both genotype and altitude of origin.
- Genotype had a significant impact on leaf morphology, but in the field there was also a significant, overriding effect of altitude. This altitude effect disappeared in glasshouse-grown plants for all morphological variables other than leaf thickness and specific leaf area.
- These results show that, while leaf length, width and area are partially controlled by genetic factors, these variables are plastic and respond to environmental influences associated with a particular altitude. Thus altitudinal trends in leaf size in *N. cunninghamii* are unlikely to be the result of adaptation.

Key words: altitude, leaf size, stomatal density, palaeoecology, genotype, *Nothofagus cunninghamii*, Southern beech.

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Introduction

The relationship between altitude and plant morphology is of great interest to plant physiologists, ecologists and palaeobotanists alike. These interests are largely caused by the fundamental influence of altitude on environmental variables such as temperature and the partial pressure of CO₂, and hence the possibility of acclimation and adaptation to particular environmental conditions (Körner, 1999). High-altitude species tend to be morphologically and physiologically distinct from closely related species from lower altitudes. Altitude also has a major effect on leaf morphology and physiology within a species. Leaves generally decrease in length, width and area with increasing altitude (Körner *et al.*, 1986), while leaf thickness increases with increasing altitude (Körner *et al.*, 1989; Roderick *et al.*, 2000), causing a reduction of specific leaf area (leaf area per unit leaf d. wt; SLA). In addition to a general thickening of the leaf lamina, increasing altitude can also lead to increases in the relative

thickness of cuticle, epidermis and, in some cases, the development of a hypodermis (Tanner & Kapos, 1982). Such morphological changes are no doubt related to environmental stresses associated with living at high altitudes.

The presence of relationships between altitude and leaf morphological and anatomical variables has resulted in the estimation of past climatic conditions from both historic and fossil leaves (Beerling & Kelly, 1997; Jordan, 1999; Rundgren & Beerling, 1999). There has also been interest in adaptation to particular altitudes, as there is evidence that plants originating from different altitudes remain different when grown at the same altitude (Körner & Diemer, 1994; Hovenden & Brodribb, 2000). Adaptation to a particular altitude is therefore a distinct possibility within a species, and altitudinal differences within species have been formalized into subspecific taxa in some instances. For example, separate subspecies of *Leptorhynchus squamatus* have recently been described based on persistent differences in leaf morphology between alpine and lowland individuals (Flann *et al.*, 2002). If plants from

across altitudinal gradients are adapted to their particular altitudinal conditions, this questions the validity of climatic reconstructions based on particular leaf morphological or anatomical criteria, as these variables may not accurately reflect the particular climatic variable as assumed in such reconstructions.

Part of the problem in interpreting the well documented relationship between altitude and leaf morphology and physiology is the confounding of environmental and genetic or microevolutionary factors. Within any one species, leaf area may decrease with increasing altitude because of direct environmental impacts on leaf development and expansion, or because of genetic controls on these processes. In other words, selection may act such that plants from higher altitudes tend to have smaller leaves independent of the environmental conditions under which the leaf developed and expanded. This has been seen in the tree species *Metrosideros polymorpha*, which showed phenotypic plasticity as well as a genetic basis for morphological variation along an altitudinal gradient (Cordell *et al.*, 1998). Cordell *et al.* (1998) argued that the combination of plasticity and genetic determination allowed this species to dominate a wide ecological distribution. A similar study showed that the extensive altitudinal distribution of *Pennisetum setaceum* was the result of ecological tolerance rather than the adaptation of specific ecotypes (Williams & Black, 1993). This confounding between potentially causative factors not only has implications for our understanding of the relationship between plants and their environment, but also interferes with the formulation of descriptive models such as those developed by Jordan & Hill (1994).

Southern beech, *Nothofagus cunninghamii* (Hook.) Oerst., is a dominant rainforest tree of cool temperate south-eastern Australia, but is most common in Tasmania. It has a large altitudinal range, occurring from sea level to over 1500 m in Tasmania, and occurs at higher altitudes in Victoria (Curtis & Morris, 1975). There is an extensive and well documented leaf fossil record for *N. cunninghamii* stretching back to the early Pleistocene (Jordan & Hill, 1994). Additionally, leaf morphological characters including leaf size, thickness and SLA are strongly influenced by altitude in this species (Jordan & Hill, 1994; Hovenden & Brodribb, 2000). However, leaf morphology of *N. cunninghamii* is also under strong genetic control (Hovenden, 2001). Thus *N. cunninghamii* presents an excellent opportunity to investigate the relative contributions of environmental and genetic factors to the relationship between leaf morphology and altitude in a species with a wide altitudinal range.

The aim of this study was therefore to investigate the influence of altitude of origin on leaf morphology of *N. cunninghamii*. This was done using a 'common-garden' experiment, in which a large number of cuttings from several trees at each of four altitudes were grown in a single glasshouse. The effects of altitude of origin and genotype were then determined for leaves expanded in both the field and glasshouse.

Materials and Methods

A single branch bearing *c.* 300 leaves was collected from each of 12–14 adult *N. cunninghamii* trees at four altitudes within Mt Field National Park, Tasmania (42°58' S, 146°28' E). All branches were collected on the northern (sunny) side of the trees and 1–4 m above the ground. The four sites, at altitudes of 1100, 780, 350 and 100 m, are located along the Lake Dobson Road and occur within *c.* 15 km of each other. As each tree sampled was single-stemmed and separate from all others, it was assumed that each tree was a genetically distinct individual, henceforth referred to as a genotype. A sample of *c.* 20 leaves was collected from each branch for measurement of field-grown leaf characters. Numerous cuttings were made from the remainder of each branch and placed in shallow, rectangular pots on a heat bed at the School of Plant Science, University of Tasmania (42°55' S, 147°20' E), 100 m above sea level. Cuttings struck from between eight and 12 genotypes from each altitude.

Those cuttings that struck successfully were planted individually into 15 cm diameter pots containing a mixture of equal volumes of sharp sand and pine bark, to which slow-release fertilizer was added (Osmocote 9 month, Scotts-Sierra Horticultural Products, Maryville, OH, USA). The plants were then transferred to a temperature- and humidity-controlled glasshouse. Temperature was controlled at 25/12°C on a 14/10 h day/night cycle. Relative humidity was controlled at 85 ± 5% day and night. Plants were watered by overhead irrigation twice daily, which was sufficient to keep the soil moist at all times.

Morphology measurements

Young, fully expanded leaves were collected from each genotype after 3 months' growth. Both field- and glasshouse-grown leaves were measured for leaf gross morphology. Leaf length, width and thickness were all measured using digital callipers. Leaf length was measured from base of petiole to leaf tip, while leaf width was recorded as maximum width of the blade. Leaf thickness was measured for the central part of the lamina, half-way between midrib and margin. Leaf area was measured using a flat-bed scanner connected to a personal computer running image analysis software. All material was dried in an oven at 70°C for 1 wk, then weighed to obtain leaf dry weight. Specific leaf area was expressed as leaf area per unit leaf d. wt.

Stomatal density measurements

Stomatal density counts were recorded from five fields of view of recently fully expanded leaves by isolating the leaf cuticle. Two leaves were taken from three plants in the glasshouse of each genotype from each altitude. Of the field plants, three leaves per genotype from each altitude were used. The cuticle

bears a lasting impression of the epidermis and is very resilient. Cuticular material is sometimes the only fossil material available, so much of the fossil data, stomatal index and density are based on leaf cuticles (Jordan, 1999; Jordan & Hill, 1999). *Nothofagus cunninghamii* is hypostomatous, so only the abaxial surfaces were examined. Each leaf, with its edges removed to allow the solution to penetrate, was placed in 5 ml 15% Cr_2O_3 solution. The leaves were left in solution for 3 d until all the mesophyll tissue had disintegrated. Cuticles were then removed from the solution, washed and mounted, unstained, in glycerin jelly, and examined under a light microscope. Mounted cuticles were examined through a light microscope and photographed with a digital camera. Each field of view was $310 \times 235 \mu\text{m}$ and contained between 20 and 50 stomata. Five fields of view were photographed per leaf, and the stomatal density estimated per field of view. Calibration was achieved using a stage micrometer.

Statistical analyses

Results were analysed by nested ANOVA using general linear model procedures in the SAS statistical program (SAS Institute Inc., 2000). Analyses were performed on natural logarithm transforms of raw leaf measurements to stabilize variances. Field data were analysed with genotype nested within altitude: (genotype(alitude)). As replicate cuttings were measured for each genotype in the glasshouse, these data were analysed with pot(genotype) as well as genotype(alitude). Where there was a significant pot(genotype) effect, genotype(alitude) effects were assessed using the mean square of pot(genotype) as the error term. Where there was a significant genotype(alitude) effect, altitude effects were assessed using the mean square of genotype(alitude) as the error term. Where significant differences occurred, means were compared using the Ryan–Einot–Gabriel–Welsch *post hoc* comparison (Day & Quinn, 1989). Relationships between field and glasshouse leaf characters were tested on a genotype basis using linear regression procedures in the SAS statistical program.

Results

Both altitude and genotype had a significant impact on leaf morphology of *N. cunninghamii* leaves grown in the field (Table 1). Thus while leaf morphology depended on genotype, there was still an overriding and highly significant effect of altitude on all features. Leaves grown in the field became smaller in length, width, overall area and weight (Fig. 1), but became thicker and hence had decreasing SLA with increasing altitude (Fig. 2).

Leaves from each altitude differed significantly from leaves from all other altitudes in length, width, area (Fig. 1) and SLA (Fig. 2). There was no significant difference in the dry weight of leaves from 350 and 780 m (Fig. 1). The leaf length : width (*L* : *W*) ratio differed only between 'high' and 'low' altitudes, with leaves from 150 and 350 m being more lanceolate than those from 780 and 1100 m (Fig. 2). Stomatal density of field-grown leaves generally increased with increasing altitude, although leaves from 780 m had a higher stomatal density than those from 1100 m (Fig. 2).

Within each altitude, there was a significant impact of genotype on all morphological characters measured (Table 1). The variation within each altitude is evident in Figs 3, 4. The degree of variation among and within genotypes within each altitude depended on the character, with less variation in leaf length among co-occurring genotypes than there was in leaf dry weight. This is supported by the results of the ANOVAs (Table 1). Despite the variation among genotypes from the same altitude, the trends are evident, particularly for leaf length and width.

When cuttings from different altitudes were grown in the glasshouse, only stomatal density and leaf thickness, and hence SLA, were significantly affected by altitude of origin (Table 1). Other leaf morphological features were unaffected by altitude of origin, but apart from leaf dry weight, all were significantly affected by genotype. There was also a significant pot effect on all characters other than stomatal density (Table 1). The lack of a significant impact of altitude of origin

Table 1 Results of ANOVA for field- and glasshouse-grown leaves of *Nothofagus cunninghamii*

Variable	Field-grown		Glasshouse-grown		
	Altitude	Genotype	Altitude	Genotype	Pot
Length	0.0001	0.0001	0.17	0.007	0.001
Width	0.0001	0.0001	0.63	0.007	0.05
Thickness	0.0001	0.01	0.01	0.003	0.001
Area	0.0001	0.0001	0.59	0.02	0.001
Dry weight	0.04	0.0001	0.57	0.09	0.0001
SLA	0.0001	0.0001	0.03	0.02	0.0001
<i>L</i> : <i>W</i>	0.0001	0.0001	0.45	0.002	0.0002
Stomatal density	0.0008	0.0001	0.0005	0.005	0.25

Values shown are $P < F$. SLA, specific leaf area (leaf area per unit d. wt); *L* : *W*, leaf length to width ratio; significant effects ($P < 0.05$) in bold.

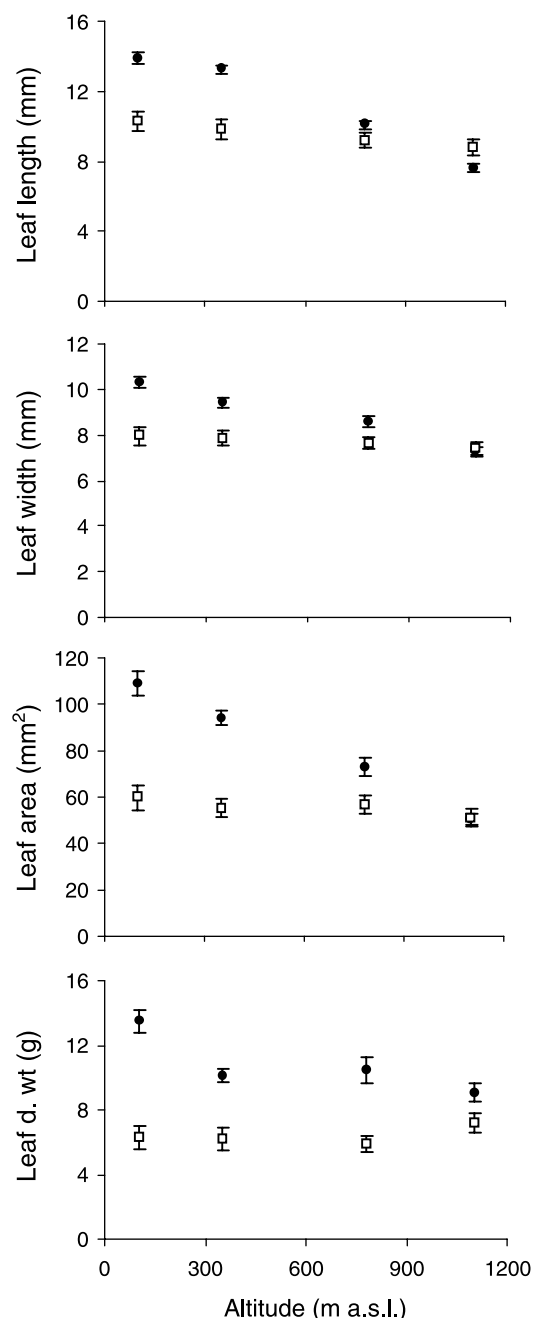


Fig. 1 Influence of altitude on leaf size in *Nothofagus cunninghamii*. Closed symbols, field-grown; open symbols, glasshouse-grown leaves. Error bars, \pm SE.

on glasshouse-grown leaves is also clear from Figs 3, 4 which demonstrate the variation among genotypes.

While there is a significant impact of altitude of origin on stomatal density, SLA and leaf thickness, the trends across the transect vary from those evident for the field-grown leaves. The SLA of field-grown leaves was greatest at 350 m, followed by 100, then 780 m, and was lowest at 1100 m (Fig. 2). The situation for glasshouse-grown leaves was different, with

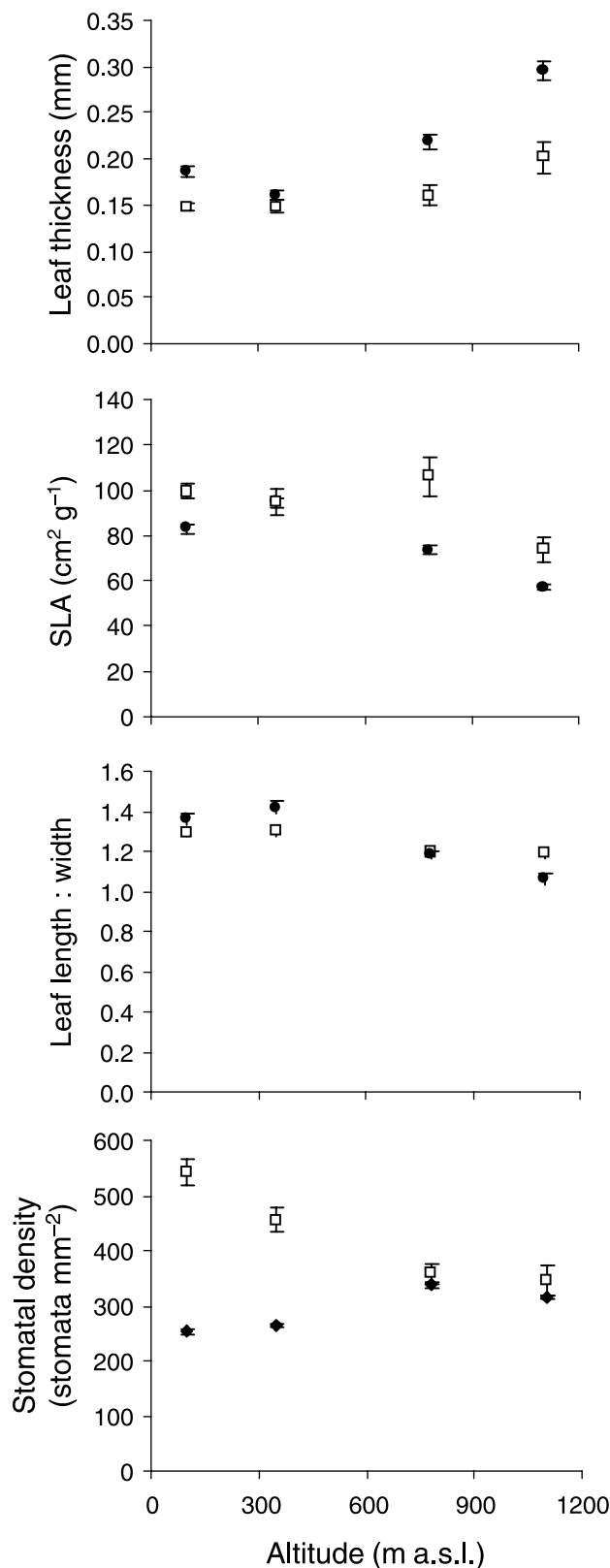


Fig. 2 Altitude effects on leaf morphology and stomatal density of *Nothofagus cunninghamii*. Closed symbols, field-grown; open symbols, glasshouse-grown leaves. Error bars, \pm SE.

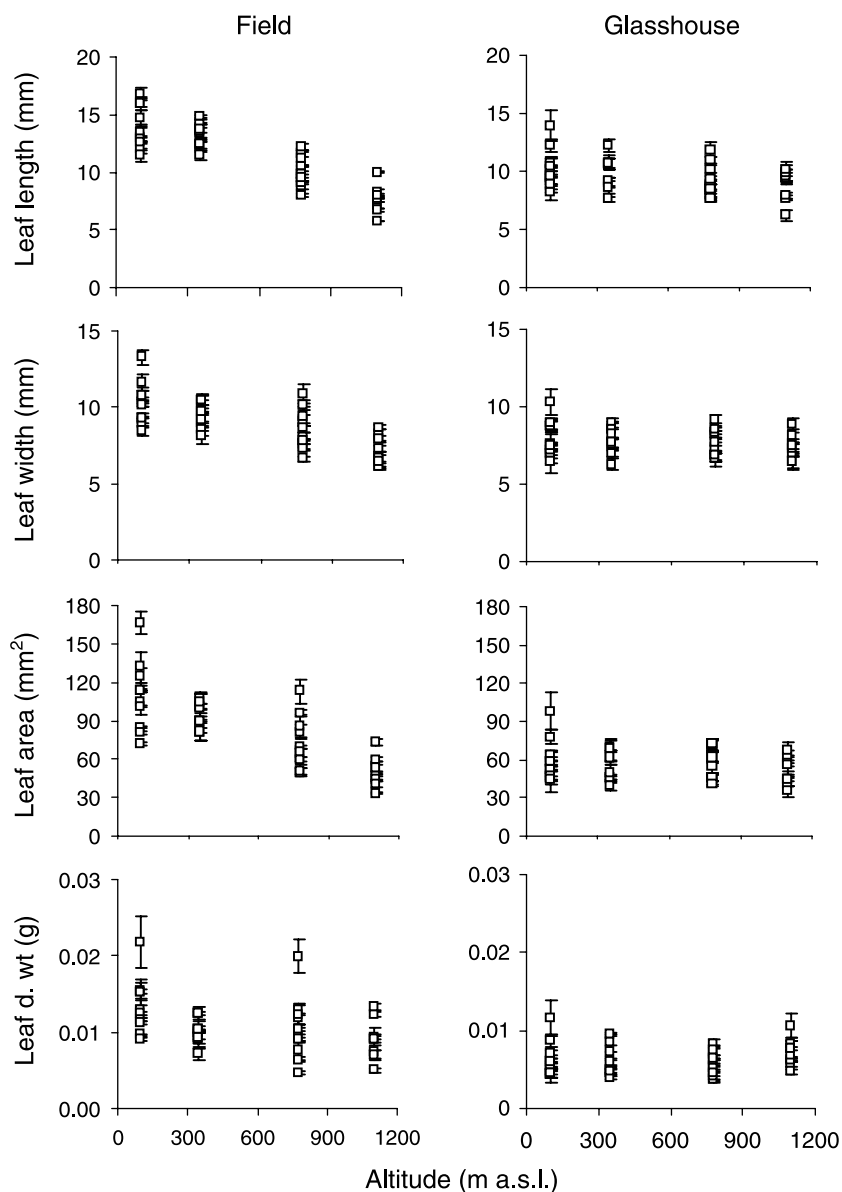


Fig. 3 Variation in leaf size among genotypes of *Nothofagus cunninghamii* from each altitude for field-grown leaves (left) and glasshouse-grown leaves (right). Each point is the mean for a genotype \pm SE.

plants from 780 m having the greatest SLA, plants from 1100 m the lowest, and plants from 350 and 100 m being intermediate between the two (Fig. 2). The altitudinal trend in stomatal density of glasshouse-grown leaves was the opposite of that for field-grown leaves. Field-grown leaves showed an overall increase in stomatal density with increasing altitude, whereas stomatal density decreased with increasing altitude of origin for glasshouse-grown leaves (Figs 2, 4).

While there was no significant impact of altitude of origin on the size of glasshouse-grown leaves (Table 1), leaf length and width declined slightly with increasing altitude of origin (Figs 1, 3). Leaf $L:W$ ratio also tended to decrease with increasing altitude of origin (Fig. 2).

Glasshouse-grown leaves were, on average, significantly smaller than those collected in the field (Fig. 1). This was

largely caused by the large size of field-grown low-altitude leaves (Fig. 1). This trend is evident in both leaf length and width, but is most pronounced in leaf area. Glasshouse-grown leaves were also thinner and lighter than field-grown leaves (Fig. 1). For instance, field-grown leaves from 100 m were 35% longer, 29% wider, had 81% greater shadow area and were 114% heavier than glasshouse-grown leaves from the same altitude of origin (Fig. 1). The differences between glasshouse-grown and field leaves were most prominent at lower altitudes, but at high altitudes there was little difference between field- and glasshouse-grown leaves. For example, the average leaf area of field-grown leaves at 1100 m was 50 mm², while that of glasshouse-grown leaves was 52 mm² (Fig. 1).

We investigated further the impact of genotype on leaf morphology of *N. cunninghamii* by regression analyses. For

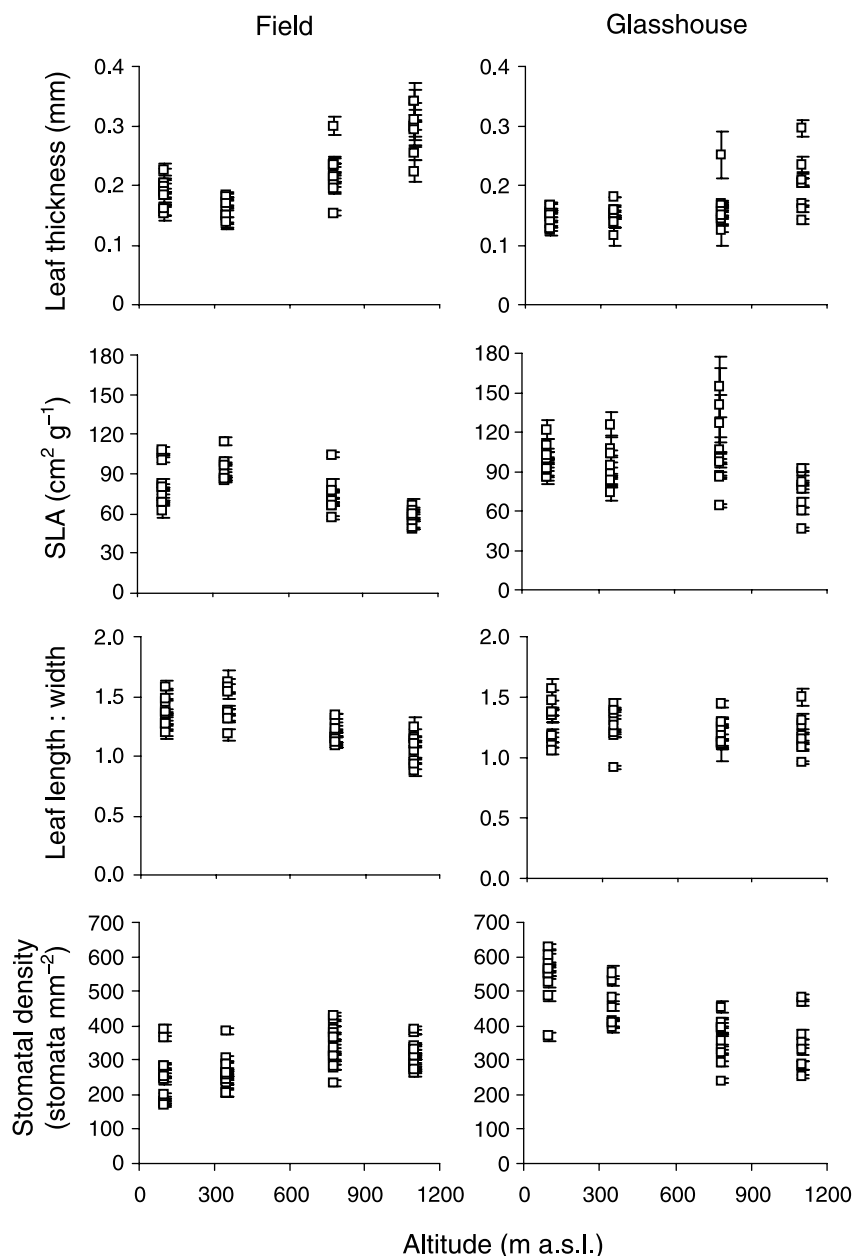


Fig. 4 Variation in leaf morphology and stomatal density among genotypes of *Nothofagus cunninghamii* from each altitude for field-grown leaves (left) and glasshouse-grown leaves (right). Each point is the mean for a genotype \pm SE.

leaf length, width, thickness, area, d. wt, SLA and $L : W$ ratio, there was a statistically significant relationship between field-grown leaves and those developed in the glasshouse when analysed on a genotype basis (Table 2). This shows that each of these characters was partially genetically controlled, but that the degree of control, while statistically significant, was generally low. The r^2 values indicate that genotype explained between 10 and 15% of the variation in leaf morphological characters, apart from leaf length and thickness, in which genotype accounted for 21 and 38% of the variation, respectively (Table 2). The stomatal density of glasshouse-grown leaves was not significantly related to the stomatal density of

field-grown leaves when analysed on the basis of genotype (Table 2).

Discussion

Leaf morphology in *N. cunninghamii* is significantly affected by altitude, in a manner similar to that of many other species (Halloy & Mark, 1996). Our results indicate that, while there is a significant genetic basis to the variation in leaf form in *N. cunninghamii*, the morphological response to the environment generally overrides the genetic influence. Thus, when plants from a range of altitudes were grown in the same

Table 2 Results of regression analyses between field- and glasshouse-grown leaves of *Nothofagus cunninghamii* analysed by genotype

Variable	r^2	$P < F$
Leaf length	0.21	0.003
Leaf width	0.14	0.015
Leaf thickness	0.38	0.0001
Leaf area	0.13	0.02
Leaf weight	0.10	0.03
SLA	0.15	0.01
$L : W$	0.11	0.03
Stomatal density	0.05	0.21

Glasshouse leaf variables were the dependent variables. SLA, specific leaf area (leaf area per unit d. wt); $L : W$, leaf length to width ratio.

environment, there was no significant impact of altitude of origin on leaf morphology, apart from leaf thickness, SLA and stomatal density. Thus in the field leaf morphology was significantly dependent on altitude, despite significant variation among plants within each altitude. When plants from a range of altitudes were grown in a common environment, this altitudinal effect disappeared while the genotype effect remained. Regression analyses indicated that the leaf morphology of a particular plant is 10–38% dependent upon its genotype.

The results shown here have important implications for our understanding of the relationship between leaf morphology and both altitude and climate. Past studies have used various leaf morphological characteristics to infer past climates from fossil and subfossil leaves (Wolfe & Upchurch, 1987; Christophel & Greenwood, 1989). The genus *Nothofagus* has been used extensively for this purpose (Hill & Read, 1987; Jordan & Hill, 1994; Jordan, 1999). Much of the supporting evidence for these inferences comes from the relationship between leaf size and altitude in extant populations (Jordan & Hill, 1994). There is well established evidence that leaves of *N. cunninghamii* decrease in length and area with increasing altitude (Jordan & Hill, 1994; Potter, 1997). However, previous work showed a significant impact of genotype on leaf morphology, which cast doubt on the reliability of the relationship between leaf morphology and altitude (Hovenden & Brodribb, 2000; Hovenden & Schimanski, 2000; Hovenden, 2001). This work confirms significant variation in leaf morphology among individuals growing at the same site, but also demonstrates that environmental variables have an overriding influence on leaf length, width, area and $L : W$ ratio. The strong environmental influence on leaf morphology is further supported by the significant pot effect in the glasshouse experiment. This shows that, for a given genotype, small differences in growth conditions associated with the placement of different clones caused significant variation in all gross leaf morphological variables (Table 1). Hence it is likely that leaf length, width, area and $L : W$ ratio are all sensitive to environ-

mental variables and therefore may be useful in palaeoclimatic reconstructions using *N. cunninghamii*.

Leaf thickness of glasshouse-grown plants was significantly affected by altitude of origin and, consequently, so was SLA (Table 1). This indicates that leaf thickness is under stronger genetic control than the other leaf morphological variables. Thus the increase in leaf thickness with increasing altitude is likely to be the result of natural selection rather than a direct phenological response to some environmental variable. This is supported by regression analyses (Table 2), which showed that nearly 40% of the variation in leaf thickness was because of genotype. Leaf thickness is important in terms of carbon assimilation as, so long as light is not limiting, thicker leaves tend to have a higher photosynthetic rate per unit leaf area (Körner & Diemer, 1994). Plants from higher altitudes have higher carbon assimilation rates per unit area (Hovenden & Brodribb, 2000), and there is a genetic basis for this difference (Körner & Diemer, 1994), which supports the proposition that thicker leaves would be selected for with increasing altitude.

Previous research using common-garden experiments has shown that adaptation to particular environmental conditions does exist within some species. For example, there is a significant and persistent effect of site of origin on the leaf size of *Populus deltoides* (Rowland, 2001) and *Alchemilla alpina* (Morecroft & Woodward, 1996). The C_4 grass species *Pennisetum setaceum* possesses a high degree of plasticity, and there is no evidence of its adaptation to specific environments in Hawaii, where the species is an invasive exotic (Williams & Black, 1993). This may be because all individuals of *P. setaceum* in Hawaii are descended from a single genotype. However, leaf physiology is also independent of environment of origin in the subarctic dune species *Leymus mollis* (Imbert & Houle, 2000). This contrasts with the situation for *Picea abies*, in which altitude of origin has a strong influence on leaf physiology (Oleksyn *et al.*, 1998). Our results show that most leaf morphological variation in *N. cunninghamii* is environmentally determined, but SLA and leaf thickness are also dependent on altitude of origin. This mix of genetic and environmental influences on morphology of *N. cunninghamii* leaves is also seen in various other species that occur along environmental gradients, including *Metrosideros polymorpha*, in which leaf morphology was largely genetically determined but leaf anatomy and physiology were environmentally determined (Cordell *et al.*, 1998). Thus it appears that the degree of environmental plasticity and adaptation is species-dependent.

Leaf epidermal characters such as stomatal index and stomatal density are increasingly being used to draw climatic inferences from fossils, most popularly to estimate $[CO_2]$ (Beerling *et al.*, 1995; Kürschner, 1996; Atchison *et al.*, 2000). Stomatal density varies with altitude in many species (Körner *et al.*, 1986; Körner & Cochrane, 1986; Woodward, 1986). Additionally, it has been argued that stomatal density is not always a good indicator of $[CO_2]$ (Körner, 1988), given the range of influences on leaf morphology, including

microsite variation, sampling, phenological and genotypic effects. This research has shown that stomatal density of *N. cunninghamii* is significantly affected by altitude (Table 1; Fig. 1), despite significant variation among genotypes within each altitude. However, while there was a significant effect of altitude of origin on stomatal density of glasshouse-grown leaves, the trend of stomatal density with altitude was markedly different in glasshouse- and field-grown leaves (Fig. 1), and there was no correlation between field and glasshouse stomatal density on a genotype basis (Table 2). Stomatal density of plants from 100 and 350 m was much higher in glasshouse-grown than in field-grown plants, whereas leaf area was much lower in glasshouse-grown than in field-grown plants. Two major factors determine stomatal density in any given leaf. The first is stomatal initiation, the specific developmental process that leads to the development of stomatal guard cells from undifferentiated epidermal cells. Stomatal initiation is estimated by calculation of the stomatal index, the ratio of stomatal pores to all epidermal cells. The second process is leaf expansion, which affects epidermal cell size and therefore the number of epidermal cells per unit area, thereby affecting stomatal density. There is, however, a third factor that may influence stomatal density: the total number of epidermal cells in the leaf. Two leaves of the same size, which have the same ratio of stomatal pores to epidermal cells, will have different stomatal densities if they have different numbers of epidermal cells on the leaf surface. Therefore stomatal density increases either when stomatal initiation is increased, as this increases the number of stomata per epidermal cell; or when leaf expansion is reduced, as this leads to an overall reduction in leaf surface area and hence the same number of stomata in a smaller area; or when the total number of epidermal cells on a leaf is increased. The results shown here indicate that stomatal density of glasshouse-grown plants from 100 and 350 m was higher than those from the field because leaf expansion was reduced in the glasshouse-grown leaves. Thus stomatal density was indirectly affected by environmental factors that altered leaf size by reducing leaf expansion. Stomatal density therefore may be a useful climatic indicator.

Previous work has shown the strong influence of genotype on leaf morphology of *N. cunninghamii* (Hovenden & Schimanski, 2000), a trend strongly supported here. Previous research on *N. cunninghamii* used few genotypes from few altitudes, and this suggested that there might have been more variation among genotypes within a site than among sites in all characters of palaeoclimatological interest. This work has shown that there is indeed a large degree of variation among genotypes within a site, but that there is still a significant effect of altitude on field-grown leaves. One of the concerns about the potential confounding of genetic and environmental controls of leaf morphology is that genetic control may mask climatic signals in the historic and prehistoric records (Hovenden, 2001). This would be particularly pronounced for long-lived species. This is unlikely to be the case for

N. cunninghamii for leaf size characteristics other than leaf thickness, which would all be useful indicators of environmental conditions.

As the evidence from this common-garden experiment indicates that the reduction in leaf size with increasing altitude is dependent on environmental factors, the challenge is to determine the environmental variable, or combination of variables, contributing to this trend. Jordan & Hill (1994) demonstrated that leaf length of *N. cunninghamii* along an altitudinal gradient was significantly related to temperature, but experimental work showed that a 5°C temperature difference had no effect on leaf expansion of well watered *N. cunninghamii* (Hovenden, 2001). This is also the case for Douglas fir, *Pseudotsuga menziesii* (Apple *et al.*, 2000). Roderick *et al.* (2000) used models and data from various species of *Eucalyptus* to demonstrate that leaf area, and hence leaf size, is influenced by a combination of nutrients and water availability. Further, McDonald *et al.* (2003) demonstrated that water availability and soil phosphorus were independently related to leaf size in 690 species at 47 sites. As water and nutrients were equally available for glasshouse-grown leaves in this experiment, the lack of any differences in leaf area between the treatments is consistent with this model. It is possible that water availability and nutrition are important determinants of leaf size in *N. cunninghamii* in the field. It is interesting that glasshouse-grown leaves from low altitudes were much smaller than those grown in the field. As the soil was always kept moist, it is unlikely that water availability was limiting leaf expansion in the glasshouse. It is possible that the plants were mildly nutrient-limited, as they were only supplied with slow-release fertilizer.

Light exposure was unlikely to have been consistent across the altitudinal transect, despite the fact that the most sun-exposed leaves were collected at each site. Shaded glasshouse-grown leaves are significantly larger than fully exposed leaves (M.J.H., unpublished), so it is possible that the low-altitude leaves were partial shade leaves. Leaves from the two high-altitude sites were definitely full-sun leaves, as the vegetation at both sites was short open woodland. At low-mid altitudes, *N. cunninghamii* occurs in tall mixed forest and hence only the uppermost canopy leaves would receive sunlight throughout the day. Field leaves from the two lower sites therefore received direct sunlight for only part of the day, despite the fact that they were collected from the edges of clearings. Thus sun exposure may be the major factor contributing to the leaf size-altitude relationship in *N. cunninghamii*. If this is the case, there are major implications for climatic reconstructions based on leaf macrofossils.

There was a large degree of genetic variability in leaf morphology of co-occurring *N. cunninghamii* individuals. Genetic variability is evolutionarily advantageous in an unreliable environment and, according to Endler (1977), is a result of a more recent mixing of genotypes. *N. cunninghamii* is poorly dispersed, so it is likely that near neighbours are

maternally related. However, *N. cunninghamii* is wind-pollinated, so it is possible that the large degree of genetic variability within a site is caused by variation in paternity. The combination of morphological plasticity and genetic variability of *N. cunninghamii* has undoubtedly contributed to its wide distribution in south-eastern Australia (Read & Busby, 1990), which includes disturbed sites and environments not generally suitable for other rainforest species.

In conclusion, both genetic and environmental processes contribute to leaf morphological trends in *N. cunninghamii*. Despite the high degree of genotypic variation seen in this and previous studies, leaf length, width, area and $L:W$ ratio are all significantly related to environmental conditions. Therefore we believe that historic and prehistoric leaf samples can be reliably used for climatic reconstructions, provided sufficient samples are acquired to account for genotypic variability. It is not clear what the exact environmental controls of leaf morphology are in *N. cunninghamii*, but future work should investigate the impact of nutrient, light and water availability on leaf morphological characters of this species.

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