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Research paper

To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech?

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The phenotypic responses of functional traits in natural populations are driven by genetic diversity and phenotypic plasticity. These two mechanisms enable trees to cope with rapid climate change. We studied two European temperate tree species (sessile oak and European beech), focusing on (i) in situ variations of leaf functional traits (morphological and physiological) along two altitudinal gradients and (ii) the extent to which these variations were under environmental and/or genetic control using a common garden experiment. For all traits, altitudinal trends tended to be highly consistent between species and transects. For both species, leaf mass per area displayed a positive linear correlation with altitude, whereas leaf size was negatively correlated with altitude. We also observed a significant increase in leaf physiological performance with increasing altitude: populations at high altitudes had higher maximum rates of assimilation, stomatal conductance and leaf nitrogen content than those at low altitudes. In the common garden experiment, genetic differentiation between populations accounted for 0–28% of total phenotypic variation. However, only two traits (leaf mass per area and nitrogen content) exhibited a significant cline. The combination of in situ and common garden experiments used here made it possible to demonstrate, for both species, a weaker effect of genetic variation than of variations in natural conditions, suggesting a strong effect of the environment on leaf functional traits. Finally, we demonstrated that intrapopulation variability was systematically higher than interpopulation variability, whatever the functional trait considered, indicating a high potential capacity to adapt to climate change.

Keywords: adaptation, altitude, common garden, functional traits, genetic variation, phenotypic variations.

Introduction

Over the last few decades, attention has increasingly focused on evolutionary responses to rapid climate change (reviews by Bradshaw and Holzapfel 2006, Parmesan 2006). One major concern in this context relates to the ability of long-lived species to cope with rapid climate change (Saxe et al. 2001, Kremer 2007, Lindner et al. 2009). Stressful environments, such as high mountain ecosystems, provide an ideal experimental setting for investigating adaptation in such species, as selection pressures along ecological gradients are increased

by climate change (Lesica and McCune 2004, Pauli et al. 2007). Habitat fragmentation may limit range shifts (Davis and Shaw 2001), but an upward shift has been reported for many species in Europe (Lenoir et al. 2008). In mountainous regions, sharp changes in abiotic factors occur over short distances, leading to major changes in the selection pressures acting on plant life-history traits; the responses to these selection pressures may differ between species (Vitasse et al. 2009a). Thus, altitudinal gradients provide an ideal experimental opportunity for studying the variation of functional traits in plants in response to environmental factors and the implications of this

variation for the capacity of populations to respond to changing environmental conditions (Körner 2007).

The maintenance of a positive carbon balance in a wide range of environments is a key feature of plant species with wide ecological distributions (Spencer et al. 1994, Sultan et al. 1998). Leaf ecophysiological traits such as photosynthetic capacity, stomatal conductance, water use efficiency, leaf size and leaf thickness have significant influence on net carbon gain, and hence on components of plant fitness (Ackerly et al. 2000, Wright et al. 2004). Both genetic diversity and phenotypic plasticity in these morphological and physiological traits may then induce differential survival rates of populations. Indeed, the response to natural selection depends on the level of genetic diversity in natural populations (Bürger and Krall 2004), as a high level of genetic variation in ecophysiological traits is being required for adaptive evolution. However, the genetic basis for evolutionary change in ecophysiological traits of tree species remains poorly understood.

Along an altitudinal gradient, the divergence between populations may be influenced by differences in the selective pressures imposed by different ecological environments, neutral evolutionary processes or both (Still et al. 2005). Elucidation of the causes of intraspecific genetic differentiation is a central theme in evolutionary biology (e.g., Reed and Frankham 2001, Latta 2003). Morphological and physiological adjustments allow individuals to persist in the stressful environment associated with high altitudes. For instance, plants growing at high altitudes usually have distinctive morphological features, including dwarfness, compact habit and small, densely pubescent leaves. Leaf physiology also changes according to altitude. The leaf mass per area (LMA) generally tends to increase with increasing altitude (Zhang and Marshall 1995, Hultine and Marshall 2000, Kao and Chang 2001), suggesting thicker leaves at high altitude. Leaf nutrient content per unit area has been shown to be higher at high altitude than at low altitude for several species, including *Picea abies* (Oleksyn et al. 1998), *Nothofagus menziesii* (Körner et al. 1986) and *Betula pubescens* spp. (Weih and Karlsson 2001). Several studies have shown that the maximum assimilation rate (A_{\max}) increases along altitudinal gradients for deciduous and evergreen tree species (Körner 1999, Kohout and Read 2006, Bresson et al. 2009, but see Zhang et al. 2005). Leaf phenology has also been widely studied along altitudinal gradients (Migliavacca et al. 2008, Vitasse et al. 2009b, Moser et al. 2010) and the phenological trends observed in natural conditions are known to be partly under genetic control (Jensen and Hansen 2008, Vitasse et al. 2009a). Abiotic factors and evolutionary processes affecting bud phenology have been extensively studied, but little is known about the factors affecting morphological or physiological leaf traits (but see Premoli and Brewer 2007).

This study addressed natural selection on ecophysiological traits of temperate tree species in southern France. We asked

whether selection on ecophysiological traits acts along environmental gradients. First, based on in situ measurements, we investigated phenotypic changes in leaf morphological and physiological traits with altitude in European beech (*Fagus sylvatica*) and sessile oak (*Quercus petraea*). Second, we assessed the extent to which these variations are under genetic control by means of a common garden experiment. We hypothesized that selection at high altitude should favor ecophysiological traits that optimize carbon assimilation to counterbalance the reduced growing season length. However, we hypothesized that leaf ecophysiological traits are more under environmental (i.e., plasticity) than genetic control, as shown for leaf phenology (Vitasse et al. 2010).

Materials and methods

Species and population sampling

In situ assessment of functional traits was conducted along altitudinal gradients in the Gave and Ossau valleys of the central and western part of the Pyrenees mountains in France (Table 1). These valleys run parallel to each other, but are separated by 30 km. Their altitude increases from north to south. This region is characterized by an oceanic mountain climate, with a mean annual temperature of 11.8 °C and a precipitation of 1102 mm (1971–2000) at low altitude (Tarbes, 43°11'N 00°00'W, 360 m above sea level (a.s.l.), Météo France). We focused on two common European tree species (*F. sylvatica* and *Q. petraea*) widely distributed in the Pyrenees. Oaks are common at low altitude, in mixed oak stands and on south-facing slopes, whereas beech is present at higher altitude and is usually associated with fir (*Abies alba*) on north-facing slopes.

We sampled 10 natural established populations of beech and 14 of oak at various altitudes in each valley (Table 1). Temperature ranges of 6 and 6.4 °C along the altitudinal gradient were recorded for the Gave and Ossau valleys, respectively (Table 1). Seven to 27 dominant trees of similar height were randomly selected from each of these 24 populations for leaf trait measurements. The minimum distance between selected trees was 20 m. We used a global positioning system receiver (GPS Pathfinder ProXR, Trimble Navigation, Sunnyvale, CA, USA) to determine the exact altitude of each population. Leaves were harvested between 5 and 7 m height in the crown on sun-exposed branches using a pole tree pruner.

Common garden experiment

Seeds from the sampled trees and populations were collected in the fall of 2004 and 2005 for both species. For oak, we sampled several altitudes in addition to those sampled in the in situ study (300, 1000 and 1300 m (± 50 m) a.s.l.; Table 1). After one growing season in the nursery, seedlings were transplanted to the lowland common garden experiment south of

Table 1. Geographic and climatic data (T_a , mean annual temperature, °C, in 2005–2007) for populations sampled along the altitudinal gradients.

	Sites	Valley	Altitude	Latitude	Longitude	Slope aspect	T_a
<i>Fagus sylvatica</i>							
100	Josbaig	Ossau	148	43°15'N	00°44'W	Flat	11.5
400	Bager	Ossau	422	43°07'N	00°32'W	North	12.3
800	Eaux Bonnes	Ossau	824	42°56'N	00°22'W	North	10.3
1200	Fabrèges	Ossau	1260	42°52'N	00°24'W	North	6.7
1600	Bious	Ossau	1551	42°51'N	00°27'W	North	6.1
100	Laveyron	Gave	131	43°45'N	00°13'W	North	12.9
400	Gaves de Pau	Gave	488	43°05'N	00°05'W	North	11.4
800	Chèze	Gave	773	42°55'N	00°02'W	North	10.2
1200	Haugarou	Gave	1190	43°00'N	00°12'W	North	6.6
1600	Barège	Gave	1604	42°53'N	00°06'E	North	6.5
<i>Quercus petraea</i>							
100	Josbaig	Ossau	259	43°13'N	00°44'W	South	13.2
400	Bager	Ossau	422	43°07'N	00°32'W	North	12.3
800	Le Hourcq	Ossau	841	42°54'N	00°26'W	North	9.0
1200	Gabas	Ossau	1194	42°53'N	00°25'W	South	9.1
1600	Artouste	Ossau	1614	42°53'N	00°24'W	South	7.2
100	Laveyron	Gave	131	43°45'N	00°13'W	Flat	12.9
300	Ibos	Gave	387	43°15'N	00°00'W	South	12.4
400	Adé	Gave	427	43°08'N	00°00'W	South	12.3
600	Pierrefites	Gave	627	42°56'N	00°03'W	South	11.8
800	Chèze	Gave	803	42°55'N	00°02'W	South	11
1000	Bourdalats	Gave	1082	42°54'N	00°06'W	South	9.8
1200	Gèdre	Gave	1235	42°47'N	00°01'E	South	9.7
1300	Gèdre haut	Gave	1349	42°47'N	00°02'E	South	8.6
1600	Péguère	Gave	1630	42°52'N	00°07'W	South	7.7

Populations in bold typeface were assessed only in the common garden experiment.

Bordeaux (44°34'N, 00°16'W, 23 m a.s.l., 1 ha). The design of the experiment included three completely randomized and continuous blocks. Seedlings were transplanted at a spacing of 3 m × 2 m. An electric deer fence was installed and one pesticide (pyrethroid) was sprayed in the spring of 2006, 2007 and 2008 to prevent damage due to phytophagous insects. The mean annual temperature in the common garden was about 13.2 °C, and the mean annual rainfall was about 836 mm (1984–2006). Planting procedures and the features of the common garden have been described in more detail elsewhere (Vitasse et al. 2009a). The common garden included 425 individuals of beech and oak in total, and the sample size of populations varied between 5 and 15 individuals. Some of the populations from the Ossau valley (400 and 1200 m for beech and from 1600 m for oak) were excluded from the common garden analysis, because they contained too few living individuals.

Gas exchanges

Leaf gas exchange measurements were carried out with a portable steady-state flow-through chamber (PLC6) connected

to an infra-red gas analyser (CIRAS-2, PP Systems, Hitchin, UK) equipped with temperature, humidity, light and CO₂ control modules. Net gas exchange was measured within a sealed 2.5 cm² cuvette, with an initial CO₂ concentration of 37.5 ± 0.3 Pa, a temperature of 25 ± 0.3 °C and a relative humidity of 70 ± 20%, controlled by regulating the flow diverted through the desiccant. For measurements of photosynthetic photon flux density in saturated light conditions, leaves were illuminated with a red–blue light source attached to the gas exchange system maintained at 1500 μmol m² s⁻¹. Maximum assimilation rate (A_{max} , μmol m² s⁻¹) and maximum stomatal conductance ($g_{s,max}$, mmol m² s⁻¹) are expressed on a one-sided leaf area basis and calculated using the equations of von Caemmerer and Farquhar (1981). Before measurement campaigns, the analyzer was calibrated in the laboratory with 400 ppm standard gas. Full CO₂ and H₂O zero (differential calibration) measurements were performed in the field after each set of four measurements.

All measurements were taken between 8:00 and 11:00 (UT), on fully expanded unshaded leaves. Leaf gas exchanges were measured for the leaves of adult beech and oak trees

(from 7 to 27 individuals according to the population considered) along the two altitudinal gradients, on 5 consecutive days at the end of July, in 2006 and in 2007. Measurements were carried out on two leaves per tree, immediately after the branch carrying the leaves was removed from the crown with a pole tree pruner, at a height of 5–7 m. In the common garden experiments, measurements were performed on two leaves per plant, on 5–13 individuals per provenance (3 or 4 years old) sampled on 12 consecutive days at the end of July, in 2008 and 2009.

Biochemical analyses

We carried out laboratory determinations of leaf nitrogen content on the samples used for gas exchange measurements from both the in situ altitudinal gradient and the common garden experiment. For the in situ analysis, between 9 and 21 leaf samples per population were dried at 70 °C, mineralized with hot sulfuric acid and analyzed colorimetrically with a Technicon auto-analyzer (Technicon II, HI System, Tarrytown, NY, USA). The determination of the leaf nitrogen content was performed in alkaline solution where ammonia reacts with sodium salicylate and chlorine to form a colored complex (O'Neill and Webb 1970). This complex was then measured at 660 nm. In the common garden experiment, between 5 and 12 dried samples were crushed to a fine powder in a grinder (MM200, Retsch GmbH, Haan, Germany) and analyzed by isotope ratio mass spectrometry.

Leaf morphological traits

For both experiments (in situ and common garden), leaf samples were collected from the same individuals as for previous measurements, from between 9 and 21 trees per population for in situ experiments and from between 5 and 13 trees per population for common garden experiments. Two fully expanded unshaded leaves were harvested and impressions of the underside of the leaf were obtained with transparent enamel nail polish (Brewer and Smith 1997). Stomata were counted under a

microscope (Leica DM2500, Microsystems, Houston, TX, USA), for three areas per leaf (1 area = 0.230 mm²) at ×100 magnification. All the areas used for stomatal counts were in the middle of the leaf, to avoid possible variation at the base and tip. These data were standardized by dividing by surface area, to obtain the number of stomata per mm² (SD, St mm²).

The leaf surface area (L_s , cm²) was measured with a planimeter (Light Box model, Gatehouse, Scientific Instruments Ltd, Norfolk, UK), on the same leaf samples subsequently used for nitrogen determination. We sampled five leaves per tree for 9–20 trees per population in the in situ altitudinal gradient study and for 6–14 trees per population for the common garden experiment. Samples were then dried at 65 °C and weighed on an electronic balance (Explorer Pro, EP114 model, Ohaus Corporation, Pine Brook, NJ, USA) for the calculation of LMA (kg m⁻²).

Statistical and data analysis

For both experiments (in situ and common garden), relationships between functional traits and altitude were assessed with a linear regression model. Analysis of variance (ANOVA) revealed that there was no valley effect. We therefore pooled the data for both valleys for subsequent analyses and graphs, for both experiments. Relationships between functional traits and altitude were assessed using weighted linear regressions on population means. The linear regression was considered as significant if the probability of statistical significance (P) was <0.05 and considered as marginally significant if $0.05 < P < 0.1$.

The coefficient of variation is a standardized measure of the variability of a trait. For each trait, the intrapopulation variability (CV_{intra}) was calculated for each population and then averaged by species. The interpopulation variability (CV_{inter}) was calculated from the between-population standard deviation and the overall mean value (Table 2).

In the common garden experiment, we used ANOVA to assess the significance of differentiation between populations. We used PROC GLM with the RANDOM statement (version 9.1, SAS

Table 2. Intra- and interpopulation variability (CV_{intra} and CV_{inter} , respectively) of functional leaf traits in the common garden experiment and along altitudinal gradients (in situ).

	<i>Fagus sylvatica</i>				<i>Quercus petraea</i>			
	Common garden		In situ		Common garden		In situ	
	CV_{intra}	CV_{inter}	CV_{intra}	CV_{inter}	CV_{intra}	CV_{inter}	CV_{intra}	CV_{inter}
L_s	0.30	0.17	0.18	0.28	0.20	0.16	0.20	0.18
LMA	0.16	0.06	0.61	0.33	0.15	0.08	0.62	0.13
SD	0.21	0.13	0.02	0.09	0.19	0.07	0.01	0.08
g_{smax}	0.26	0.09	0.29	0.28	0.27	0.16	0.30	0.45
A_{max}	0.26	0.09	0.33	0.25	0.42	0.39	0.21	0.26
N_a	0.22	0.1	0.01	0.34	0.18	0.1	0.01	0.16

L_s , leaf size (cm²); LMA, leaf mass per area (kg m⁻²); SD, stomatal density (St mm⁻²); g_{smax} , maximum stomatal conductance (mmol m² s⁻¹); A_{max} , maximum assimilation rate (μmol m² s⁻¹); N_a , leaf nitrogen content (g m⁻²).

Institute, Cary, NC, USA). We checked the assumptions of homogeneous variance and normal distribution for physiological and morphological data before carrying out the analyses. The ratio of the among-population variance component to total variance was estimated with PROC VARCOMP, using the restricted maximum likelihood method. These analyses used the following statistical model: [1] $Y_{ijk} = \mu + P_i + b_j + (Pb)_{ij} + \varepsilon_{ijk}$, where Y_{ijk} is the observation of individual seedling k for one of the analyzed characters from provenance i and block j , μ is the overall mean of the analyzed characters, P_i is the random effect of provenance i , b_j is the fixed effect of block j , $(Pb)_{ij}$ is the interaction between provenance i and block j , and ε_{ijk} is the residual variation, including the effect of tree k belonging to combination ijk . Variances of the random effects (population σ_p^2 , interaction σ_{bp}^2 , residual σ_ε^2) were also calculated. The overall differentiation (D) between populations was calculated as $D = \sigma_p^2 / (\sigma_p^2 + \sigma_{bp}^2 + \sigma_\varepsilon^2)$. D is an analog of Q_{st} (Spitze 1993), the genetic differentiation of quantitative traits $Q_{st} = \sigma_p^2 / (\sigma_p^2 + 2\sigma_\lambda^2)$, where σ_λ^2 is the within-population additive variance. In our study, σ_λ^2 could not be estimated, so the denominator of D is the overall phenotypic variance rather than the overall genetic variance as for Q_{st} . All the analyses were performed using SAS 9.2 software (SAS, version 9.2, SAS Institute).

Results

Phenotypic variation along the altitudinal gradient (in situ experiment)

Oak trees generally displayed higher values than beech trees for morphological and physiological traits. The mean LMA along the altitudinal gradient was significantly higher for oak ($0.080 \pm 0.003 \text{ kg m}^{-2}$) than for beech ($0.064 \pm 0.004 \text{ kg m}^{-2}$) ($P = 0.0347$). Maximum photosynthetic rates were 25% higher for oak than for beech ($P = 0.0281$), on average (mean $A_{\max} = 12.5 \pm 0.8$ and $9.3 \pm 0.6 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for oak and beech, respectively), whereas maximum stomatal conductance values were not significantly different between the two species ($P = 0.0545$; mean $g_{\text{smax}} = 180 \pm 17$ and $123 \pm 12 \text{ mmol m}^{-2} \text{ s}^{-1}$ for oak and beech, respectively). Conversely, nitrogen content was higher in oak leaves than in beech leaves ($P = 0.0242$) with $N_a = 1.8 \pm 0.1$ and $1.3 \pm 0.1 \text{ g m}^{-2}$ for oak and beech, respectively.

For all traits and both species, we found a significant effect of altitude ($0.0053 < P < 0.0001$ and $8.08 < F < 211.5$). Overall, we found significant linear trends along the altitudinal gradient for almost all functional traits (except for SD for oak), acting in the same direction for both species (Figure 1). L_s decreased with increasing altitude by $1.1 \text{ cm}^2 \text{ 100 m}^{-1}$ for beech and $0.9 \text{ cm}^2 \text{ 100 m}^{-1}$ for oak, whereas LMA increased with increasing altitude by $3.1 \times 10^{-3} \text{ kg m}^{-2} \text{ 100 m}^{-1}$ for beech and $1.6 \times 10^{-3} \text{ kg m}^{-2}$ for oak. SD only increased for beech by

$2.3 \text{ stomata mm}^{-2} \text{ 100 m}^{-1}$ (Figure 1). Leaf nitrogen content (N_a) and maximum photosynthetic rates (A_{\max}) increased linearly with increasing altitude. A_{\max} increased to the same extent for both species ($0.3 \mu\text{mol m}^{-2} \text{ s}^{-1} \text{ 100 m}^{-1}$ increase in altitude). Likewise, N_a increased by $7.8 \times 10^{-2} \text{ g m}^{-2} \text{ 100 m}^{-1}$ for beech and by only $4.7 \times 10^{-2} \text{ g m}^{-2} \text{ 100 m}^{-1}$ for oak. Maximal stomatal conductance slightly increased with increasing altitude by $3.7 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ 100 m}^{-1}$ for beech and $7.9 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ 100 m}^{-1}$ for oak (Figure 1), but altitudinal trends were only marginally significant. No significant trend was found for the instantaneous water use efficiency (i.e., $\text{WUE} = A_{\max}/g_{\text{smax}}$) ($P = 0.276$ and $P = 0.877$ for beech and oak, respectively).

CV_{inter} was lower than the CV_{intra} for LMA and photosynthetic capacity (g_{smax} and A_{\max}) for beech and for L_s and LMA for oak (Table 2). For the other traits, CV_{inter} was higher than CV_{intra} .

Genetic differentiation in the common garden experiment

Overall, as in the in situ experiment, oak trees displayed higher values of morphological and physiological traits. Mean LMA along the altitudinal gradient was higher for oak ($0.06 \pm 0.006 \text{ kg m}^{-2}$) than for beech ($0.013 \pm 0.0007 \text{ kg m}^{-2}$; $P < 0.0001$). Leaf nitrogen content was much higher ($P < 0.0001$) in oak leaves than in beech leaves (mean $N_a = 1.5 \pm 0.02$ and $0.3 \pm 0.10 \text{ g m}^{-2}$ for oak and beech, respectively). The two species had similar mean maximal stomatal conductance values ($P = 0.0662$) ($g_{\text{smax}} = 150 \pm 14$ and $169 \pm 15 \text{ mmol m}^{-2} \text{ s}^{-1}$ for oak and beech, respectively). Mean assimilation rate was higher for beech than for oak ($P = 0.0002$) ($A_{\max} = 8.0 \pm 0.9$ and $11.7 \pm 1.1 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for oak and beech, respectively).

For both species, we found no significant trends for L_s whereas a significant positive linear correlation between LMA and altitude was highlighted for beech ($1.2 \times 10^{-4} \text{ kg m}^{-2} \text{ 100 m}^{-1}$). For both species, no significant trend was found for SD, A_{\max} , g_{smax} (Figure 1) and the instantaneous water use efficiency (data not shown, $P = 0.4112$ and $P = 0.2920$ for beech and oak, respectively). For physiological traits, only N_a showed a linear altitudinal trend, increasing by $3 \times 10^{-3} \text{ g m}^{-2}$ per 100 m for beech and by $17 \times 10^{-3} \text{ g m}^{-2}$ per 100 m for oak. Overall, mean values obtained in the common garden experiment tend to be lower than those measured in situ, except for L_s and SD.

In the common garden experiment, CV_{intra} was higher than CV_{inter} (from 38 to 68% for beech and from 8 to 65% for oak), for all traits and both species (Table 2). Significant genetic differentiation between populations was observed for L_s in beech and for L_s , SD, g_{smax} and A_{\max} in oak (Table 3). Overall, significant differentiation (Δ) between populations of different provenances was found for L_s in both species: 13.4 and 27.6% for beech and oak, respectively. Significant differentiation was also

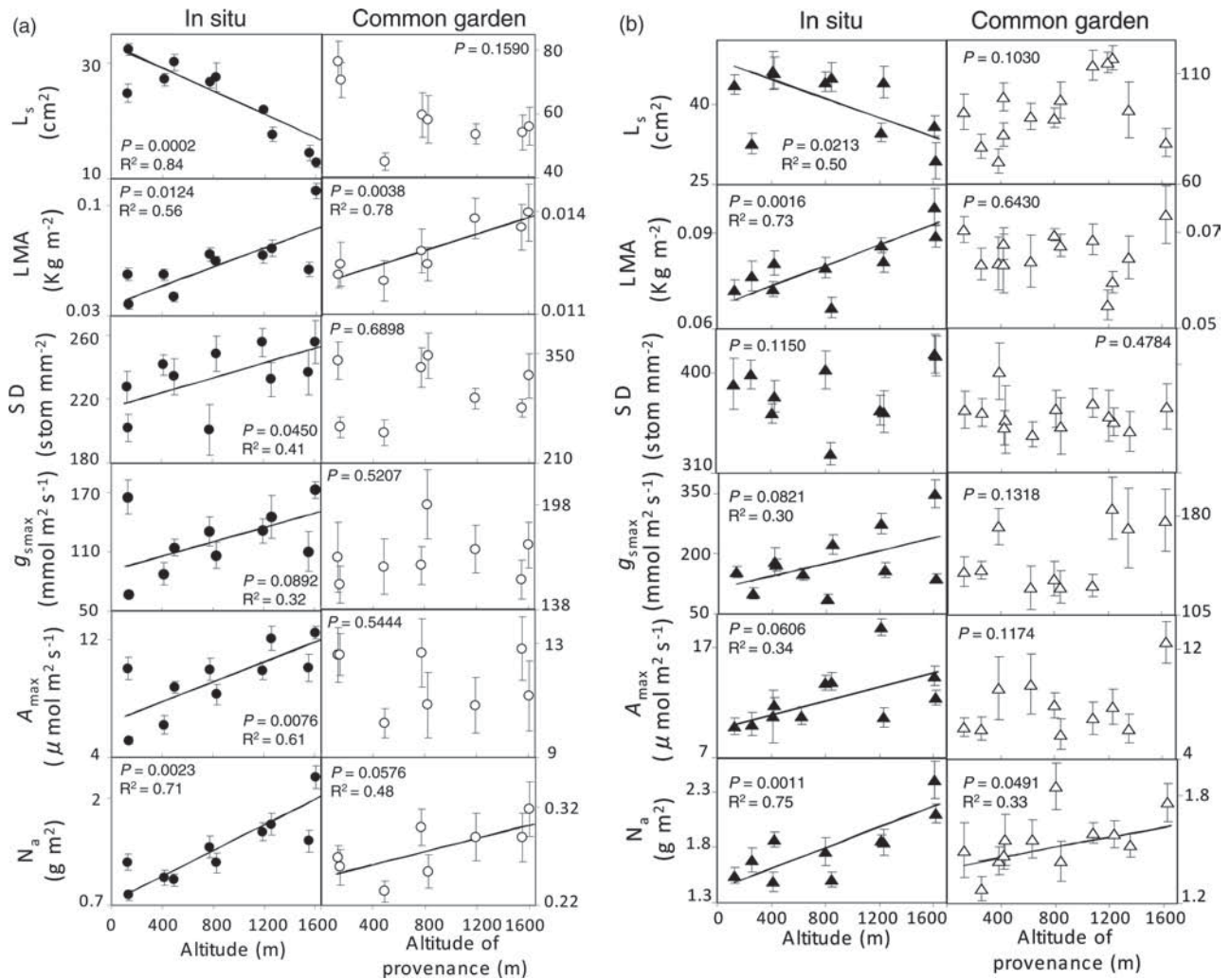


Figure 1. Functional leaf traits according to altitude (m) or altitude of provenance origin (a) for beech and (b) for oak, in situ (left) and in the common garden experiment (right). L_s , leaf size (cm^2); LMA, leaf mass per area (kg m^{-2}); SD, stomatal density (stom mm^{-2}); $g_{s\max}$, maximum stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$); A_{\max} , maximum assimilation rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); N_a , nitrogen leaf content (g m^{-2}). Only slopes significantly different from zero are shown on the graph. Circles and triangles indicate measurements for beech and oak, respectively. Closed and open symbols indicate measurements carried out in situ and in the common garden, respectively.

observed for $g_{s\max}$ and A_{\max} in oak ($D = 15$ and 20% , respectively, see Table 3).

Discussion

Leaf morphological and physiological traits of trees measured in natural conditions have been rarely investigated in concert with measurements of their progeny in common garden experiments (Cordell et al. 1998, Premoli and Brewer 2007). Our study therefore provides an unusual opportunity to determine to what extent variation of tree ecophysiological traits along an altitudinal gradient is under environmental and/or genetic control. It highlights high levels of phenotypic variability for leaf functional traits along altitudinal gradients. Overall, populations growing at high altitude had substantially higher LMA, N_a and photosynthetic capacity and smaller L_s than

those at low altitude. Between-population genetic differentiation in ecophysiological traits has been documented for various grass species (Chapin and Oechel 1983, Roy and Lumaret 1987, Carver and Nevo 1990), but tree species have been poorly investigated so far (but see Dang et al. 1994, Rowland 2001). In the common garden experiment, few traits displayed significant differentiation between provenances originating from different altitudes. Furthermore, we observed less between-population variation in the common garden experiment than in the in situ experiment, suggesting strong environmental determinism due to altitude and/or a population–altitude interaction. Nevertheless, the high CV_{intra} of functional traits (three times higher, on average, than the CV_{inter} for all traits and both species) suggests substantial genetic diversity within populations, which could potentially facilitate rapid adaptation.

Table 3. Genetic differentiation (D) between populations from different altitudes, for beech and oak, for six functional leaf traits measured in the common garden experiment.

	<i>Fagus sylvatica</i>			<i>Quercus petraea</i>			
	F	P	D	F	P	P	D
Morphological traits							
L_s	8.69	0.0019**	13.4	7.73	<0.001***		27.6
LMA	2.17	0.1440 ^{ns}	4.4	1.93	0.1069 ^{ns}		0
SD	2.86	0.0866 ^{ns}	5.6	5.87	0.0218*		0
Physiological traits							
g_{smax}	0.42	0.7910 ^{ns}	0	2.60	0.0451*		15
A_{max}	0.88	0.5172 ^{ns}	0	2.93	0.0238*		20.1
N_a	3.53	0.0565 ^{ns}	8.3	0.54	0.8129		3.3

Note: D is the ratio (%) of the variance component of provenance to total variance estimated by analysis of variance ($\sigma_p^2 / (\sigma_p^2 + \sigma_{bp}^2 + \sigma_\epsilon^2)$); F , Fisher's test value (significance levels of the population effect: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant).

L_s , leaf size (cm^2); LMA, leaf mass per area (kg m^{-2}); SD, stomatal density (St mm^{-2}); g_{smax} , maximum stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$); A_{max} , maximum assimilation rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); N_a , leaf nitrogen content (g m^{-2}).

Leaf size (L_s) decreased significantly with increasing altitude for both species. This trend is consistent with the findings of previous studies (Zhang and Marshall 1995). In the common garden experiment, no significant trend was observed for L_s , suggesting that phenotypic variability along the gradient is due to environmental effects. In contrast, genetic variation between populations from contrasting environments has been reported for L_s in *Populus deltoides* (Rowland 2001) and *Alchemilla alpina* (Morecroft and Woodward 1996), suggesting that diversifying selection with altitude may be responsible for L_s differentiation. We also found that LMA significantly increased with increasing altitude for both species: at high altitude leaves were thicker and/or denser than at low altitude. This result is also consistent with most previous findings (Zhang and Marshall 1995, Cordell et al. 1998, Hultine and Marshall 2000, De Lillis et al. 2004, Li et al. 2004, Scheepens et al. 2010), although the study by Schoettle and Rochelle (2000) highlighted a decrease in LMA with increasing altitude in *Pinus flexilis*. The higher LMA probably results from lower temperatures at higher altitudes (Meinzer et al. 1985, Friend et al. 1989, Kao and Chang 2001). It has been pointed out that leaves with high LMA generally contain more photosynthetic machinery per unit area (Vitousek et al. 1990), increasing water use efficiency and photosynthetic capacity at high altitude (Hultine and Marshall 2000). For beech, LMA increased significantly with altitude of provenance, reflecting genetic adaptation to native habitats. Few studies have shown for tree species (Schulze et al. 2006, Martin et al. 2007) and herbaceous species (Agrawal et al. 2008, Scheepens et al. 2010) that the in situ phenotypic variability of LMA may result partly from the local genetic adaptations of populations over the altitudinal gradient. In our study, in situ between-population variation was always greater than that observed in the common garden experiment, demonstrating the importance of environmental factors.

Our results also showed an increase in leaf N_a with increasing altitude, consistent with the findings of several previous studies (Sparks and Ehleringer 1997, Oleksyn et al. 1998, Cordell et al. 1999, De Lillis et al. 2004). However, several studies have highlighted a decrease in N_a (Zhang et al. 2005) or the absence of a significant trend with increasing altitude (Premoli and Brewer 2007). High leaf nitrogen content at high altitude may be a response to low temperature rather than nutrient concentration in smaller leaves (i.e., high LMA) (Weih and Karlsson 2001). Leaf nitrogen content is less diluted under cold conditions than under warm conditions (Körner 1999), particularly if low temperatures and short growing seasons restrict shoot growth more than N uptake by roots, resulting in a higher concentration of nitrogen in leaves. Alternatively, plants growing at high altitude may adopt a strategy of adaptation to lower resource availability (Chapin et al. 1990) and higher abiotic stress levels (cold hardiness). In the common garden experiment, N_a followed a trend similar to that in natural conditions for beech, although the absolute values were lower and the range was narrower. Therefore, our results evidenced both genetic and environmental effects in leaf nitrogen content (Reich et al. 1996, Schulze et al. 2006).

Maximum assimilation rate (A_{max}) also increased with increasing altitude for both species. This positive trend has been widely documented in various other species (*Nothofagus pumilio* (Premoli and Brewer 2007), *Nothofagus* sp. (Kohout and Read 2006) and *Pinus merkusii* (Koskela 2001)), but the opposite trend was found for *Quercus pannosa* (Zhang et al. 2005). The increase in A_{max} with increasing altitude may result from the higher leaf nitrogen content at high altitude (Friend et al. 1989). Photosynthetic capacity generally increases with leaf nitrogen content per unit area, because photosynthetic enzymes, such as ribulose-1,5-bisphosphate carboxylase, contain large quantities of nitrogen (Field and Mooney 1986,

Evans 1989). Nitrogen content therefore has a positive impact on the rates of carboxylation (Cheng and Fuchigami 2000, Manter et al. 2005) and assimilation (Peterson et al. 1999, Cheng and Fuchigami 2000, Warren et al. 2000). A higher A_{\max} value at high altitude may enable trees to compensate for a short growing season (Körner 2003). In the common garden experiment, no trend was found for A_{\max} in both species, suggesting that this trait is essentially under environmental determinism. Although few studies measured gas exchange on trees in common garden experiments, A_{\max} has always increased with increasing altitude of provenance origin (Cordell et al. 1998, Oleksyn et al. 1998, Premoli and Brewer 2007). These studies have concluded that this trait was under both genetic and environmental control, but with the environment making a larger contribution than genetics.

Stomatal density increased significantly with increasing altitude only for beech. No consensus concerning SD has yet been reached: some authors have reported a decrease in SD with increasing altitude (Hultine and Marshall 2000, Schoettle and Rochelle 2000) whereas others have reported an increase (Körner and Mayr 1981, Hovenden and Brodribb 2000, Kouwenberg et al. 2007, Premoli and Brewer 2007), and no clear trends for the change in SD with altitude were identified in any of the four species examined by Holland and Richardson (2009). A decrease in SD might reflect a decrease in the relative humidity of the air with increasing altitude (Schoettle and Rochelle 2000), whereas an increase in SD might reflect acclimation, counteracting the limited photosynthetic potential due to the decrease in CO_2 partial pressure (Körner and Mayr 1981, McElwain 2004). Körner (1999) also proposed an alternative hypothesis, attributing the altitudinal cline in SD to changes in foliar light interception. Incident photosynthetically active radiation could increase with altitude due to reduced atmospheric scattering and absorption over a shorter distance; previous studies have reported more stomata on leaves when they are more intensely exposed to light (Hanson 1917, Ashton and Berlyn 1994). Moreover, temperature has been shown to have no significant influence on SD in growth chamber experiments (Kouwenberg et al. 2007).

Maximum stomatal conductance (g_{smax}) slightly increased with altitude in our study for both species. Maximum stomatal conductance to water vapor diffusion has been examined as a function of altitude and has generally been reported to increase with altitude (Körner et al. 1986, but see Richardson and Berlyn 2002). Plants with high g_{smax} values may be able to assimilate carbon more rapidly than those with a lower conductance. In the common garden experiment, for both species, no between-population variation of SD and g_{smax} was observed, implying strong environmental determinism for this trait. Common garden studies have reported a lack of population differentiation for both SD and g_s in two *Nothofagus* species, suggesting environmental control (Hovenden and

Vander Schoor 2006, Premoli and Brewer 2007), but exceptions have been found (Hovenden and Brodribb 2000).

The extent to which tree populations are able to adapt to environmental changes will depend on the level and distribution of genetic variation within and between tree populations and phenotypic plasticity. Comparison of field and common garden morphological and physiological traits for these tree species populations evidenced the relative importance of plasticity in determining the phenotypic variation of traits compared with genetic variations. However, our results show that, despite gene flow between populations due to their proximity (Alberto et al. 2010), tree populations may nonetheless display genetic differentiation for several functional traits due to diversifying selection along the steep environmental gradient. These small, consistent differences among populations could still have far-reaching ecological and evolutionary importance. As large within-population differentiation has been reported for all traits, due to selection occurring during past natural warming (Kremer et al. 2010), we suspect that substantial adaptive evolutionary changes are likely to occur in response to current climate change. The upward altitudinal migration of montane species already recorded (Lenoir et al. 2008) will be facilitated by the extensive genetic diversity within populations and phenotypic plasticity (Kramer 1995, Chuine and Cour 1999, Vitasse et al. 2010). However, the lack of systematic, long-term monitoring of species distribution, morphology, physiology and other critical responses of montane species is seriously hampering our ability to predict the impact of climate change on mountain biodiversity. Studies at local scales are required to provide insight into the specific factors determining the patterns of species distribution associated with steep altitudinal gradients. We observed similar trends for two geographically distant transects, indicating that local drivers of functional leaf traits may be consistent at the regional scale. For further inference and large-scale generalization, investigations will be required over several climatic and geographic regions (latitudinal gradients).

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