

Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden

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Abstract: The aim of the study was to determine whether there are genetic variations in growth and leaf phenology (flushing and senescence) among populations of six woody species (*Abies alba* Mill., *Acer pseudoplatanus* L., *Fagus sylvatica* L., *Fraxinus excelsior* L., *Ilex aquifolium* L., and *Quercus petraea* (Matt.) Liebl.) along altitudinal gradients, using a common-garden experiment. We found (i) significant differences in phenology and growth among provenances for most species and (ii) evidence that these among-population differences in phenology were related to the annual temperature at the provenance sites for ash, beech, and oak. It is noteworthy that along the same climatic gradient, species can exhibit opposing genetic clines: beech populations from high elevations flushed earlier than those from low elevations, whereas we observed the opposite trend for ash and oak. For most species, significant altitudinal clines for growth were also revealed. Finally, we highlighted the fact that both phenology timing and growth rate were highly consistent from year to year. The results demonstrated that despite the proximity of the populations in their natural area, differences in altitude led to genetic differentiation in their phenology and growth. These adaptive capacities acting along a natural climatic gradient could allow populations to cope with current climate change.

Résumé : L'objectif de cette étude était de déterminer à l'aide d'un test de provenances, s'il existe des variations génétiques de la croissance et de la phénologie foliaire (feuillaison et sénescence) entre des populations issues de gradients altitudinaux, chez six espèces ligneuses (*Abies alba* Mill., *Acer pseudoplatanus* L., *Fagus sylvatica* L., *Fraxinus excelsior* L., *Ilex aquifolium* L. et *Quercus petraea* (Matt.) Liebl.). Pour la plupart de ces espèces, des différences significatives ont été observées entre les provenances pour la phénologie et la croissance. Ces différences phénologiques sont corrélées avec la température annuelle des sites de provenances chez le frêne, le hêtre et le chêne. Il est important de noter que les espèces présentent des clines opposés alors que les populations étudiées proviennent d'un même gradient climatique : les populations de hêtre provenant de hautes altitudes présentaient une feuillaison plus précoce que celles issues de basses altitudes, alors que le frêne et de chêne montraient des clines opposés. Des clines altitudinaux significatifs ont également été trouvés pour la croissance de la plupart des espèces. Nous avons également mis en évidence que la phénologie et le taux de croissance étaient stables d'une année sur l'autre. Les résultats de notre étude montrent que le gradient altitudinal a induit une différenciation génétique de ces populations pour leur croissance et leur phénologie, malgré la proximité des populations étudiées dans leur milieu naturel. Ces mécanismes adaptatifs, qui ont eu lieu le long d'un gradient climatique naturel, pourraient permettre aux populations de faire face au changement climatique actuel.

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Introduction

Palynology and genetic studies have highlighted the occurrence of very large changes within the geographical range of tree species during the present interglacial period (Brewer et al. 2002; Petit et al. 2003). This suggests that the principal response to climate warming may be migration rather than in-situ evolution. However, trees growing today,

and those growing in the future, will face unprecedented rates of climate change compared with the natural warming that occurred during the last postglacial period (Intergovernmental Panel on Climate Change 2007). In addition, forest habitats are highly fragmented at present, resulting in geographical barriers to gene flow between tree populations (Young et al. 1996; Lowe et al. 2005). Both the rate of climate change and this fragmentation will test the capacity of trees to cope with these changes (Thomas et al. 2006). Thus, the present challenge is to ensure that species and genotypes that are living in naturally established forests or are being proposed for planting today will be able to adapt to the changing climate. There are two mechanisms by which trees accommodate to climatic changes: they change their genetic makeup as a result of natural selection induced by environmental change, or they modify their gene expression. The former mechanism is driven by genetic diversity and the latter by phenotypic plasticity (Palmroth et al. 1999; Pigliucci

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et al. 2006). However, little is known concerning the inherent adaptive capacities that permit tree species to cope with new environmental conditions (i.e., how their genetic diversity facilitates their adaptation), and therefore how populations of different tree species will respond to the predicted changes.

For temperate-zone trees, the growth rate can have a strong impact on survival, biotic interactions, and long-term establishment (Coomes and Allen 2007). Growth traits are therefore relevant to the study of inherent capacities to adapt to climate change. Adaptation to winter cold by temperate- and boreal-zone trees involves complex genetic, physiological, and developmental processes (Howe et al. 2003). It is generally assumed that there is a trade-off between adaptation to stress and growth, especially in cold climates. Indeed, in cold climates, such as at high altitudes, physiological traits that increase cold-resistance will be selected, to the detriment of growth (Körner 2003). Under favourable conditions, a higher growth rate increases species' competitive ability, survival, and long-term success. Growth rate is therefore a crucial trait, especially in the first years of seedling establishment.

For survival success of trees, one of the most important characteristics that contributes to fitness, other than growth rate, is the timing of leaf phenological events (unfolding and senescence dates). In temperate climates, early-flushing trees or later hardening trees are more susceptible to spring or fall frost damage. Spring frost may kill the young leaves and shorten the overall growing season. This damage considerably affects a tree's fitness and reduces its yearly growth (Lechowicz 1984; Leinonen and Hanninen 2002). In fall, frost damage can reduce carbohydrate storage and consequently affect the next year's growth rate (Norby et al. 2003; Skomarkova et al. 2006). Further, frost may kill the flower primordia that were initiated in the bud. In the event of neither late frost in spring nor early frost in fall, trees that are able to flush earlier and (or) grow later in the season are likely to outcompete their neighbours. Besides changes in growth potential and susceptibility to frost, phenological changes may further modify tree fitness via a shift in synchronization with defoliating insects (Visser and Holleman 2001). Overall variation of phenological traits induced by temperature regimes or biotic factors will have a profound impact on the fitness of trees. Because of the strong relationship between phenology and fitness, natural populations growing under contrasting temperature regimes are expected to undergo diversifying selection for dates of leaf unfolding and leaf senescence (Worrall 1983; Howe et al. 2003; Ohsawa and Ide 2008).

High-altitude environments impose severe constraints on the phenology (reproduction, frost damage to young leaves) and establishment of plants, owing to the short growing season, low temperatures, and persistence of snow cover (Premoli 2003; Körner 2007). As a consequence, species display a wide range of morphological and physiological variations along altitudinal gradients (Oleksyn et al. 1998; Körner 2003), particularly in leaf phenology (Vitasse et al. 2009b). However, we do not know to what extent genetic variations are driving these phenological patterns. Therefore, studies assessing both the genetic and the environmental components of phenological patterns are crucial to making

progress in our predictions of the impact of climate warming on tree phenology and distribution (Savolainen et al. 2007). The best way to test whether there are genetic differences among populations within a species is to conduct common-garden studies (Rehfeldt et al. 2002). Although there have been a lot of common-garden experiments that examined phenotypic differentiation among populations of a single species (especially to improve growth for the timber industry), none of these tests considered among-population differentiation of several species coexisting along the same climatic gradient. This among-population approach using co-existent species may improve our understanding of local adaptations to environmental factors and sharpen our predictions of the impact of climate change on tree fitness and community composition. By assessing genetic variation under common-garden conditions, we explored whether steep temperature gradients have induced genetic gradients and whether the latter gradients resulted in different clines for six co-occurring woody species (four deciduous and two evergreen). More specifically, this study assesses altitudinal effects on the survival, growth, and phenology of these six species. We aim to answer the following questions. (i) Are populations sampled along altitudinal gradients differentiated in terms of these traits? (ii) To what extent can differentiation among populations be explained by the temperature at the provenance site? (iii) How could changes in phenology affect growth? In addition, we aim to test the stability of the differentiation among populations by comparing phenology and growth measurements obtained over 2 years in the common-garden experiment.

Methods

Studied species and seed sources

Two of the four deciduous species studied, sessile oak (*Quercus petraea* (Matt.) Liebl.) and European beech (*Fagus sylvatica* L.), are widely distributed in the Pyrénées (Pyrenees Mountains) at low and mid altitudes, respectively. The other two species, common ash (*Fraxinus excelsior* L.) and sycamore maple (*Acer pseudoplatanus* L.), are mainly riparian species that are also frequent along the altitudinal gradient considered (except sycamore, which is not present at the lowest elevations). The two evergreen species studied have different ecological requirements: holly (*Ilex aquifolium* L.) is an oceanic understorey species, more common at low elevations, whereas silver fir (*Abies alba* Mill.) grows at high elevations (above 800 m) and is more frequent on north-facing slopes.

Seeds were collected in the Pyrénées at different elevations from 130 to 1630 m. Sites were located along two elevational transects corresponding to two parallel valleys extending north-south on the French side of the mountains (see Table 1). We collected seeds of sycamore, ash, beech, fir, and holly in fall 2004, whereas seeds of oak were harvested in fall 2005 because seed production was low in 2004. We sampled seeds from nine populations per species, on average, depending on the species (for details see Table 1). For each selected population, seeds were collected from at least 10 mature trees. A further description of the sites where seeds were collected is available in Vitasse et al. (2009b). Throughout the paper, the term population refers

Table 1. Elevations, mean annual air temperatures, and provenance sites for the six tree species studied in the common-garden experiment.

	Gave valley			Ossau valley		
	Elevation (m)	<i>n</i>	<i>T_a</i> (°C)	Elevation (m)	<i>n</i>	<i>T_a</i> (°C)
<i>Abies alba</i>	840	37	10.31	824	8	10.37
	1190	19	6.70	1186	11	7.44
	1604	29	6.56	1551	24	6.16
<i>Acer pseudoplatanus</i>	481	54	10.72	450	36	11.43
	885	11	9.86	824	27	10.37
	1250	14	8.12	1186	25	7.44
<i>Fagus sylvatica</i>	1533	41	6.70	—	—	—
	131	11	12.89	148	32	11.52
	488	15	11.37	422	5	12.27
<i>Fraxinus excelsior</i>	773	27	10.24	824	35	10.25
	1190	37	6.59	—	—	—
	1604	28	6.46	1551	26	6.05
<i>Ilex aquifolium</i>	130	35	12.79	148	17	11.58
	481	23	10.72	450	35	11.43
	885	24	9.86	824	8	10.37
<i>Quercus petraea</i>	1250	10	8.12	1186	11	7.44
	1533	7	6.70	—	—	—
	131	6	13.04	148	6	11.58
<i>Quercus petraea</i>	427	7	12.48	422	32	12.43
	815	27	9.73	824	30	10.37
	1190	29	6.70	1186	30	7.44
<i>Quercus petraea</i>	131	39	12.89	259	38	13.15
	387	36	12.45	—	—	—
	427	36	12.33	422	22	12.27
<i>Quercus petraea</i>	627	39	11.83	—	—	—
	803	26	10.96	841	34	9.04
	1082	38	9.82	—	—	—
<i>Quercus petraea</i>	1235	35	9.73	1194	27	9.04
	1349	37	8.55	—	—	—
	1630	21	7.68	—	—	—

Note: *n*, number of individuals used for phenological observations; *T_a*, annual air temperature (2005–2006 for fir, sycamore, ash, and holly, and 2005–2007 for beech and oak).

to individuals of a given species whose seeds were harvested in the same valley and at the same altitude, and the term provenance refers to the location of origin of a tree population.

Planting procedures used in the common-garden experiment

In spring 2005, after a cold treatment that varied according to the species, seeds collected in 2004 from five species were sown in a nursery at the INRA Pierroton station (44°44'N, 00°46'W), whereas oak seeds were sown 1 year later. In January 2006, seedlings of beech, maple, and ash were transplanted in the common-garden plantation. Seedlings of the other three species, oak, fir, and holly, were transplanted in February 2007, owing to their lower growth rate. The common-garden experiment was located at sea level in southwest France (Toulonne Gironde INRA station, 44°34'N, 00°16'W, 23 m a.s.l.). The mean annual temperature is 13.2 °C and mean annual rainfall is 836 mm (1984–2006). During the experiment (2006–2007) the mean annual temperature was 13.6 °C and mean annual rainfall was 781 mm. The soil of the common garden is a sandy loam

type. The design of the experiment included three completely randomized and contiguous blocks. Each block contained 510 ± 1 plants, including all the tested provenances. Seedlings were transplanted at a spacing of 3 m × 2 m. On average, each provenance contained 25 individuals (Table 1). The common garden comprised 1529 individuals in total. Phenological observations were made on the total number of surviving plants (1317; Table 1). Wood fibre mulch was placed around the base of each seedling to limit competition from herbaceous plants and conserve soil moisture. An electric deer fence was installed and one pesticide (pyrethroid) was sprayed in spring of 2006, 2007, and 2008 to prevent damage by herbivores.

Phenological observations

The timing of leaf unfolding and senescence was monitored from spring 2006 to spring 2008. We examined each seedling every week from March to May for bud development and every week from September to December for leaf senescence. In spring we recorded the development stage of the apical buds from bud dormancy to leaf unfolding, using an intermediate grading scale of 3–5 according to the spe-

cies (see methods in Vitasse et al. 2009a). For fir, beech, holly, and oak, we used the criterion “at least one leaf unfolding” on the apical buds to judge that the leaf-unfolding date for one plant had been reached. For the fast-growing species (ash and sycamore) we considered that the leaf-unfolding date had been reached for one plant when 50% of the buds had met this criterion. In spring 2006 some apical buds were damaged by phytophagous insects and these individuals were removed from the analysis. In fall, we calculated the percentage of missing leaves and the percentage of remaining leaves on the seedling that were coloured. We considered that the senescence date had been reached for one seedling when 50% of its leaves were non-functional, i.e., either coloured or fallen, according to eq. 1:

$$[1] \quad x_t = \frac{\alpha_t(100 - \beta_t)}{100} + \beta_t$$

where x_t is the percentage of nonfunctional leaves on the selected seedling at date t , α_t is the percentage of coloured leaves at date t , and β_t is the percentage of missing leaves at date t . Then, for each seedling, the exact dates of leaf unfolding and senescence were estimated by linear interpolation between two consecutive observations.

In this study we chose the term growing-season length to characterize the period between leaf-unfolding and leaf-senescence dates for each seedling, which corresponds to the canopy duration (physical growing season) (White and Nemani 2003). At the population scale, the leaf-unfolding and leaf-senescence dates and growing-season length correspond to the mean for all individuals belonging to the same provenance.

Growth measurements

Height and stem diameter of all individuals were measured in January of 2005, 2006, and 2007. Stem diameter was measured using an electronic caliper to 0.01 mm accuracy, and height was measured by means of a graduated pole to 0.01 m accuracy. To characterize altitudinal differentiation in growth among populations, we used final height and stem diameter (2007) to minimize the transplantation effect. To test correlations between growth and phenological traits, we used annual height increment and phenology measured in 2007.

Meteorological measurements

Air temperature was measured using data loggers (HOBO Pro RH/Temp, Onset Computer Corporation, Bourne, Massachusetts, USA) in all provenance sites. Sensors were set 1.5 m above the ground using a pole located at the two plot extremities and were protected by a white plastic shelter to prevent exposure to rain or direct sunlight. Data were recorded hourly from 1 January 2005 to 31 December 2007. Annual temperatures of seedling provenances ranged from 4.7 to 6.9 °C for sycamore and beech (see Table 1).

Statistical analyses

We used analysis of variance (ANOVA) to test the significance of the differentiation among populations, using PROC GLM with the RANDOM statement (SAS Institute Inc. 2004). Before the ANOVA was performed, phenologi-

cal and growth data were examined and found to conform to assumptions of homogeneity of variance and normal distribution. The ratio of the population variance component to total variance was estimated using PROC VARCOMP with the method of restricted maximum likelihood (REML). These analyses used the following statistical model:

$$[2] \quad Y_{ijk} = \mu + P_i + b_j + (Pb)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the observation of individual seedling k for one of the analysed characters from provenance i and block j , μ is the overall mean of the analysed 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 random effects (population σ_p^2 , interaction σ_{bp}^2 , residual σ_e^2) were also computed. The overall differentiation (D) among populations was calculated as $\sigma_p^2 / (\sigma_p^2 + \sigma_{bp}^2 + \sigma_e^2)$. D is an analog of Q_{st} (Spitze 1993), which is the genetic differentiation of quantitative traits ($Q_{st} = \sigma_p^2 / (\sigma_p^2 + 2\sigma_A^2)$, where σ_A^2 is the within-population additive variance. In our study, σ_A^2 could not be estimated, so the denominator of D is the overall phenotypic variance rather than the overall genetic variance as it is for Q_{st} .

To test whether genetic variations in growth and leaf phenology are explained by the local climate of the provenances, we used a linear regression model with (i) mean annual temperature of seedling provenances for phenology (because temperature is the best environmental factor for explaining phenological variations), and (ii) altitude of seedling provenances for growth and survival (because growth and survival could be affected by other factors such as rainfall). All analyses were performed using SAS version 9.1 (SAS Institute Inc. 2004).

Results

Overall differences among populations

Significant differences in growth and leaf-unfolding date among populations were detected for all species except fir, whereas among-population differences in leaf-senescence date were smaller and significant only in 2007 (Table 2). Overall differentiation in leaf-unfolding date among populations amounted to 36% and 45% for oak and ash, respectively (Table 2), and was weaker for the other species (<15%). For oak, there was a provenance effect on both leaf phenology (spring and fall) and growth ($p < 0.0001$; Table 2). For leaf-senescence date, overall differentiation was always less than 12% (Table 2). For growth (height and diameter), we found strong among-population differentiation for all species except sycamore; among-population variations in height accounted for 14% and 31% of total variance for oak and holly, respectively (Table 2).

The ANOVA also indicated no significant interactions between block and provenance for leaf phenology or growth for any species in any year (data not shown). Nevertheless, we found a significant block effect on leaf-unfolding date for sycamore and ash in 2007 and on leaf-senescence date for beech in 2006 and sycamore in 2007. Significant block effects on height were also found for ash and sycamore.

Table 2. Genetic differentiation (*D*) among populations of the six tree species.

	<i>Abies alba</i>		<i>Acer pseudoplatanus</i>		<i>Fagus sylvatica</i>		<i>Fraxinus excelsior</i>		<i>Ilex aquifolium</i>		<i>Quercus petraea</i>	
	<i>D</i>	<i>F</i>	<i>D</i>	<i>F</i>	<i>D</i>	<i>F</i>	<i>D</i>	<i>F</i>	<i>D</i>	<i>F</i>	<i>D</i>	<i>F</i>
Flushing date												
2007	8	2.2 ns	6	3.0**	9	3.4**	29	8.0***	14	2.9*	36	18.3***
2008	0	0.7 ns	8	4.4***	11	3.6***	45	15.8***	5	2.2*	36	21.0***
Senescence date												
2006	—	—	2	1.2 ns	6	1.8 ns	6	1.6 ns	—	—	—	—
2007	—	—	9	4.3***	8	3.3**	8	2.5*	—	—	11	4.8***
Growth												
Height	15	5.1***	4	2.3*	20	5.2***	21	4.2***	31	5.9***	14	5.0***
Stem diameter	31	11.7***	2	1.5 ns	15	5.0***		1.3 ns	10	3.1**	8	2.9***

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

Trends with altitude and temperature of provenance sites

Survival and height

Most populations exhibited high survival rates, particularly sycamore, ash, and oak (Fig. 1a). The only significant cline between survival rate and provenance elevation was found for fir, with provenances from the highest elevations having the lowest survival rates ($R^2 = 0.80$, $p = 0.02$; Fig. 1a). No significant altitudinal cline was found for any of the other species.

Population growth followed a clinal trend: height declined linearly with increasing altitude of provenance sites for all species (Fig. 1b). This altitudinal cline was significant for ash, oak, holly, and beech, with a linear decrease in height between the lowest and highest populations of about 32%, 35%, 39%, and 52%, respectively. In addition, species exhibited different growth rates: fir had the lowest growth rate (the average height in 2007 was below 0.25 m), ash and sycamore had the highest (the average height in 2007 was above 1.80 m), and the other species had intermediate rates.

Phenological traits

In 2007, leaf unfolding occurred, on average, around day 114 (24 April) but varied according to species. Fir was the earliest species to flush, around 16 April, and holly the latest, around 9 May. We found a significant correlation between leaf-unfolding date and temperature at provenance sites for two of the six species studied: beech and ash. For ash, populations from the coldest climate (high elevations) flushed much later than populations from the warmest one, by $-1.9 \text{ days}\cdot\text{C}^{-1}$ at the provenance site ($R^2 = 0.89$, $p < 0.001$; Fig. 2). We found the opposite cline for beech: populations from the coldest climate flushed slightly earlier, by about $-0.43 \text{ days}\cdot\text{C}^{-1}$ ($R^2 = 0.48$, $p = 0.04$; Fig. 2). No significant trend was found for the other species, but there was a marginally significant trend for oak populations to exhibit the same cline as ash populations, i.e., populations from high elevations tended to flush later ($R^2 = 0.29$, $p = 0.06$; Fig. 2). We found the same significant clines for leaf-unfolding date in 2008 (ash: $R^2 = 0.83$, $p < 0.001$; beech: $R^2 = 0.56$, $p = 0.02$, data not shown).

In fall there was a significant clinal trend towards a correlation between leaf-senescence date and temperature at prov-

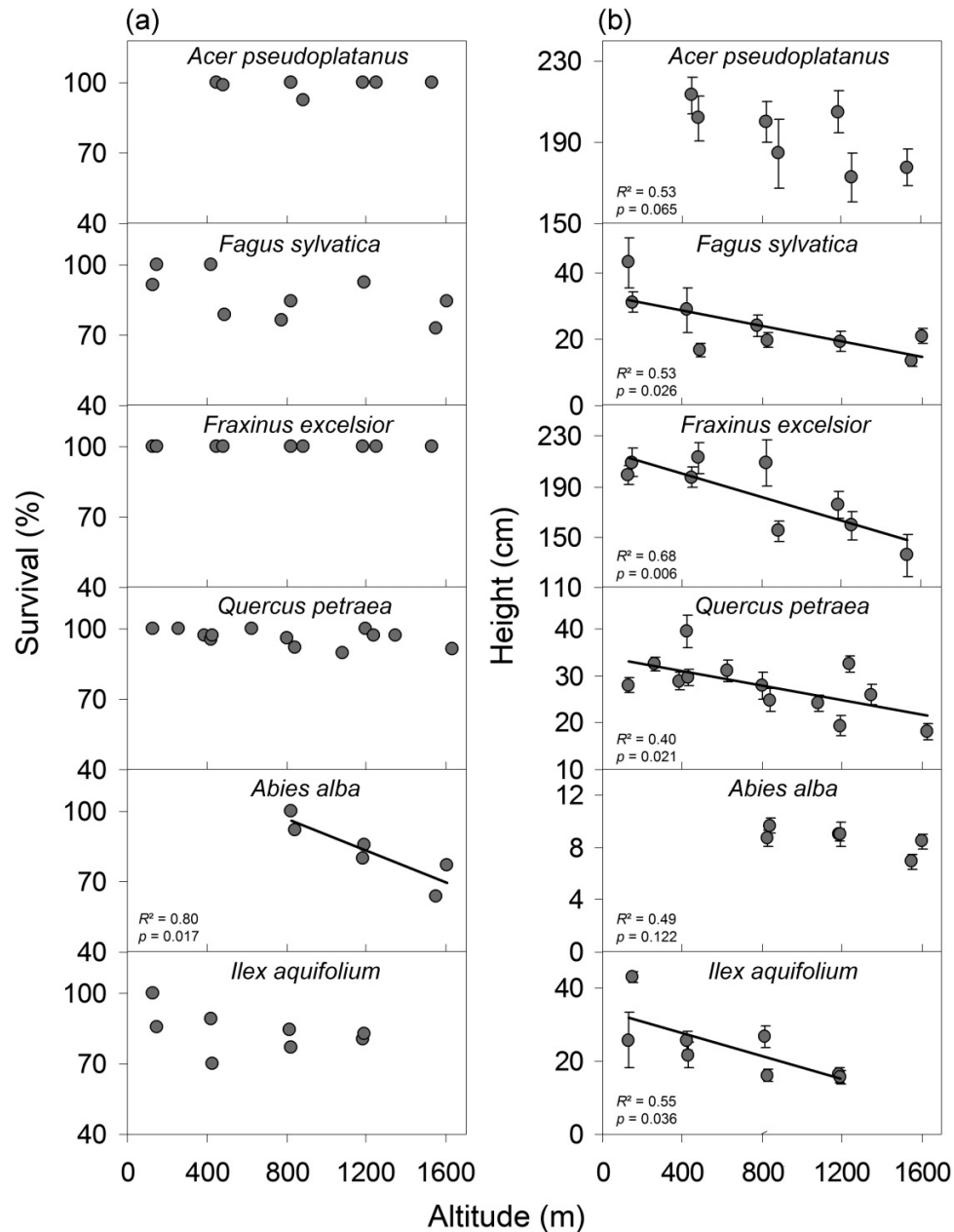
enance sites for only one of the four deciduous species (oak). For this species, senescence occurred later for populations from the coldest provenances ($-1.2 \text{ days}\cdot\text{C}^{-1}$, $R^2 = 0.34$, $p = 0.04$; Fig. 2). Beech tended to exhibit the opposite clinal trend ($R^2 = 0.42$, $p = 0.06$; Fig. 2), while no trend was found for the two other species.

For deciduous species, the length of the growing season in 2007 varied strongly among species: about 172, 189, 197, and 201 days for beech, ash, sycamore, and oak, respectively (Fig. 2). Finally, we found a significant clinal trend towards a correlation between growing-season length and temperature at provenance sites for ash, with an increase of $2.1 \text{ days}\cdot\text{C}^{-1}$ ($R^2 = 0.65$, $p = 0.009$, Fig. 2). This represents an extension of the growing season of about 9% from the highest to the lowest elevation. No significant clinal pattern was found for the other species. Of the deciduous species, beech was the latest to flush and the earliest to start senescence, and thus had the shortest growing season (Table 1).

Correlation between phenological traits and growth rate

Overall, we found strong correlations between phenology and growth rate, but the correlations differed in sign. For fir and holly, seedlings exhibiting earlier leaf unfolding had a higher growth rate (significant negative Pearson's correlation coefficient; Table 3). In contrast, for sycamore, individuals that flushed later had a higher growth rate ($r = 0.32$, $p < 0.001$; Table 3). No significant correlation was found for oak, ash, or beech. The timing of leaf senescence was significantly positively correlated with seedling growth rate for sycamore and beech (Table 3). This means that individuals exhibiting late leaf senescence had a higher growth rate. Oak showed the opposite trend ($r = -0.16$, $p = 0.001$) and no correlation was found for ash. Finally, we found a significant positive correlation between growing-season length and growth rate (height increment) for beech and oak (Table 3). No significant correlation was found for the other two deciduous species. We also found a positive correlation between leaf phenological events in spring and fall for oak and sycamore. Indeed, for these two species, individuals that flushed earlier also started leaf senescence earlier. For the four deciduous species we found a higher correlation between the duration of the growing season and the timing of leaf senescence than with the timing of leaf unfolding.

Fig. 1. Survival rates (a) and mean heights in winter 2007 (b) of populations of the six species studied in the common-garden experiment versus elevation of provenance sites (R^2 , coefficient of determination; p , significance levels of the p values of the slope).



Stability of phenological traits from year to year

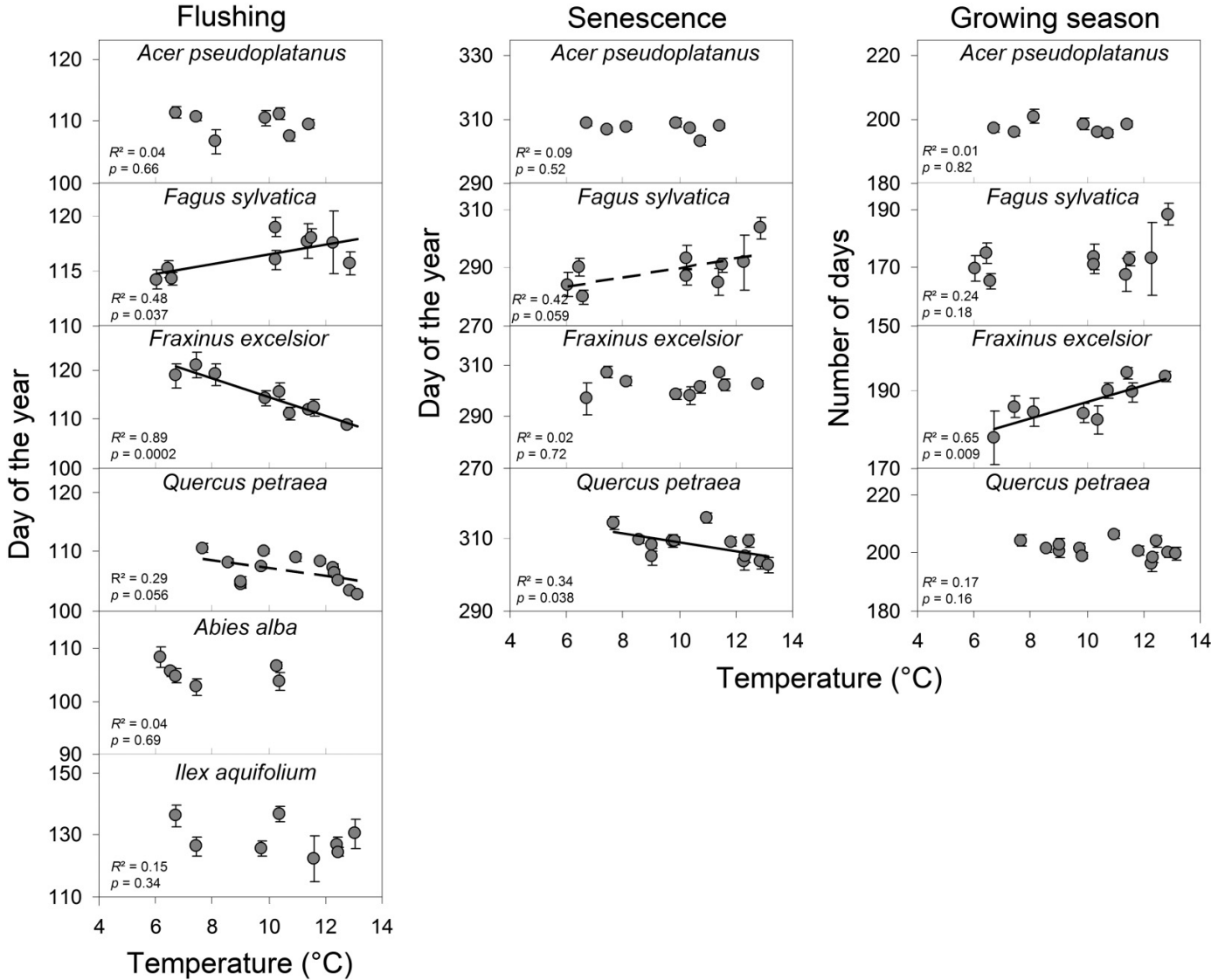
With regard to the timing of leaf phenology, there was a strong positive correlation between years, especially for flushing date (Table 4). Indeed, phenotypic correlations were significant for leaf-unfolding dates between the two years recorded for all species ($0.26 < r < 0.69$ for fir and ash) except holly. When we computed correlations among population mean values within species, we found significant positive correlations only for beech, ash, and oak ($r > 0.81$). Then, using all the individuals, we found a significant correlation between the two years when leaf senescence was monitored for sycamore and ash only, whereas at the population scale, the only significant correlation was found for beech. For growing-season length, overall we found positive correlations between the two years, but correlations were

significant only for beech at the population scale. Finally, for these three species, we found year-to-year stability of growth rates at the phenotypic level ($r > 0.20$, $p < 0.001$) but not at the population scale.

Discussion

Our common-garden study showed that tree populations from different altitudes were significantly differentiated with regard to phenology (especially the deciduous species) and growth rate. For some species this differentiation followed a clinal trend according to the temperature at the provenance site. Overall, populations from low-elevation sites had higher growth rates than those from high-elevation sites. In contrast, for phenological traits we found positive or

Fig. 2. Dates of flushing and leaf senescence and length of the growing season in 2007 for populations of the six species studied in the common-garden experiment plotted against annual temperature at provenance sites (R^2 , coefficient of determination; p , significance levels of the p values of the slope).



negative clines depending on the species. Finally, we highlighted the fact that both phenology and growth rate were highly consistent among years, particularly at the individual scale.

Relationships between growth and phenology

In our study we showed that earlier flushing or senescence could either positively or negatively affect growth, depending on the species. Even though these correlations between growth and phenology were not necessarily causal when provenances from different altitudes were compared, they could be explained by a trade-off between survival and productivity. Indeed, earlier flushing in spring allows carbon assimilation to increase, but also increases exposure to late frost (Lechowicz 1984; Leinonen and Hanninen 2002). The timing of leaf senescence may also affect growth because it is associated with remobilization of nutrients, particularly nitrogen, and storage of photosynthates (Norby et al. 2003; Lim et al. 2007). Thus, prolonged senescence in fall can re-

sult in increased storage of photosynthates but can also increase the risk of incomplete nutrient remobilization, owing to frost damage to functional leaves (Keskitalo et al. 2005).

Differentiation in early survival and growth

We found that genetic differentiation among populations tended to follow an altitudinal cline along which populations from low altitudes had, overall, higher growth rates than populations from high altitudes. This contributed the most to the strong population effect on growth in the ANOVA. Similar results were found in a number of studies conducted in other mountain ranges (Rehfeldt 1994; Oleksyn et al. 1998; Saenz-Romero et al. 2006; Rweyongeza et al. 2007). This pattern was likely due to differential selection pressure along the altitudinal gradient. Indeed, populations from low altitudes tend to be adapted to the mild climate under which selection has favoured a high allocation to growth and competitive ability, whereas populations from high altitudes display lower growth rates and greater cold-tolerance (Körner

Table 3. Pearson's correlations coefficients between leaf phenology and growth rate in 2007 for all seedlings of a given species.

	Trait	Flushing date	Senescence date	Growing-season length
<i>Abies alba</i>	Growth rate	-0.26**	—	—
<i>Acer pseudoplatanus</i>	Growth rate	0.32***	0.18*	-0.08 ns
	Growing-season length	-0.55***	0.69***	—
	Flushing date	—	0.22**	—
<i>Fagus sylvatica</i>	Growth rate	-0.13 ns	0.58***	0.59***
	Growing-season length	-0.30***	0.96***	—
	Flushing date	—	-0.03 ns	—
<i>Fraxinus excelsior</i>	Growth rate	-0.01 ns	0.02 ns	0.02 ns
	Growing-season length	-0.58***	0.78***	—
	Flushing date	—	0.06 ns	—
<i>Ilex aquifolium</i>	Growth rate	-0.46***	—	—
<i>Quercus petraea</i>	Growth rate	-0.05 ns	-0.16**	0.15**
	Growing-season length	-0.20***	0.93***	—
	Flushing date	—	0.17***	—

Note: "Growth rate" refers to the height-growth increment from January 2007 to January 2008 (*, $0.01 < p \leq 0.05$; **, $0.001 < p < 0.01$; ***, $p < 0.001$; ns, not significant).

2003). The maternal effect may have played a role in this pattern, especially through seed mass, which can affect the growth rate of seedlings during the first years (Oleksyn et al. 1998). We found a significant correlation between seed mass and altitude of the seed provenance for oak only, with seeds from high altitudes being lighter than seeds from low altitudes ($R^2 = 0.65$, $p < 0.0001$; data not shown). These differences in seed mass among populations could affect seedling growth in the first years and may have strengthened the cline measured in our common garden. Most species exhibited a high survival rate, except for fir, whose survival decreased with increasing elevation of the provenance sites. This may indicate genetic adaptation to low temperature and (or) vapour-pressure deficit. Indeed, these two variables, which were relatively high in the low-elevation common garden seemed to negatively affect populations from high elevations. The low survival rates of high-elevation populations observed here suggest that natural populations growing at high elevations will suffer the most from climate warming.

Differentiation in leaf phenology

Most studies on genetic variation in phenology show high heritability of the timing of flushing and leaf senescence or bud set (review in Howe et al. 2003). Our study also showed that both phenology and height growth were consistent from year to year, which demonstrates the high degree of genetic control of these characters, especially flushing and growth.

The strong and significant effects of provenance on flushing date (except for fir) clearly indicated population differentiation in these traits. Our results for six species showed that altitudinal clines in spring phenology due to temperature gradient were strongly species-specific. Moreover, relationships between fitness and spring phenology probably varied among species, resulting in different adaptations to temperature. Indeed, to our knowledge, our study is the first to show opposing clinal differentiation between species along the same climatic gradient (all populations were sampled along the same altitudinal transects). Beech populations

from high elevations flushed earlier than those from low elevations, which is consistent with the results of other studies on this species (vonWuehlisch et al. 1995; Chmura and Rozkowski 2002). In contrast, for ash and oak we found the opposite clinal trend: populations from high elevations flushed later than those from low elevations. Our results contradict those of previous studies on sessile oak by Deans and Harvey (1995) and Ducouso et al. (1996). However, in those two studies, the altitudinal gradients were much shorter than in our study (all populations were naturally growing below 500 m a.s.l., where temperature inversion occurs) and were intermingled with latitudinal variations. A lot of phenological studies show population differentiation in flushing dates along latitudinal and longitudinal gradients (Morgenstern 1996; Chmura and Rozkowski 2002). Altitudinal differentiation among populations is less documented but, generally, common-garden experiments have shown that populations from high elevations generally flush earlier than those from low elevations (Worrall 1983; vonWuehlisch et al. 1995; Acevedo-Rodriguez et al. 2006), as was the case here for beech only. This cline could be due to the fact that lower thresholds of temperature accumulation are needed for bud break in high-elevation provenances (Worrall 1983; vonWuehlisch et al. 1995). This could be the result of adaptation to lengthening of the short growing season at high elevations. In contrast, only one study has reported the opposite altitudinal cline to that shown for ash and oak in our study. Indeed, Barnett and Farmer (1980) found that populations of *Prunus serotina* from low elevations exhibited earlier leaf unfolding than those from high elevations. For this species, natural selection seems to have selected individuals exhibiting later flushing at high elevations to avoid frost damage to leaves. On the other hand, for three out of the six species no phenological cline was detected among populations according to their provenance climate. This absence of a cline was also reported in previous studies of a few species (for a review see Morgenstern 1996), though this could be explained by the high phenological variation usually observed within populations (Kleinschmit 1993).

Table 4. Interannual correlations between phenological traits and height growth, using all individuals and population mean values for each species.

	Flushing date (2007 × 2008)		Senescence date (2006 × 2007)		Growing-season length (2006 × 2007)		Growth rate (2006 × 2007)	
	Individuals	Populations	Individuals	Populations	Individuals	Populations	Individuals	Populations
<i>Abies alba</i>	0.26**	0.52	—	—	—	—	—	—
<i>Acer pseudoplatanus</i>	0.49***	0.57	0.22**	0.31	0.03	0.47	0.39***	-0.39
<i>Fagus sylvatica</i>	0.52***	0.81**	0.13	0.91***	0.11	0.84**	0.20**	0.36
<i>Fraxinus excelsior</i>	0.69***	0.95***	0.19*	0.01	0.09	0.64	0.21**	0.60
<i>Ilex aquifolium</i>	0.20	-0.07	—	—	—	—	—	—
<i>Quercus petraea</i>	0.63***	0.93***	—	—	—	—	—	—

Note: For individuals, Pearson's correlations were computed over all seedlings within a given species (i.e., phenotypic correlations); for populations, Pearson's correlations were calculated among population mean values. Leaf-senescence dates are given for the four deciduous species. "Growth rate" refers to the height-growth increment (*, 0.01 < p ≤ 0.05; **, 0.001 < p < 0.01; ***, p < 0.001).

With regard to leaf senescence, we observed a significant cline only for oak: populations from low elevations started to senesce earlier than those from high elevations. In contrast, there was a tendency for beech populations to exhibit the opposite clinal trend, as was found by Chmura and Rozkowski (2002) also. The end of the growing season is known to be difficult to assess in field trials (Deans and Harvey 1995) and therefore little is known about genetic differentiation in leaf senescence or growth cessation among natural tree populations, particularly along altitudinal gradients (Chmura 2006). Moreover, in common-garden experiments, the genetic component of leaf senescence can be difficult to demonstrate because of natural biases due to environmental effects such as low temperature, which can cause early leaf fall for all provenances simultaneously. In addition, the end of the growing season (for which measurements of bud set often provide a surrogate) is reported to respond to latitude because of variations in day length: populations from high latitudes generally start to senesce or end their growth earlier than populations from low latitudes (Hänninen et al. 1990; Deans and Harvey 1995; Mimura and Aitken 2007; Jensen and Hansen 2008). In our study the provenance sites of low- and high-altitude populations were very close to each other (within a 30 km radius), with consequently no variation in day length among sites. We may conclude, therefore, that the altitudinal clinal patterns of leaf senescence observed for oak and beech were more likely to have been induced by adaptation to temperature rather than to differences in day length.

The potential for adaptation to current climate change also depends on dispersal rates, establishment rates, and phenotypic plasticity and not only on genetic diversity of fitness-related traits. From in-situ assessments of phenological variations, Vitasse et al. (2009b) demonstrated that mature populations in the same sites showed very large variations in phenology between the lowest and highest elevations (up to 59 days for oak flushing and 61 days for beech senescence). These phenotypic differences observed in situ reflect both environmental and genetic variations, while the variations observed in the common-garden experiment reflect genetic variation only. Therefore, we stress that in-situ variability is more likely due to high phenotypic plasticity than to local adaptations. However, local adaptations may increase phenotypic variability in situ, as for ash flushing (altitudinal clines in the common garden follow the same clines in situ), or decrease it, as for beech flushing (i.e., opposite clines in the common garden and in situ).

Conclusion

From the perspective of climate change, the present results demonstrate the importance of considering existing genetic variation among populations when recommending seed transfers for forest-management planning. The opposing clines that were observed among species reveal the different relationships that exist between traits and fitness in the different species. As a result, recommendations regarding seed transfer have to be made on a species basis, taking into account also the different traits in combination. However, genotype × environment interactions can lead to different results in different environments. Because of genotype × en-

vironment interactions, the genetic rank of populations might change, so the population best suited to one environment might not be the best population in another environment. Therefore, several common gardens at different altitudes are needed to assess genotype \times environment interactions and confirm our conclusions regarding recommending seed transfers at a large scale. In natural forests, migration of species and populations to new areas is one of the possible responses to future climate change (Davis and Shaw 2001). The altitudinal distribution of mountain species is already changing as a result of climate warming (Lenoir et al. 2008). However, climate change may be too rapid for natural migration to cope with (Aitken et al. 2008). Thus, under rapid climate change, phenological plasticity and (or) genetic diversity will certainly play a key role in allowing trees to respond immediately to temperature changes (Kramer 1995; Chuine and Cour 1999). Our results showed that despite the probably intense gene flow between populations because of their proximity, considerable differentiation in adaptive traits such as phenology and growth can arise among tree populations, owing to diversifying selection along the steep environmental gradient. These adaptive mechanisms could allow populations to cope with current climate change.

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