

Elevational adaptation and plasticity in seedling phenology of temperate deciduous tree species

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Abstract Phenological events, such as the initiation and the end of seasonal growth, are thought to be under strong evolutionary control because of their influence on tree fitness. Although numerous studies highlighted genetic differentiation in phenology among populations from contrasting climates, it remains unclear whether local adaptation could restrict phenological plasticity in response to current warming. Seedling populations of seven deciduous tree species from high and low elevations in the Swiss Alps were investigated in eight common gardens located along two elevational gradients from 400 to 1,700 m. We addressed the following questions: are there genetic differentiations in phenology between populations from low and high elevations, and are populations from the upper elevational limit of a species' distribution able to respond to increasing temperature to the same extent as low-elevation populations? Genetic variation of leaf unfolding date between seedlings from low and high populations was detected in six out of seven tree species. Except for beech, populations from high elevations tended to flush later than populations from low elevations, emphasizing that phenology is likely to be under evolutionary pressure. Furthermore, seedlings from high elevation exhibited lower phenological plasticity to temperature than low-elevation

provenances. This difference in phenological plasticity may reflect the opposing selective forces involved (i.e. a trade-off between maximizing growing season length and avoiding frost damages). Nevertheless, environmental effects were much stronger than genetic effects, suggesting a high phenological plasticity to enable tree populations to track ongoing climate change, which includes the risk of tracking unusually warm springs followed by frost.

Keywords Leaf unfolding · Budset · Common garden · Genecology · Elevational gradients

Introduction

Deciduous tree species in a temperate climate need to adapt their phenology to optimize the timing of growth and reproduction according to local biotic and abiotic factors (Polgar and Primack 2011). Genetic diversity within and among populations and phenotypic plasticity of phenological key events, such as the emergence of leaves or flowers, play crucial roles in adaptation, and thus in species survival and their competitive abilities in the context of the rapid ongoing climate change (Savolainen et al. 2007). In fact, tree phenology fulfills the three criteria required to be considered as adaptive, that is, being able to evolve in response to selective pressures. First, there is a high phenological variation within populations (e.g. Baliuckas et al. 2005; Alberto et al. 2011), second, phenological traits show a high level of heritability (e.g. Billington and Pelham 1991; Howe et al. 2000), and third, phenological traits are tightly related to individual performance/fitness through biotic (Tikkanen and Julkunen-Tiitto 2003; van Asch and Visser 2007; Wesolowski and Rowinski 2008) and abiotic (Augsburger 2008; Chuine 2010) interactions. Temperature

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is assumed to be the main abiotic selective driver leading to clinal variation of diverse adaptive traits along elevational gradients (Oleksyn et al. 1998; Premoli et al. 2007; Bresson et al. 2011), whereas in semi-arid regions, moisture availability can exert similar selective pressure (Broadhead et al. 2003). Variations of functional phenotypic traits along environmental gradients result from three major components which can be partitioned by conducting analyses of variance (Schlichting 1986). The three sources of variance include phenotypic variance due to genotypes (V_G), phenotypic variance due to environments (V_E), and variance due to the interaction of genotypes with the environment ($V_G \times V_E$). The two latter components represent the phenotypic plasticity, with V_E considered as the environmental component of the plasticity, and $V_G \times V_E$ as the phenotypic plasticity due to genetic differences among genotypes/provenances. The $V_G \times V_E$ interaction reflects genetic diversity in plasticity and is, thus, assumed to represent the heritable component of phenotypic plasticity (Schlichting 1986; Thompson 1991). So, some genotypes/provenances may show more and others less phenotypic plasticity along the same environmental gradient.

Over the last century, numerous experiments using common gardens have shown genetic differentiation of tree phenology among populations from different elevational or latitudinal provenances (review in Langlet 1971; Morgenstern 1996; with references therein). While these experiments were mostly established to provide information for forest management, there is now renewed interest to assess the ability of tree populations to cope with ongoing climate change (Aitken et al. 2008; Lindner et al. 2010). One question that remains open is whether tree populations growing in contrasting climates will have the same capability to respond to environmental change such as the ongoing climatic changes (Vitasse et al. 2009b; Doi et al. 2010; Hoffmann and Sgro 2011).

At the upper elevational limit of tree species, cold conditions strongly constrain tree phenology: late frosts in spring are frequent, and bud burst timing must be optimized to minimize the danger of frost damage, while at the same time maximizing the length of the growing season to ensure growth and the production of viable seeds. Tree populations growing at their upper elevational limits may therefore undergo a strong selective abiotic pressure and could be well differentiated from those growing at lower elevations, provided there has been sufficient time for genetic differentiation and sufficient genetic isolation. Leading edge populations are usually smaller and more isolated than populations inhabiting the center of the distribution range, leading to a lower genetic diversity (Willi et al. 2007) and possibly to a weaker phenotypic plasticity to cope with new environmental conditions. On the other hand, these populations could also benefit from gene flow

from populations inhabiting warmer areas, which would introduce alleles (pre)-adapted to warmer climate and thus allow leading edge populations to cope with rapid climate warming (Lindner et al. 2010; Kremer et al. 2012). Phenotypic plasticity can be directly enhanced by natural selection in heterogeneous environments (Thompson 1991; Schlichting and Pigliucci 1993), and has been proposed as a catalyst in evolutionary processes for local adaptation (Pigliucci et al. 2006). Yet, although numerous studies have reported genetic differentiation in phenology among tree populations from contrasting climates, only a few studies have addressed whether these populations also diverge in their phenotypic plasticity, including the two components V_E and $V_E \times V_G$ (but see Rehfeldt et al. 2002; Baliuckas and Pliura 2003; Williams et al. 2008; Vitasse et al. 2010). The reason is that most of the studies used only one common garden, and, hence, a single common climate which can be more suitable for some provenances than others. Since both genetic- and environment-related factors affect plant phenotype, results from a single common garden (commonly established at a warm location) can give misleading results and do not allow a proper assessment of the response of the tested populations to environmental changes.

Elevational gradients provide a unique “in situ experiment” for exploring the impact of temperature on tree phenology (Körner 2000). The relative short distance between elevations with markedly different temperatures in mountain terrain enables the analysis of temperature effects on plants at otherwise similar conditions. We used common gardens established at different elevations in two main valleys in the Swiss Alps, including seven tree species widely distributed in Europe, to examine genetic differentiation between low- and high-elevation populations and their plastic responses in leaf unfolding and timing of budset to temperature changes. For each species, seedlings from seeds harvested at low and high elevation within each of the two studied areas were investigated. The objectives of this study were to quantify (1) whether populations of deciduous trees growing at their upper elevational limits in the Swiss Alps are genetically adapted in their phenology to face low temperature conditions; (2) the extent to which leaf phenology is influenced by genetic factors (provenance) and temperature; and (3) to compare phenological plasticity between populations from low and high elevations. We hypothesized that (1) cold temperatures have led to genetic differentiation in leaf phenology between tree populations growing at high and lower elevations, assuming that at high elevation, populations undergo a strong directional selection by freezing temperatures, favouring late flushing individuals; and (2) that populations naturally growing close to their upper elevational limits exhibit a lower phenological plasticity to temperature.

Materials and methods

Study species and seed sources

Seven deciduous tree species having a wide distribution range in Europe were selected. *Fraxinus excelsior* L., mainly distributed at low elevation up to about 1,500 m in the Swiss Alps; *Fagus sylvatica* L. and *Prunus avium* L., occurring from low to mid-elevation up to about 1,700 m; *Laburnum alpinum* (Mill.) Bercht. & J. Presl. and *Acer pseudoplatanus* L., two predominantly mid-montane species found up to about 1,900 m; and *Sorbus aria* L. and *Sorbus aucuparia* L. distributed between lower montane level and elevations higher than 1,900 m, in particular *S. aucuparia* which also occurs at the alpine treeline. The precise elevation limits of these species in the two study areas are provided in Vitasse et al. (2012).

For each species, seeds were collected from June to November 2009, according to their maturation stage, at both high and low elevations in two regions of the Swiss Alps, 200 km apart: the region around St-Maurice, VS (west) and the region around Haldenstein, GR (east) (see Kollas et al. 2012 for further details). Seeds of *L. alpinum* were obtained in the western region only because this species does not occur naturally in the eastern region of the Swiss Alps. The western and eastern regions were considered as replicates to test low against high elevational provenances. Within regions and species, the elevational difference between populations from low and high elevation ranged from 660 m for *P. avium* to 1,360 m for *A. pseudoplatanus*, and the average distance between low and high populations was on average 15 km (Table 1). For each species and both regions, mature trees occur more or less continuously between the selected low and high populations (see fig. 2 in Vitasse et al. 2012 for further details). For each selected population and species, seeds were collected from five randomly sampled trees which were at least 50 m apart but within an elevational band of 50 m.

Growth conditions

Seeds were sown in an unheated open-window greenhouse at Witterswil (47°29'35"N, 7°31'14"E) after a cold treatment in spring 2010 (see Kollas et al. 2012 for further details). Immediately after germination, each seedling was replanted in a square container (14 cm wide × 23 cm deep) containing three litres of the following substrate: 30 % pumice stone, 20 % bark compost, 20 % quartz sand, 16 % cocofiber, 10 % turf and 4 % clay. A fertilizer was applied in each pot in a slow release tablet form (Plantacote® Plus 6M) containing all essential elements and the full range of trace elements needed for optimal growth (7.5 g containing 14 %N, 3.9 %P, 11.7K, 1.2 %Mg + trace elements). The alternative of using similarly reciprocal native soils or inocula was dismissed after mycorrhiza expert consultation. Such a treatment would have exerted uncontrolled bias (site × provenance × soil source interactions). About 1 week after repotting, seedlings were moved outside the greenhouse in order to acclimate them to cooler conditions. A total of 4,442 containers were then brought to all common garden sites by trucks during the second week of June 2010 for the eastern region and during the fourth week of June 2010 for the western region.

Common garden sites and experimental design

Common gardens were established at 437, 1,058, 1,522 and 1,708 m a.s.l in the western region and at 606, 1,002, 1,251 and 1,400 m a.s.l in the eastern region (Table 2) within the two seed collection areas in the Swiss Alps. The western common gardens were located near St-Maurice, VS (46°12'48"N, 7°00'09"E) on a south-west facing slope, the eastern gardens were located near Haldenstein, GR (46°52'44"N, 9°31'32"E) on a south-east facing slope (Table 2). All common gardens were installed in open conditions. Each common garden was divided into three separate blocks, except the highest gardens in each of the

Table 1 Elevation range of the four sampled provenances for the seven species included in the study (West Low, West High, East Low, East High) with the elevational difference ($\Delta_{\text{Elevation}}$) and the average distance (Δ_{Distance}) between low and high provenances within each region

Species	West		East	
	Low–high ($\Delta_{\text{Elevation}}$) (m)	Δ_{Distance} (km)	Low–high ($\Delta_{\text{Elevation}}$) (m)	Δ_{Distance} (km)
<i>F. excelsior</i>	420–1,440 (1,020)	16.2	570–1,230 (660)	23.4
<i>P. avium</i>	356–1,075 (719)	27.7	575–1,235 (660)	12
<i>L. alpinum</i>	690–1,770 (1,080)	1.2	Species not present	
<i>A. pseudoplatanus</i>	400–1,760 (1,360)	16.1	590–1,430 (840)	9.6
<i>S. aria</i>	570–1,750 (1,180)	7.4	620–1,290 (670)	14.1
<i>S. aucuparia</i>	950–2,060 (1,110)	15.5	930–1,870 (930)	27
<i>F. sylvatica</i>	540–1,240 (700)	5.2	610–1,280 (670)	17.4

two regions that consisted of only two blocks because fewer individuals were available (Online Resource 1). One block consisted of two plots each of 1.50 m × 3 m. Each block contained all species randomly distributed within the block with the four provenances (East Low, East High, West Low, and West High) randomly distributed within species' subplots. Within blocks, each provenance was represented by ten individuals corresponding to five mother trees sampled per population (called hereafter "family") and two replicate seedlings per family. The same systematic design was applied in each of the eight gardens. Thus, a maximum of 30 individual containers per seed provenance were grown in each common garden for all species (five families × two replicates × three blocks). Depending on the success of seed germination, some provenances were represented by fewer individuals (see Online Resource 1). Each block was delimited by a wooden frame filled with a 10-cm sand layer separated from the underlying ground by a permeable plastic foil layer. The containers were placed on top of the sand layer with additional sand filling up the gaps between containers in order to insulate the root zones from short-term atmospheric temperature changes. A shade cloth (40–45 % reduction of incoming solar radiation) was installed in each garden at the beginning of the growing season in spring 2010 and 2011, after leaf emergence for all species in order to reduce evapotranspiration and full sun-exposure (seed beds are not commonly under full light in natural forest seedlings). A fence of 2 m height around the blocks prevented browsing of large herbivores. To avoid water limitation later in the season, plants were watered in all gardens maximally three times a week when the weather was hot and dry, by a local person assigned to each area during the growing season 2010 and 2011. Thus, we aimed to avoid any effects of limiting soil nutrient and water availability on tree phenology in order to focus on the effect of different temperatures induced by the elevational

gradient. At the beginning of the second growing season, the slower-growing species *F. sylvatica* and *S. aria* were moved to the southern side of each block to reduce shading effects from the other (taller) species. A few individuals died during the experiment (<3 %) and a few were damaged by mice (<1 %). Damaged or poorly growing individuals were not included in the analyses. Thus, an average of 531 individuals per common garden were included in statistical analyses (Online Resource 1). Throughout the paper, the term provenance is used to refer to individuals of a given species coming from seeds sampled at a specific elevation in a specific region (i.e. East Low, East High, West Low, and West High).

Meteorological data

Air temperature was recorded hourly in each common garden at 0.5 m above the ground using small data loggers (TidBit v.2 UTBI-001; Onset computer, Bourne, MA, USA) positioned under a white double-layered plastic shelter to prevent any exposure to rain or to direct sunlight. In early spring, data loggers in one common garden (Falera, 1,251 m a.s.l in the eastern region) stopped working. In this common garden, early spring temperatures were thus interpolated using linear regression between hourly data of the closest common garden (Arella, 1,002 m a.s.l, about 20 km distant) and data recorded in Falera during the period May–June ($R^2 > 0.92$). The temperature lapse rate in early spring (March–April) was about 0.49 K for every 100 m increase of elevation in the western region and about 0.37 K in the eastern region (see Table 2). A late spring frost occurred at the highest gardens of both study regions in mid-April 2011 when leaves of the majority of species were unfolded (Online Resource 2). These events caused substantial frost damage only in *F. excelsior* (Online Resource 3).

Table 2 Location, elevation, slope aspect, and temperatures that occurred before leaf flushing began for the eight common gardens

Region (close city, exposure)	Site	Elevation (m)	Latitude	Longitude	T_1	T_2	T_3	T_4	T_5	Spring onset ^a
West (St-Maurice, SW)	Eslex	437	46°11'52"N	7°01'33"E	2.2	3.9	8.2	14.1	16.6	14 February
	Les Posses	1,058	46°16'26"N	7°02'37"E	−0.5	2.4	5.1	11.1	13.7	5 March
	Rosseline	1,522	46°12'55"N	7°02'37"E	−2.1	1.1	2.9	8.1	10.6	24 March
	Les Martinaux	1,708	46°12'53"N	7°03'03"E	−1.6	0.7	2.7	7.6	10.5	25 March
East (Haldenstein, SE)	Böfel	606	46°52'18"N	9°31'06"E	1.2	2.7	7.5	13.4	15.8	21 February
	Arella	1,002	46°52'25"N	9°30'31"E	0.6	2.9	6.7	12.7	14.3	25 February
	Falera	1,251	46°48'14"N	9°14'23"E	−0.5	1.8	5.6	11.7	13.6	5 March
	Nesselboden	1,400	46°52'09"N	9°29'24"E	−1.8	0.6	3.7	9.1	10.7	21 March

T_1 , T_2 , T_3 , T_4 , T_5 , monthly mean temperature (°C) of January, February, March, April and May 2011, respectively

^a Spring onset values were derived from linear regressions between monthly mean temperatures from January to May 2011 and time, as the date when the threshold of 5 °C was reached. These values are used as a proxy for the elevational trends of the advance of spring phenology

Phenological observations

The timing of leaf emergence and budset was monitored in all gardens in 2011. In spring 2011, each seedling was observed weekly by one observer assigned to each region to assess the timing of leaf unfolding. These observers were trained at the beginning of the growing season by one of the authors (Y.V.) to minimize observer bias. The stage of the apical bud development was recorded using a categorical scale from 0 (no bud activity) to 4 (leaves out and flat). At stage 1, buds were swollen and/or elongating; at stage 2, buds were open and leaves were partially visible; at stage 3, leaves had fully emerged from the buds but were still folded, crinkled or pendant, depending on species; at stage 4, at least one leaf was fully unfolded. Species and provenance were compared using the average date when leaves of seedlings reached stage 3 which was estimated by linear interpolation when necessary (i.e. when this stage occurred in between two monitoring dates). Seedlings that suffered from severe frost damage occurring around the date of bud break were recorded.

Furthermore, we examined each individual to assess the budset, on 20 and 21 July 2011 in the western region and on 26 and 27 July 2011 in the eastern region. The apical bud was regarded as formed (score 1) when it was clearly visible in axils of the uppermost leaves. A score of 0 was attributed to seedlings continuing to produce new leaves or when no winter bud was visible.

Data analysis

Genetic differentiation in leaf phenology as well as its phenological plasticity to temperature between low and high provenances were tested using general linear model ANOVAs. For the date of leaf unfolding, the statistical model accounted for the following sources of variation: the region of common gardens (west vs. east), the elevation of common gardens nested within the region of common gardens, the region of origin (west vs. east), the elevation of origin nested within the region of origin (low vs. high), the interaction between the region of origin and the elevation of common gardens and the interaction between the elevation of origin and the elevation of common gardens. Although the region of origin could have been considered as random effect by nature, we decided to treat it as fixed effect due to the low number of factor levels (only two regions) which does not allow reliable estimation of the associated variance term when considered as random effect (Bolker et al. 2009). Additionally, the design did not allow the quantification of the seed family effect within provenance because the number of replicates per mother tree was too small and too variable among provenances and gardens to allow for a proper estimation of this effect. Because all

the seedlings in each common garden shared the same soil conditions with no limitation in water availability and nutrients, we assumed that a significant main effect of the region or the elevation of common garden on phenology indicates temperature-induced phenotypic plasticity (V_E). Similarly, a significant interaction between the region or the elevation of origin and the elevation of the garden indicates that the magnitude of the phenological response is dependent on the origin of the population which is the result of genetically based phenotypic plasticity ($V_E \times V_G$). Here, it is important to notice that we are considering plasticity at the population level, as an average across individuals from each provenance, rather than in the strict sense, i.e. at the genotype level. The budset score was analyzed for each region of common gardens separately because the monitoring date was different in each region.

The data for spring phenology were examined for assumptions of homogeneity of variance and normal distribution and the binomial data (budset data) were examined for over-dispersion before carrying out the analyses and were found to conform to model requirements. Spring temperature was calculated for each species as the mean temperature from 30-day before the date of leaf unfolding of the earliest provenance to the leaf unfolding date of the latest provenance. Hence, this mean spring temperature differs among species according to their phenology. Linear regressions were used to assess the effect of spring temperature on timing of leaf unfolding for each provenance. These linear regressions represent the reaction norm of the leaf unfolding date of each provenance to temperature changes and allow the comparison of the phenological plasticity among provenances within species and among species. All analyses were performed using R 2.12.2 (R Development Core Team 2011).

Results

Leaf unfolding date

Phenological plasticity due to temperature (V_E)

In both regions, leaf emergence was significantly delayed with increasing elevation of the common gardens for all species (Fig. 1), with elevation explaining from 41 % (*S. aucuparia*) to 86 % (*P. avium*) of the total variance in leaf unfolding date (Table 3). Individuals located in the western gardens generally flushed later than those in the eastern gardens (Fig. 1; Table 3; *P* value highly significant for all species except *F. sylvatica*). The region effect of gardens was principally due to the difference in elevation between the two highest common gardens of the two regions (290 m higher in the western region), which resulted in colder

Fig. 1 Average date of leaf unfolding for each provenance of the seven species examined in the eight common gardens established at different elevations. *W-low* populations from western part of Switzerland and low elevation, *W-high* populations from western part of Switzerland and high elevation, *E-low* populations from eastern part of Switzerland and low elevation, *E-high* populations from eastern part of Switzerland and high elevation

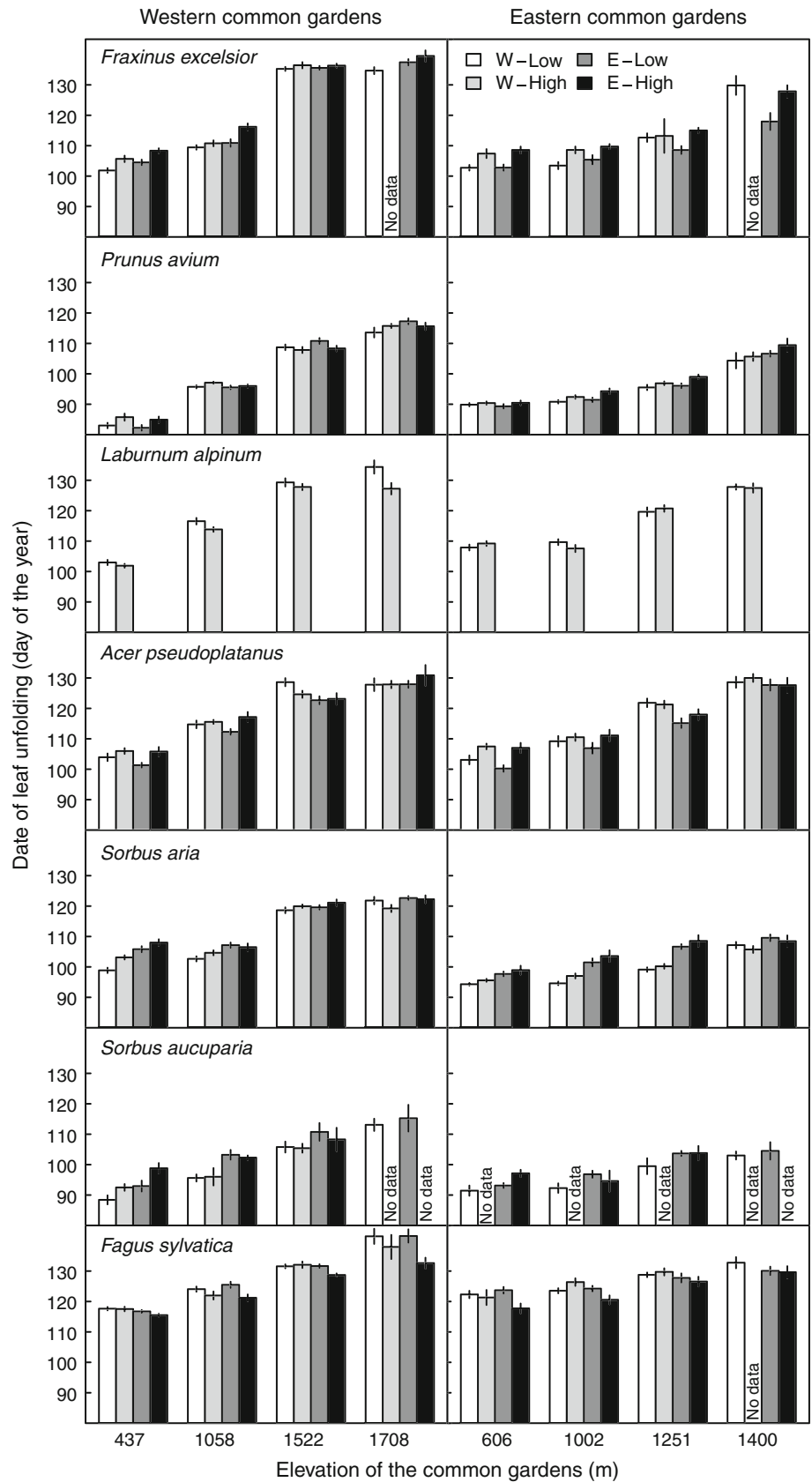


Table 3 ANOVA results for data of leaf unfolding for seedlings of the seven deciduous tree species planted in eight common gardens in two regions of the Swiss Alps

Source of variation	<i>F. excelsior</i>			<i>P. avium</i>			<i>L. alpinum</i>			<i>A. pseudoplatanus</i>			<i>S. aria</i>			<i>S. aucuparia</i>			<i>F. sylvatica</i>			
	df	F	VC	df	F	VC	df	F	VC	df	F	VC	df	F	VC	df	F	VC	df	F	VC	
Environmental effects (gardens)																						
Region (R_g)	1	468.4 ***	1.2	1	273.2 ***	<0.1	1	26.4 ***	<0.1	1	39 ***	<0.1	1	955.1 ***	34.8	1	20.2 ***	<0.1	1	1.6	<0.1	
Elevation (E_g)	6	376.6 ***	79.6	6	599.0 ***	86.1	6	204.0 ***	77.9	6	215.1 ***	71.7	6	245.8 ***	41.4	6	38.5 ***	55.8	6	138.5 ***	61.7	
Block	14	0.8	<0.1	14	1.1	<0.1	14	2.8 ***	2.1	14	1.8 *	0.7	14	1.3	0.2	14	0.7	<0.1	14	1.7 *	1.0	
Genetic effects (origin)																						
Region (R_o)	1	10.7 **	<0.1	1	3.4	<0.1	-	-	-	1	18.9 ***	0.1	1	138.4 ***	5.9	1	25.5 ***	9.0 (*)	1	12.8 ***	<0.1	
Elevation (E_o)	2	35.1 ***	2.1	2	5.7 **	0.3	1	6.2 *	0.5	2	11.4 ***	1.9	2	6.5 **	0.3	2	0.1	<0.1	2	20.1 ***	3.5	
Environmental × genetic effects																						
$R_o \times E_g$	7	4.6 ***	1.3	7	1.6	<0.1	-	-	-	7	1.6	0.1	7	6.2 ***	1.6	7	0.6	<0.1	7	0.6	<0.1	
$E_o \times E_g$	12	1.9 *	0.7	14	1.9 *	0.5	7	2.3 *	1.1	14	1.5	0.8	14	1.4	0.4	7	0.7	<0.1	13	1.9 *	0.4	
Residuals	612	-	15.8	699	-	13.6	322	-	18.4	560	-	25.6	686	-	15.9	173	-	35.2	552	-	33.7	

All sources of variation were treated as fixed factors. The elevation of common garden (E_g) was treated as a factor nested in region of common gardens (R_g), elevation of origin (E_o) nested in region of origin (R_o) and block nested in common garden and region of common gardens

df degree of freedom, F values from Fisher test, VC variance component

Significant results in bold: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, (*) $P < 0.1$

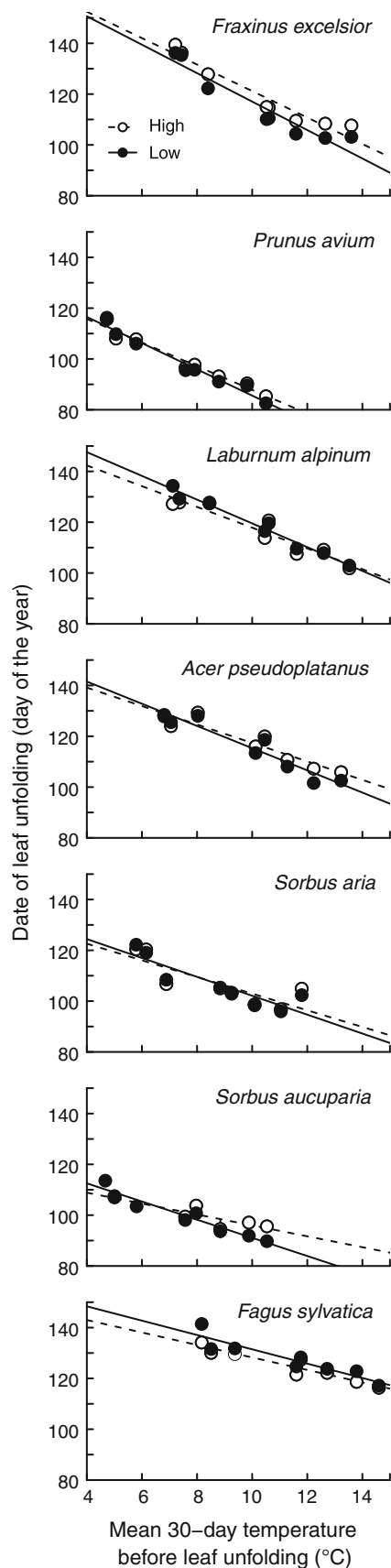


Fig. 2 Relationship between mean 30-day air temperature recorded in the common gardens prior to the flushing period and leaf unfolding dates for low and high elevation provenances of the seven studied tree species. The leaf unfolding date corresponds to the mean of all individuals per common garden for low and high elevation provenances (both regions of origin pooled) with standard errors and regression lines. For each species, the temperature corresponds to the mean air temperature during 30 days before the leaf unfolding date of the earliest provenance until the unfolding date of the latest provenance. *Black circles and solid lines* correspond to populations from low elevations; *white circles and dashed lines* correspond to populations from high elevations. All linear regressions were significant at the $P < 0.05$ level

spring temperatures in the western gardens ($\Delta T = 1.6$ K, calculated for T_{2-4} ; see Table 2). The date of leaf unfolding occurred earlier with increasing temperatures (i.e. with decreasing elevation) and was linearly related to temperature across the eight gardens for all species (Fig. 2). However, the magnitude of the phenological plasticity (slopes of regression lines of unfolding date on temperature) differed dramatically among species. For example, *F. excelsior* and *P. avium* exhibited the highest plasticity in response to temperature changes with an average shift of -5.4 ± 0.4 days K^{-1} (slope value ± 1 SD) and -4.8 ± 0.2 days K^{-1} , whereas *F. sylvatica* showed the lowest plasticity with -2.6 ± 0.2 days K^{-1} (Table 4). The percentage of the total variance observed in leaf unfolding date due to environmental factors (both region and elevation effects) was 86 % for *P. avium* and was more than 50 % for all other species (Fig. 3).

Genetic differentiation among provenances (V_G)

Overall, both the elevation and the region of origin had a significant effect on the timing of leaf unfolding for the majority of tree species, indicating a clear genetic differentiation between low and high provenances as well as between provenances from the western and the eastern part of the Swiss Alps (Table 3). In *A. pseudoplatanus*, *F. excelsior*, *P. avium* and *S. aria*, populations originating from low elevations flushed significantly earlier than did populations originating from high elevations, especially in the lower common gardens. In addition, eastern provenances flushed slightly later than western provenances (Figs. 1, 2). This region of origin effect was strongest in *S. aria*, explaining 6.2 % of the total variance (Fig. 1; Table 3). For *F. sylvatica*, both the effect of elevation and region of origin were significant, but the genetic cline of differentiation was opposite to the other species, i.e. high elevation provenances or eastern provenances tended to flush earlier than those from low elevations or from the western region. In contrast, no genetic differentiation was found between low and high provenances for *S. aucuparia* and between eastern and western region of origin for

P. avium (Table 3). The percentage of variance explained by genetic effects was smaller than the percentage of variance explained by environmental effects, accounting from 0.3 to 9 % for *P. avium* and *S. aucuparia*, respectively. Interestingly, both *Sorbus* species exhibited strong phenological differentiation among provenances that accounted for more than 6 % of the total variance (Fig. 3).

Genetic differences in phenological plasticity ($V_G \times V_E$)

A significant interaction in leaf unfolding date between the region of origin and the elevation of the gardens was only found for *F. excelsior* and *S. aria* (Table 3). For these two species, provenances from western Alps tended to have a greater plasticity to temperature than provenances from the eastern Alps (Fig. 1). A significant interaction in leaf

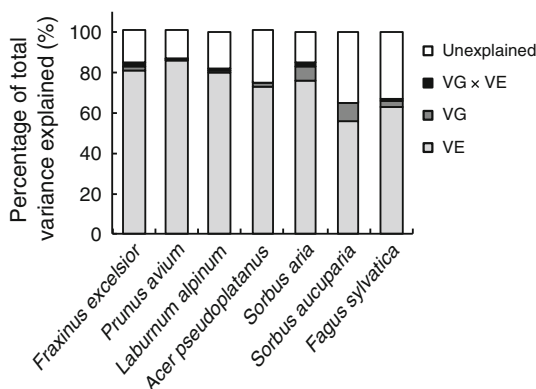


Fig. 3 Contribution (expressed in percentage) of each genetic and environmental component explaining the variability of the leaf unfolding date observed across the common gardens. V_E , proportion of the variance due to environmental factors; V_G , proportion of the variance due to genetic effects; $V_E \times V_G$, proportion of the variance due to the interaction between genetic and environmental effects. The proportions were directly extracted from the variance component analysis reported in Table 3

unfolding date between the elevation of provenances and the elevation of the gardens was found for four out of seven species, namely *F. sylvatica*, *F. excelsior*, *L. alpinum* and *P. avium* (Table 3).

Within region of origin, populations from low elevations exhibited systematically greater plasticity of leaf unfolding date in response to temperature changes than populations from high elevations (Table 4; Fig. 2). On average, the slope of the linear regressions between the leaf unfolding date and spring temperature was 0.59 ± 0.10 days K^{-1} greater for low provenances than for high provenances (paired *t* test: $t = -5.73$, $P < 0.001$). However, within species, no significant difference was found among provenances in their phenological shift to changing temperatures (tested by analysis of covariance: $P > 0.05$). The variance component due to interactions between environment (region or elevation of the gardens) and provenances (region or elevation of origin) was much smaller than V_E and V_G , reaching a maximum of only 2 % for *S. aria* and *F. excelsior* (Fig. 3).

Budset timing

Plasticity due to environment (V_E)

Within a region, elevation of gardens significantly affected bud formation in all species (Table 5; Fig. 4). Budset tended to occur later in gardens located at high elevation, irrespective of the provenance. This was particularly visible in the western gardens, where the two highest gardens were situated above 1,500 m a.s.l (Fig. 4).

Genetic differentiation among provenances (V_G)

All species except *L. alpinum* and *F. excelsior* exhibited genetic differentiation in the timing of budset between

Table 4 Response of the date of leaf unfolding to mean 30-day temperature occurring before leaf unfolding for each provenance of the seven examined tree species (slope of the linear regressions, days $K^{-1} \pm 1$ SD)

Provenance	W-Low	W-High	E-Low	E-High	Overall
<i>F. excelsior</i>	-6.0 ± 0.6	-5.0 ± 1.0	-5.4 ± 0.9	-5.1 ± 0.6	-5.4 ± 0.4
<i>P. avium</i>	-4.7 ± 0.3	-4.5 ± 0.4	-5.3 ± 0.4	-4.7 ± 0.4	-4.8 ± 0.2
<i>L. alpinum</i>	-4.7 ± 0.3	-4.1 ± 0.5	–	–	-4.4 ± 0.3
<i>A. pseudoplatanus</i>	-4.3 ± 0.6	-3.6 ± 0.6	-4.4 ± 0.6	-3.7 ± 0.5	-4.0 ± 0.3
<i>S. aria</i>	-4.2 ± 0.8	-3.4 ± 0.9	-3.3 ± 0.8	-2.9 ± 0.9	-3.5 ± 0.4
<i>S. aucuparia</i>	-3.6 ± 0.5	–	-3.5 ± 0.4	-2.1 ± 0.7	-3.0 ± 0.3
<i>F. sylvatica</i>	-2.9 ± 0.5	-2.6 ± 0.6	-2.7 ± 0.5	-2.4 ± 0.3	-2.6 ± 0.2

For each species, temperature corresponds to the mean 30-day temperature before the leaf unfolding date of the earliest provenance to the leaf unfolding date of the latest provenance

W-Low provenance from western region and low elevation, *W-High* western region and high elevation, *E-Low* eastern region and low elevation, *E-High* eastern region and high elevation

All slopes were significantly different from 0 ($P < 0.05$)

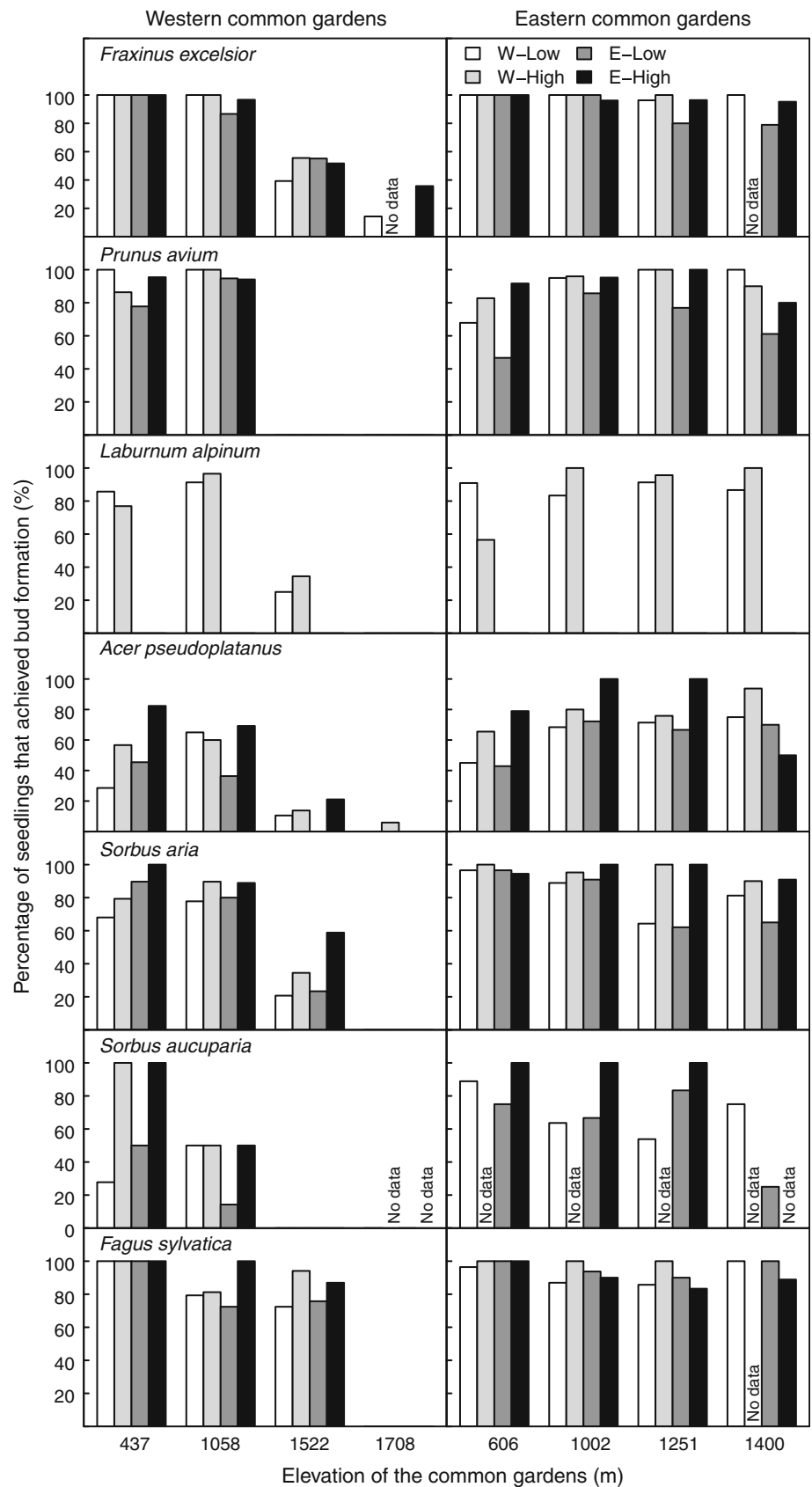
Table 5 Results of ANOVAs of budset scores for seedlings of the seven deciduous tree species planted in eight common gardens in two regions of the Swiss Alps

	<i>F. excelsior</i>		<i>P. avium</i>		<i>L. alpinum</i>		<i>A. pseudoplatanus</i>		<i>S. arica</i>		<i>S. aucuparia</i>		<i>F. sylvatica</i>	
	<i>df</i>	χ^2	<i>df</i>	χ^2	<i>df</i>	χ^2	<i>df</i>	χ^2	<i>df</i>	χ^2	<i>df</i>	χ^2	<i>df</i>	χ^2
Region West														
Environmental effects (gardens)														
Elevation (E_g)	1	177.1***	1	207.8***	1	57.1***	1	47.6***	1	137.1***	1	31.0***	1	93.5***
Block	9	8.0	9	101.4***	9	47.0***	9	55.2***	9	88.4***	9	19.2*	9	84.4***
Genetic effects (origin)														
Region (R_o)	1	<0.1	1	3.6(*)	–	–	1	<0.1	1	3.8(*)	1	0.1	1	0.2
Elevation (E_o)	2	3.2(*)	2	7.7*	1	0.3	2	16.0***	2	12.2**	2	8.9*	2	10.4**
Environmental \times genetic effects														
$R_o \times E_g$	1	3.2	1	1.9	–	–	1	3.5(*)	1	3.3(*)	1	6.0*	1	0.2
$E_o \times E_g$	2	0.2	2	0.5	1	0.8	2	2.1	2	<0.1	2	7.5*	2	5.7(*)
Residuals	312	–	269	–	159	–	283	–	351	–	99	–	316	–
Region East														
Environmental effects (gardens)														
Elevation (E_g)	1	14.8***	1	8.5**	1	8.5**	1	9.3**	1	20.3***	1	3.1(*)	1	4.1*
Block	9	5.8	9	30.7***	8	9.7	9	39.8***	9	22.9**	8	8.0	9	17.5*
Genetic effects (origin)														
Region (R_o)	1	3.5(*)	1	6.8**	–	–	1	0.5	1	2.3	1	0.8	1	<0.1
Elevation (E_o)	2	5.7(*)	2	19.5***	1	1.4	2	15.8***	2	21.6***	1	8.9*	2	5.3(*)
Environmental \times genetic effects														
$R_o \times E_g$	1	0.6	1	2.6	–	–	1	<0.1	1	<0.1	1	<0.1	1	1.8
$E_o \times E_g$	2	0.6	2	3.7	1	8.7**	2	0.8	2	2.7	1	<0.1	2	0.1
Residuals	306	–	294	–	120	–	274	–	331	–	60	–	228	–

All sources of variation were treated as fixed factors with binomial error distribution. The elevation of origin (E_o) was treated as a factor nested in region of origin (R_o) and block nested in common garden

E_g Elevation of the garden, *df* degree of freedom, χ^2 corresponds to the difference in deviance
Significant results in bold: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, (*) $P < 0.1$

Fig. 4 Percentage of individuals per provenance reaching budset at the end of July 2011 for each of the four provenances studied within each species in the eight common gardens established at different elevations. Symbols as in Fig. 1



provenances originating from low and high elevations (Table 5). Formation of apical buds tended to occur earlier for provenances originating from high elevations irrespective of the elevation of the garden (Fig. 4). This effect was quite strong for *A. pseudoplatanus* and both *Sorbus* species. No significant effect of the region of origin was detected in budset score, except for *P. avium* in the eastern gardens, where populations from the western region of origin tended to set buds earlier than populations from the eastern region of origin.

Genetic differences in phenological plasticity ($V_G \times V_E$)

Only few significant interactions between environmental and genetic effects were detected for budset score (Table 5). Interactions between elevation of origin and elevation of the gardens were detected in budset timing for *S. aucuparia* in the western gardens and for *L. alpinum* in the eastern gardens. An interaction between the region of origin and the elevation of the gardens was also detected in *S. aucuparia* in the western gardens. For all other species no significant interaction was found between environmental and genetic effects.

Discussion

The common gardens used in this study enabled the partitioning of environmental and genetic components responsible for tree seedling phenological patterns observed in situ. Furthermore, the experimental set-up allowed us to test exclusively the effect of temperature on tree seedling phenology as seedlings shared the same growth history and soil, and because they grew without water limitations and under standardized nutrient supply throughout the test period. Overall, our results showed that tree seedlings exhibited a strong phenological plasticity in their leaf unfolding date in response to temperature with substantial variation among species. Further, we demonstrated that the high elevation provenances exhibited smaller phenological plasticity, irrespective of the species and that phenological patterns along elevational gradients are, to a large extent, controlled by environmental conditions and to a much lesser extent by genetic differentiation among populations and interactions between these two drivers.

Plasticity of the leaf unfolding date (V_E and $V_E \times V_G$)

This study highlighted high inter-specific variations in the degree of phenological plasticity to changing temperatures, and that climate (in essence temperature) is the main driver of phenological plasticity in tree seedlings in spring. The

shifts of leaf unfolding date with elevation or spring temperature found here were close to the ones found in situ on mature trees along temporal or spatial gradients in other mountain areas (Lebourgeois et al. 2010; Davi et al. 2011; Cufar et al. 2012). For example, Vitasse et al. (2009b) reported along elevational gradients in Pyrenees (France) a comparable shift of 1.9 days K^{-1} for *F. sylvatica*, 5.0 days K^{-1} for *A. pseudoplatanus*, and 6.6 days K^{-1} for *F. excelsior* with a similar ranking among these species. Hence, our results suggest that, under continued global warming, tree species having high phenological plasticity in response to temperature (e.g., *F. excelsior* or *A. pseudoplatanus*) will likely respond to a greater extent than species with low phenological plasticity (e.g., *F. sylvatica* or *S. aucuparia*). Different responses of phenology to temperature change among species could ultimately affect their competitive abilities and elevational distributions. Species exhibiting the highest phenological plasticity might be temperature-dependent only and at risk of frost damage, while species with low plasticity might have a dual control of bud burst by temperature and photoperiod and be less at risk to track unusual weather. The role of the photoperiod in modulating bud burst dates has been previously demonstrated in *F. sylvatica* growing under controlled conditions (Wareing 1953; Heide 1993; Caffarra and Donnelly 2011; Basler and Körner 2012). Accordingly, *F. sylvatica* exhibited the lowest phenological plasticity to temperature in this study, which might reflect a photoperiodically controlled delay of bud burst at the lowest elevation gardens. Interestingly, and in line with our hypothesis, the genetic differentiation between low and high provenances affects the magnitude of the plasticity, given that for all species low elevation provenances exhibited greater phenological plasticity than high elevation provenances to temperature changes induced by elevational gradients used for the experimental gardens. This pattern might have been induced either by a directional selection for reduced temperature sensitivity or a more stringent interaction with photoperiodism, which together may reduce the risk of damage by unpredictable late spring frost events after warm weather episodes. This interaction between provenance and environment has crucial implication for modeling future phenological responses of temperate tree species. Variation in responses to temperature among populations might reduce the predictive power and the transferability of phenological models when they are calibrated on genetically differentiated populations. Nevertheless, we found that the environmental component of the plasticity was far stronger than genetic effects, highlighting that phenotypic variations of leaf phenology observed in situ for tree seedlings are mainly the consequence of plasticity due to environmental variation rather than genetic effects. This is in line with the few studies that

have adequately separated and quantified genetic and environmental contributions on leaf traits related to tree fitness (Kramer 1995; Baliuckas and Pliura 2003; Vitasse et al. 2010; Bresson et al. 2011). However, genetic differentiation among tree populations might be expected to be more pronounced along latitudinal gradients than along elevational gradients, because of the greater distance between core and leading edge populations that could mitigate gene flow. Directional selection may therefore more easily lead to genetic differentiation along latitudinal gradients (Jump et al. 2009), which, on the other hand, would reduce the genetic potential of populations growing on their upper latitudinal to respond to ongoing climate warming.

Genetic differentiation in the leaf unfolding date between low and high provenances

Genetic differentiation among populations of tree seedlings can be due to differences in chilling and forcing requirement to break bud dormancy, or in their different sensitivity to photoperiod. For the majority of the species, our results showed that provenances from high elevations tended to flush later than provenances from low elevations, similarly to the phenotypic cline observed in situ along elevational gradients (co-gradient variation). Although a genetic differentiation of the date of leaf unfolding was often found among provenances from different elevations in common garden experiments, the genetic cline can be opposite to the phenotypic cline observed along environmental gradients (counter-gradient variation). For example, seedling populations from high elevations were found to flush/sprout earlier than those from lower elevations in *Abies amabilis*, *Abies lasiocarpa*, *Pseudotsuga menziesii* and *F. sylvatica* (Worrall 1983; vonWuehlisch et al. 1995; Chmura and Rozkowski 2002; Acevedo-Rodriguez et al. 2006; Vitasse et al. 2009a; Gomory and Paule 2011), whereas they exhibited delayed flushing in *Prunus serotina*, *F. excelsior* and *Quercus petraea* (Barnett and Farmer 1980; Vitasse et al. 2009a; Alberto et al. 2011), or no significant difference in an array of species (Sharik and Barnes 1976; Vitasse et al. 2009a). These contrasting results demonstrate that species react differently but consistently to the evolutionary forces occurring along elevational gradients. The genetic clines occurring along environmental gradients have been generally thought as a consequence of divergent selection among populations due to either biotic or abiotic selection pressures. However, a recent study suggested that interaction between gene flow and assortative mating occurring along strong environmental gradients may lead to such co-variation of genetic and phenotypic clines among populations, without any divergent selection (Soularie and Kremer 2012). Nevertheless,

divergent selection may have shaped both co-gradient and counter-gradient variation among species co-existing along a same environmental gradient, depending on their sensitivity to a given selective pressure. In particular, along elevational gradients the balance between maximizing growth and minimizing the risk of frost damage might be a crucial evolutionary force leading to genetic differentiation in phenology between populations from low and high elevations. The short duration of the growing season at high elevations can be critical for the development of mature seeds and hardened tissues and buds for winter. Early flushing, thus, lengthens the period for growth, and subsequently increases competitive ability and the chance of producing mature seeds, while it simultaneously increases the risk of being exposed to late frosts that could damage flowers, leaves and meristem tissues. Thus, at the elevational limit of a species' range, two contrasting "strategies" might be selected for, possibly resulting in different clines among species co-existing along the same climatic gradient. In line with other studies, we found that genetic and phenotypic clines vary in opposite directions for seedlings of *F. sylvatica*, that is, seedlings from high elevations flushed earlier than those from low elevations when planted in a common garden (vonWuehlisch et al. 1995; Chmura and Rozkowski 2002; Vitasse et al. 2009a, 2010; Gomory and Paule 2011). Natural selection may have favored individuals exhibiting earlier leaf unfolding at high elevation, suggesting that for this species developmental constraints restrict fitness more than the risk of frost damage does. However, because it has been shown that significant genetic variation within populations can be preserved in traits under strong selective pressure (Howe et al. 2003), further investigations are needed to assess the genetic variation of phenological traits existing within populations along elevational gradients.

Environmental control of budset timing and genetic differentiation between low and high elevation provenances

Maturation of winter buds in late summer and rising frost hardness in autumn must be initiated before occurrence of frosts and are therefore assumed to play a key role in tree fitness (Hurme et al. 1997; Charrier and Ameglio 2011). Until recently, photoperiod (detected by the phytochrome receptor) was thought to be the only environmental control that determines budset timing of the majority of temperate tree species (Tanino et al. 2010). Yet, recent experimental studies demonstrated that, under the same photoperiod, temperature has a modulating role on the endogenous program of summer/autumn phenology, with low temperatures advancing the onset of growth cessation in several deciduous tree species (Molmann et al. 2005; Tanino et al. 2010; Heide 2011; Rohde et al. 2011). Although the date of

monitoring budset in this study was too late to unambiguously detect and disentangle both environmental and genetic effects, we found that, within the region of the gardens, the elevation of the gardens significantly affected bud formation in all species. Further, bud formation occurred later in the two highest elevation gardens, irrespective of provenances.

Except for *L. alpinum* and *F. excelsior*, a genetic differentiation between low and high elevation provenances was found, with low elevation provenances tending to achieve budset later than high elevation provenances. Among the few common garden experiments which investigated the time of growth cessation or budset with respect to the elevation of provenances, the same trend was reported for *F. sylvatica* (Chmura and Rozkowski 2002) and for two *Betula* species (Sharik and Barnes 1976). Genetic differentiation between low and high elevation provenances is likely the result of directional selection favoring individuals having earlier budset and leaf senescence at high elevation, and, thus, higher freezing tolerance in early autumn (Charrier and Ameglio 2011).

Conclusions

The ability of populations to thrive under contrasting climates depends on their genetic differentiation across climatic gradients and the genetic variation within populations (Aitken et al. 2008; Wang et al. 2010). In agreement with our first hypothesis, this study demonstrated that seedlings from tree populations growing at their upper elevational limits exhibit genetic adaptation in leaf phenology. Directional selection seems to operate in spite of the short distance among populations along elevational gradients and a presumably abundant gene flow across such gradients (Alberto et al. 2010). Our results also underlined that seedlings of tree populations in the Alps have high phenological plasticity in response to temperature changes, with substantial inter-specific differences. Interestingly, seedlings from high elevation provenances exhibited a lower phenological plasticity to temperature than those from low elevation provenances. Due to the large environmental effect and the small genetic effect on leaf unfolding date, our results suggest that both seedlings of populations of deciduous tree species growing at the upper elevation margin and of lower elevation populations have a high phenological plasticity to track ongoing climate change. Seedlings are known to exhibit more opportunistic phenology than adult trees, given that their success is not only constrained by a short thermal season but also by canopy closure, and thus light limitation (Augsburger 2008). Hence, the dominance of phenotypic over genotypic responses (in the sense of tracking concurrent

temperatures) might be seedling-specific. We thus expect genotypic responses to be more pronounced in the adult life stage.

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