

Local adaptation at the range peripheries of Sitka spruce

M. MIMURA & S. N. AITKEN

Forest Sciences and Centre for Forest Conservation Genetics, University of British Columbia, Vancouver, BC, Canada

Keywords:

gene flow;
local adaptation;
phenology;
phenotypic plasticity;
Picea sitchensis;
range-wide.

Abstract

High-dispersal rates in heterogeneous environments and historical rapid range expansion can hamper local adaptation; however, we often see clinal variation in high-dispersal tree species. To understand the mechanisms of the species' distribution, we investigated local adaptation and adaptive plasticity in a range-wide context in Sitka spruce, a wind-pollinated tree species that has recently expanded its range after glaciations. Phenotypic traits were observed using growth chamber experiments that mimicked temperature and photoperiodic regimes from the limits of the species realized niche. Bud phenology exhibited parallel reaction norms among populations; however, putatively adaptive plasticity and strong divergent selection were seen in bud burst and bud set timing respectively. Natural selection appears to have favoured genotypes that maximize growth rate during available frost-free periods in each environment. We conclude that Sitka spruce has developed local adaptation and adaptive plasticity throughout its range in response to current climatic conditions despite generally high pollen flow and recent range expansion.

Introduction

Understanding local adaptation at a species' range limits is critical for studying the evolution of the species' niche, and the potential for range shifts in response to global climate change. Peripheral populations at the boundaries of a heterogeneous range may have different evolutionary potentials than central populations as the regimes of natural selection in marginal environments are likely different (Kawecki, 2008). As changes in climate will pressure species to shift ranges to track climatic niches (e.g. Parmesan & Yohe, 2003), peripheral populations may play key roles for a species that is facing and responding to rapid environmental changes (Lesica & Allendorf, 1995; Hampe & Petit, 2005).

While peripheral populations have the potential to further expand species range through adaptation to marginal environments, in comparison with core populations, they tend to have less genetic diversity, more inbreeding and more genetic drift (reviewed by Eckert *et al.*, 2008), are smaller and more isolated from core

populations (Brown, 1984; Brown *et al.*, 1996; Sagarin *et al.*, 2006), and are more likely to be demographical sinks (e.g. Manier & Arnold, 2005; Angert, 2006). Population genetic theories predict that a high-dispersal among populations will hamper local adaptation in heterogeneous environments, and low dispersal and strong selection will promote local adaptation (Brown & Pavlovic, 1992; Spichtig & Kawecki, 2004). While gene flow is crucial for maintaining population size and genetic variation, to allow sink populations to adapt to local environments (Holt & Gomulkiewicz, 1997; Barton, 2001; Alleaume-Benharira *et al.*, 2006), negative effects of high-dispersal may be enhanced at a species' range peripheries because of asymmetric gene flow from the core to peripheral populations (Haldane, 1956; Mayr, 1963; Lande, 1972; Holt, 1996; Kirkpatrick & Barton, 1997; Lenormand, 2002). Thus, theories predict that peripheral populations tend to be maladapted unless isolated from gene flow, although little empirical evidence is available for plant species. Predominantly outcrossed, wind-pollinated forest tree species; however, generally exhibit moderate to steep genetic clines along climatic gradients for phenology and growth traits (Morgenstern, 1996; Howe *et al.*, 2003) despite high, pollen-mediated dispersal, as suggested by little among-population differentiation at neutral markers (Hamrick

Correspondence: Makiko Mimura, Environment and Information Sciences, Yokohama National University, 79-7 Tokiwadai, Yokohama, Kanagawa 240 8051 Japan.
Tel.: +81 45 339 4375; fax: +81 45 339 4375;
e-mail: mimura@ynu.ac.jp

et al., 1992). For instance, growth cessation is a trait that exhibits clinal variation in wind-pollinated forest tree species including *Populus tremula* (Ingvarsson *et al.*, 2006; Hall *et al.*, 2007), *Picea abies* (Oleksyn *et al.*, 1998; Collignon *et al.*, 2002) and *Picea mariana* (Beaulieu *et al.*, 2004). These species are expected to have experienced past range shifts during glaciations. This suggests that local adaptation seems to be achieved in such forest tree species despite high gene flow, even in expanding populations. Kawecki & Ebert (2004) also pointed out that local adaptation can be favoured only when there is little cost or other constraints on adaptive plasticity. In this study, we explore local adaptation and adaptive plasticity in three simulated environments across the species range of Sitka spruce *Picea sitchensis* (Bong., Carr.), which is known to have a high, pollen-mediated gene flow rate, high genetic diversity within populations and rapid northward range expansion during the last deglaciation.

Sitka spruce is a wind-pollinated conifer with a long, narrow distribution from California to Alaska along the Pacific coast of North America (Fig. 1). The species' range extends from 39° to 61°N latitude, and tapers in width

towards the southern and northern range limits. The environmental variation across the range is generally linearly heterogeneous, and the species gradually disappears at the northern and southern limits of the range. Like other forest species, an outdoor common garden experiment revealed strong population differentiation in bud set timing ($Q_{ST} = 0.89$), with northern populations ceasing primary growth, setting terminal buds and developing cold hardiness months earlier than the southern populations (Mimura & Aitken, 2007a). This species also exhibits little population differentiation among neighbouring populations; however, shows significant isolation by distance (Mimura & Aitken, 2007a), and strong core-peripheral population structure in rare allele frequencies (Gapare *et al.*, 2006), spatial genetic structure (Gapare & Aitken, 2006) and mating system parameters (Mimura & Aitken, 2007b).

Our primary objectives were to quantify the extent of local adaptation and phenotypic plasticity to climates at the southern and northern range limits of Sitka spruce. To accomplish this, we grew seedlings from populations sampled across the species range in growth chambers simulating the temperature and photoperiodic regimes of two peripheral environments (southern and northern peripheries) and of one environment typical of the centre of the species range. We characterized genetic clines across the species range, expressed in each environment and compared the performance among range-wide populations.

Materials and methods

Experimental design

The sampling strategy was designed to allow for a characterization of range-wide patterns of variation in Sitka spruce expressed in phenotypic traits. We obtained open-pollinated seeds, collected from 10 to 20 seed parents in each of these 17 natural populations, including geographically isolated populations at the northern (Kodiak Island, AK) and southern (Fort Bragg, CA) species range margins, and at the centre of the range (Queen Charlotte Islands, BC) (Fig. 1; Table 1). The details of geographical and climatical origins of these populations are described in Mimura & Aitken (2007b). All seeds were collected from low elevations near the ocean to eliminate elevational effects on adaptive traits, and to avoid sampling populations with introgression from *P. glauca*, a species adapted to more continental climates inland (Bennuah *et al.*, 2004).

Seeds from all 17 populations were soaked in water for 48 h, stratified at 4 °C for 3 weeks to break dormancy, and germinated on filter paper in Petri dishes. Germinals were transplanted into Ray Leach Cells® (Stuewe & Sons, Inc., Tangent, OK) and grown in a greenhouse at the University of British Columbia (UBC), Vancouver, Canada. First-year seedlings were moved outside in the

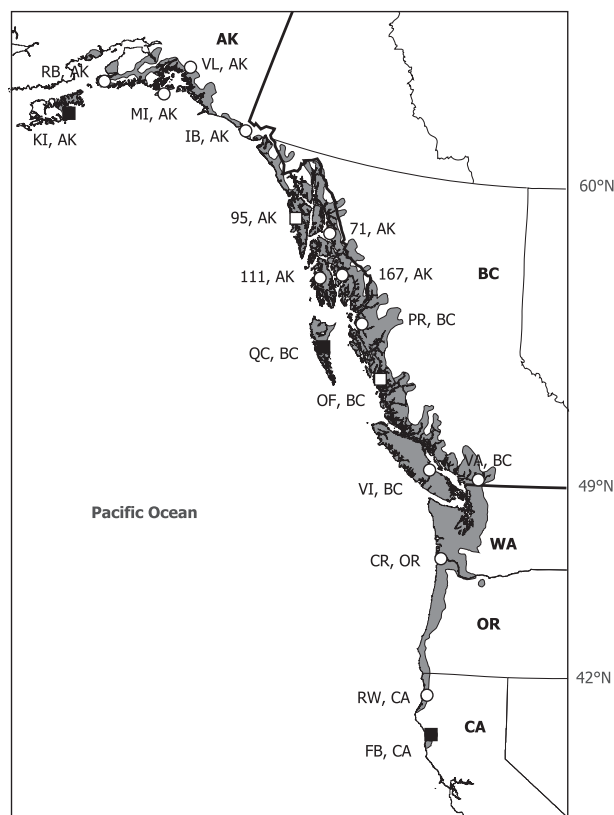


Fig. 1 Natural range of Sitka spruce (*Picea sitchensis*) (in grey) showing locations of sampled populations. Codes of populations for simulated environments (■) and all other populations (○) are defined in Table 1.

Table 1 Description of populations studied.

Population	Location	Original climate (°C)			No. seedlings tested			Distance from the centre (km)
		MAT	MWMT	MCMT	AK	BC	CA	
Kodiak Island (KI), AK*	57°N/153°W	4.7	12.8	−1.28	30	29	28	2041
Rocky Bay (RB), AK	58°N/151°W	4.1	12.7	−2.17	30	27	28	1888
Montague Is. (MI), AK	61°N/147°W	3.9	12.5	−4.11	34	33	34	1620
Valdez (VL), AK	62°N/146°W	3.5	12.9	−5.61	29	30	27	1477
Icy Bay (IB), AK	60.5°N/141°W	4.2	12	−3.44	27	29	29	1273
Lot 71 (71), AK	57.5°N/135°W	7.2	14	1.61	14	13	13	683
Lot 95 (95), AK	57°N/133°W	5.8	14.5	−3.00	16	14	9	583
Lot 167 (167), AK	56°N/131.5°W	6.6	14.2	−1.17	21	21	19	472
Lot 111 (111), AK	56°N/133.5°W	6.7	14	−0.11	20	17	18	428
Prince Rupert (PR), BC	53.5°N/130°W	7.1	13.5	1.30	26	25	26	0
Queen Charlotte Is. (QC), BC*	53°N/132°W	8	16.8	3.20	26	30	28	−180
Ocean Falls (OF), BC	52.5°N/128°W	8.3	15	−1.00	30	28	29	−223
Vancouver Is. (VI), BC	49.5°N/125°W	9.7	17.6	3.00	30	27	27	−412
Vancouver (VA), BC	49°N/123°W	10	17.1	3.60	28	29	28	−553
Columbia River (CR), OR	47°N/124°W	10.6	16	5.78	29	30	29	−911
Redwood (RW), CA	42°N/124°W	11.6	14.8	8.83	29	27	27	−1360
Fort Bragg (FB), CA*	39°N/123°W	11.8	14.7	9.11	27	23	22	−1652
Total					446	432	421	

Climatic variables are: mean annual temperature (MAT), mean warmest month temperature (MWMT), and mean coldest month temperature (MCMT). Origins are AK (Alaska, US), BC (British Columbia, Canada), OR (Oregon, US), and CA (California, US). Geographical origins of all populations are indicated in Fig. 1.

*The regions used to simulate chamber environments.

autumn of 2002 to experience sufficient chilling to break bud dormancy. Plants were then moved into three Conviron E15 growth chambers (Controlled Environments, Inc., Winnipeg, MB, Canada) on 7 April 2003, with each chamber programmed to simulate the climate of one geographical location: southern periphery, range centre and northern periphery. Climate data were obtained from Daily Climate Normals (1971–2000) for: (i) Fort Bragg, CA (southern environment); (ii) Sandspit, Queen Charlotte Islands, BC (central environment); and (iii) Kodiak Island, AK (northern environment). Climate variables at population origins were obtained from monthly climate normals (from 1971 to 2000) (National Climate Data Center of US and Environment Canada; Table 1). Temperatures and photoperiods were averaged for each 2-week period, based on climate normals of a growing season from 7 April to 10 December, and the averages were used to set the growth chamber conditions for 2-week intervals. Daily temperatures were ramped from the daily normal minimum at midnight to the daily normal maximum at noon. The isolated and nearest continuous provenances at each range periphery included in this study had very similar summer climate variables (Table 1) and growth chamber conditions averaged these for simulated temperature and photoperiod regimes.

As only three growth chambers were available, true replication of treatments was not possible; thus, each chamber can be considered a separate experiment. A complete randomized design was used for individuals within the chambers. A small number of seedlings had

buds or new shoots injured during measurement; these were left in the chamber until the experiment was completed but were excluded from analyses. Seedlings were kept well-watered and were fertilized regularly in all chambers. At the end of the experiment, each chamber contained a total of 421–446 seedlings for the analysis, from the 17 populations, with an average of 24.8–26.2 seedlings per population. There was no mortality during the experiments where only growing season conditions, which did not fall below 4 °C were simulated.

The measured phenotypic traits included second-year height increment (height), date of bud break (bud break), date of bud set (bud set), days of active height growth between bud break and bud set (growth period), mean daily growth rate (height increment divided by growing period = growth rate) and total biomass at the end of the second growing season. Bud break date and bud set date were expressed as number of days from 7 April, the first day of the experiment.

Statistical analysis

To examine whether the species range distribution along the coast is associated with climate variables, regression analysis for geographical distance from the central population along the coast was performed with climate variables. Phenotypic traits were analysed separately for each chamber and the ANOVA was performed to test the effects of population on trait variation. Geographical

clines of individual phenotypic values were regressed on distance from the centre of the range to assess the among-population divergence in the traits. All analyses were performed using R 2.5.1 (R Development Core Team, 2007).

Autumn frost information from climate data

Measuring the lifetime fitness and reproductive success of long-lived conifers is extremely difficult, as decades may pass before they reach a reproductive age, and they can reproduce for centuries. While all of the measured traits were assessed for patterns of clinal variation, we focused on bud phenology and juvenile growth traits as there is evidence of these traits showing strong local adaptation in temperate tree species (Howe *et al.*, 2003; Savolainen *et al.*, 2007). This largely represents a tradeoff between maximizing height growth to compete for light in a given environment, and synchronizing growth phenology with the local climate to avoid frost injury. To assess the seasonal fit between climate and growth rhythm, we used estimations of the first frost date from climate normals for 1971–2000 as the first date in the autumn with a 50% probability of temperatures below 0 °C. These dates were: 30 September, in the northern environment (Kodiak Island and Kitoi Bay, AK, data from the Western Regional Climate Center of US); 3 November, in the central environment (Sandspit, BC, BC Ministry of Forests and Range); and 11 December, in the southern environment (Fort Bragg, CA, Western Regional Climate Centre of US). The date for the 50% probability of first frost, based on the climate normal, meant a 50% chance of frost by that date in the autumn of a year. A seedling would have a high chance of encountering frost by that date, within a few years, which would be expected to cause critical damage to juvenile seedlings, if it had not yet set a terminal bud. Geographical areas that are near the simulated planting locations also have similar dates of first frost: 8 October, in the south Alaska environment (Seward, AK, at the northernmost continuous range of the species); 4 October, in the southeast Alaska environment (Juneau, AK, close to the '95' population, which is at a latitude similar to the Kodiak populations); and 14 December, in the southern continuous environment (Eureka, BC; close to the Redwood population).

Results

Geographical distance from the centre of the range along the coast had a strong and significant relationship with climate variables; especially, mean annual temperature and degree-days above 5 °C, based on climate data from 1971 to 2000 [$F(1,15) = 198.9$; $R^2 = 0.93$; $P < 0.001$ and $F(1,15) = 174.4$; $R^2 = 0.92$; $P < 0.001$ respectively; Fig. 2]. The distance from the centre of the range showed a weaker but significant trend with mean warmest month temperature [$F(1,15) = 15.99$; $R^2 = 0.48$; $P =$

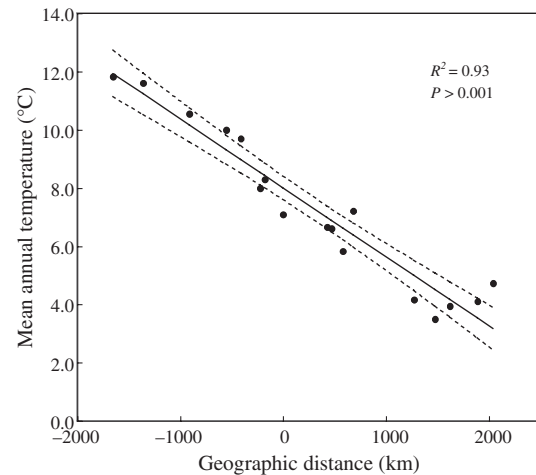


Fig. 2 Relationship between mean annual temperature and geographical distance along the Pacific coast for provenances sampled within the range of Sitka spruce. Dashed line indicates 95% confidence intervals. The x-axis is geographical distance (km) from the Prince Rupert population (PR), which is located approximately at the geographical centre of the species range.

0.001] and mean coldest month temperature [$F(1,15) = 53.37$; $R^2 = 0.77$; $P < 0.001$], but the regression with precipitation was not significant [$F(1,15) = 1.48$; $R^2 = 0.03$; $P = 0.24$].

Most of the quantitative traits showed significant among-population variation (Table 2), and a clinal pattern of variation with distance along the coast (Table 3, Fig. 3). Bud set timing exhibited strong clinal variation along the coast in all chamber environments in the regression analyses (Table 3; Fig. 3a). Generally, bud set occurred earlier in the northern populations and later in the southern populations. It also occurred earlier in the southern environment and later in the central and northern environments, likely because the critical night-length for terminal bud formation was reached earlier under the simulated southern photoperiod. Variation among populations was responsible for 75.9–79% of all

Table 2 Results of analysis of variance of among-population differences in phenotypic traits (d.f. = 16).

Traits	Southern environment		Central environment		Northern environment	
	F-value	P-value	F-value	P-value	F-value	P-value
Bud set (days)	86.51	0.000	107.16	0.000	99.39	0.000
Bud break (days)	4.66	0.000	3.27	0.000	4.29	0.000
Growth period (days)	72.74	0.000	56.47	0.000	40.06	0.000
Height (mm)	19.77	0.000	2.43	0.002	3.85	0.000
Biomass (mg)	13.96	0.000	7.73	0.000	13.85	0.000
Daily growth rate (mm)	1.4	0.137	4.71	0.000	1.96	0.015

Table 3 Regressions of individual phenotypic variation in observed quantitative traits.

Traits	Southern environment			Central environment			Northern environment			Q_{st}^*
	Gradient	R^2	P	Gradient	R^2	P	Gradient	R^2	P	
Bud set (days)	3.28	0.73	< 0.0001	1.99	0.51	< 0.0001	2.6	0.68	< 0.0001	0.89
Bud break (days)	0.01	0.01	0.0184	0.09	0	0.08	0.24	0.03	0.0003	0.29
Height (mm)	2.83	0.37	< 0.0001	0.09	0	0.53	1.12	0.09	< 0.0001	0.55–0.79
Biomass (g)	0.1	0.31	< 0.0001	0.05	0.15	< 0.0001	0.07	0.29	< 0.0001	N/A
Daily growth rate (mm)	−0.0008	0	0.62	−0.008	0.11	< 0.0001	−0.003	0.01	0.0122	0.28
Growth period (days)	3.19	0.71	< 0.0001	1.89	0.48	< 0.0001	2.35	0.55	< 0.0001	0.87

Gradients (based on regression coefficients) are expressed as estimated changes in phenotypic traits with every 100 km from north to south along the coast.

* Q_{st} values were estimated by Mimura & Aitken 2007 using an outdoor common garden experiment at University of British Columbia, BC, Canada, are shown as references.

variation in bud set within chambers. Bud break timing varied less among populations, compared with bud set, and was highly variable within populations (Fig. 3b). Bud break was generally earlier in the southern environment, intermediate in the central environment, and later in the northern environment, reflecting differences in rate of heat sum accumulation. It also showed weak clinal variation among populations in the southern and northern environments (Fig. 3b). Growth period, calculated as the number of days between bud break and bud set, showed strong among-population variation (76%, 68% and 57% in southern, central, and northern environments, respectively). It generally increased from the southern to the northern simulated environments in all populations (Table 3; Fig. 3c). Variation in growth period largely reflected variation in bud set timing; thus, bud set was chosen as the phenological trait of interest in subsequent analyses.

A significant cline was seen along the coast in height (second-year height increment in the growth chamber environment) in the southern environment. The cline in height was significant but weaker in the northern environment and not significant in the central environment (Table 3; Fig. 3d). The majority of variation in height was within-population (56%, 95% and 89% in southern, central and northern environments, respectively). Biomass had moderate among-population variation (25–37.2%) and displayed a significant cline along the coast in all chamber environments (Table 3; Fig. 3e). The southern populations had slightly less biomass in the northern, compared with southern environment, while the northern populations did not differ substantially for biomass among the chambers (Fig. 3e).

Surprisingly, the central populations were slightly shorter in their original central environment than in the other environments. The southern and central populations both showed reduced biomass in the central environment, compared with that in both the northern and southern environments. After the experiment was completed, the chamber used for the central environment was found to be exchanging air at a different rate, compared with the other chambers, so the seedlings may

have been carbon dioxide deprived. In any case, as this did not appear to influence the timing of bud set, the results were included, though they should be interpreted with some caution.

The majority of variation for daily growth rate was also found to be within populations (88–97%). All populations had a high growth rate in the southern environment with no clinal trend expressed (Fig. 3f). Growth rate showed a clinal response along the coast in the central and northern environments (Table 3; Fig. 3f). The growth rates of southern populations were dramatically lower in the two more northern environments, compared with those of the southern environment, while the northern populations showed comparatively lower reduction in growth rate from the southern to the northern environments (Fig. 3f).

Among the observed traits, height increment (Fig. 4a) and growth rate (Fig. 4b) showed genotype-by-environment interaction, while bud phenology (Fig. 3c,d) did not. The local population tended to have a greater height increment and greater growth rate per day (height increment divided by growing days) than nonlocal populations. Seedlings from southern populations grew less in the cooler environments (central and northern) than in the southern environment, while the northern populations grew more in the cooler environments than in the southern environment (Fig. 4a). The first autumn frost date, with a 50% probability, is estimated to come earlier than the dates when the southern and central populations actually set bud in the northern environment (Fig. 4c). The estimated autumn first frost dates in the environments were calculated based on actual climate data, and may not be directly comparable with the observed bud set dates, based on the simulated common garden experiment using chambers controlled only for temperature and photoperiod. Nonetheless, only the northern genotypes tended to set buds before the frost risk increased in the northern environments.

While genotype-by-environment interaction is evident in height increment and growth rate between two extreme environments, bud set and bud burst timing showed no genotype-by-environment interaction. Each

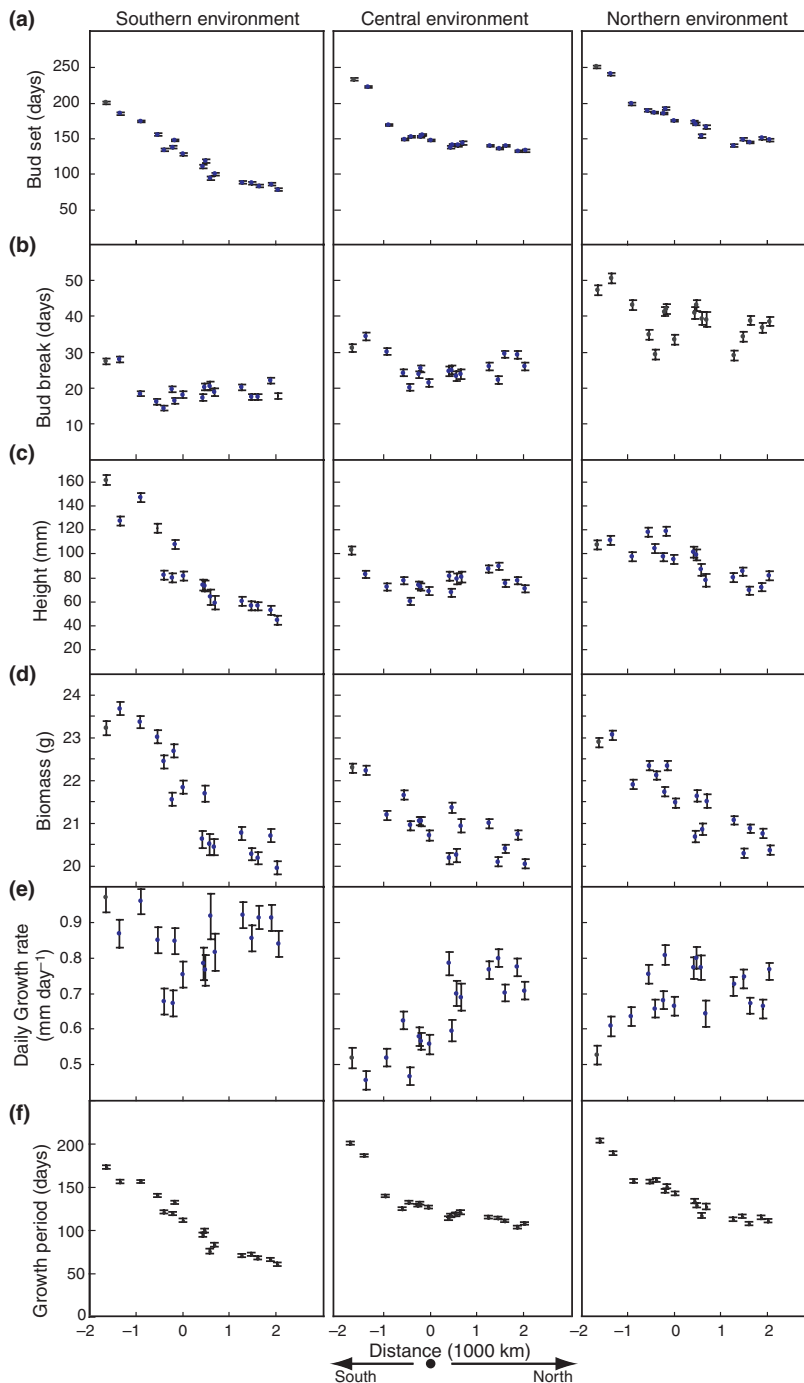


Fig. 3 Phenotypic clines in selected growth response variables in three growth chamber environments by population provenance along the coast. The x-axis is geographical distance (km) from the Prince Rupert population (PR), which is located approximately at the geographical centre of the species range. Chamber names indicate the geographical location simulated for temperature and photoperiod conditions during the growing season. Traits include: (a) bud set; (b) bud break (days after 7 April 2003); (c) height; (d) biomass; (e) daily growth rate; and (f) growth period. Error bars indicate standard errors of the population means.

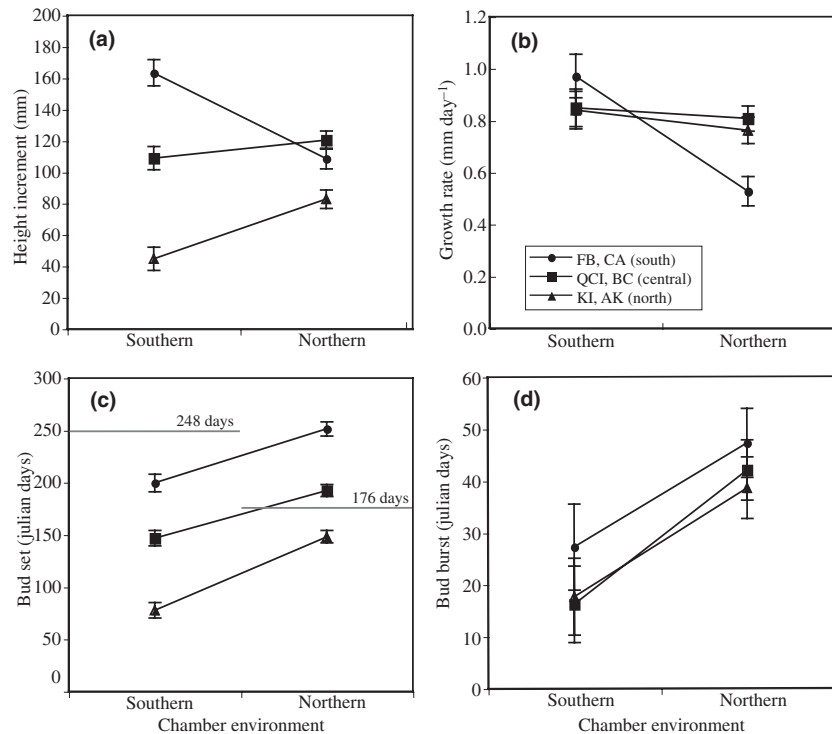
population seems to have parallel reaction norms in the bud phenology. While bud set timing was delayed in the cooler and longer photoperiod northern environment during the summer season, which increases the risk of frost injury during active growth, buds flushed earlier in the warmer and shorter photoperiod southern environment, in which the risk of cold injury ends earlier in spring, allowing an earlier start to the growing season.

Discussion

Local adaptation and phenotypic plasticity

Adaptive plasticity is an evolutionary alternative to local adaptation (Kawecki & Ebert, 2004). Local adaptation, in the presence of natural selection, may evolve with a limited dispersal rate (e.g. Kisdi, 2002), stronger spatial variation

Fig. 4 Genotype-by-environment interaction of three populations representative of two simulated peripheral environments, as well as a central environment: Fort Bragg, CA (southern population native to the southern simulated environment); QCI, BC (central population; central environment not shown); and Kodiak, AK (northern population native to the northern simulated environment). Each plot shows differences in phenotypic values in height increment (a), growth rate per day (b), bud set timing (c) and bud burst timing (d). Bud set and bud burst phenology are recorded as number of days since the experiment started (7 April). Plot (c) indicates the number of days since the experiment started with a 50% probability of first autumn frost estimated from climate normals (1971–2000).



than temporal variation and also in the absence of cost or constraints to adaptive phenotypic plasticity (Kawecki & Ebert, 2004). Local adaptation can also be seen in the phenotypic tradeoffs that occur between populations in different environments (i.e. genotype-by-environment interaction).

In Sitka spruce, bud phenology (specifically, the timing of bud set) shows well-developed adaptive divergence in this species (Mimura & Aitken, 2007a). The growth period is likely optimized to fit the frost-free period in the local climates, especially for northern populations where cold hardiness is critical for growth and survival of this species. The timing of bud break is determined by heat sum accumulation once adequate autumn chilling has been received (Howe *et al.*, 2003). In contrast, growth cessation and terminal bud set are triggered by a species- and population-specific critical night length (Howe *et al.*, 2003). Night length is shorter in northern, rather than southern environments, until the autumnal equinox, which likely explains why bud set dates were later for all populations in the northern environment. An extended photoperiod in northern environments would delay growth cessation in all populations of this species. These findings imply that bud set timing is under strong selection that favours local genotypes because of the trade-off between maximizing growth and avoiding frost injury, i.e. bringing northern genotypes to the south can result in a short period of active growth that does not fully utilize the longer frost-free period; and bringing southern genotypes to the north can result in later bud

set and an increased risk of exposure to frost during active growth. This would explain the high among-population divergence for this trait.

On the other hand, reaction norms in bud burst seem to reflect phenotypic plasticity. Bud burst requires heat sum accumulation after adequate chilling. Many *Picea* species do not have an absolute chilling requirement (Nienstaedt, 1967; Worrall & Mergen, 1967). A provenance test in Norway spruce found a substantial latitudinal cline in degree-days to bud burst in the absence of chilling; however, chilling treatment reduced differences among provenances (Søgaard *et al.*, 2008). Seedlings in our study were all chilled in the same outdoor environment before the growth chamber experiment commenced. Thus, if there is adaptive divergence in the intrinsic heat sum requirement for bud burst with limited or no chilling, it may be masked in this study. Nonetheless, our study showed that buds flushed earlier in warmer temperatures where the growth season is extended. The nonapparent constraint of adaptive plasticity on local adaptation may also have resulted in little clinal variation for this trait.

Growth rate responds to temperature, but populations may differ in their thresholds or optimal growth temperatures (Chuine *et al.*, 2001). Growth rate is likely a key trait for local adaptation in temperate tree species to fit the seasonal cycle and to reach a maximum growth (Rehfeldt, 1989; Mimura & Aitken, 2007b). This trait also shows strong evidence for local adaptation in this species, in that local genotypes had higher growth rates than

nonlocal genotypes in a local environment, considering the timing of frost, although we assumed that bud set timing in our experiments under simulated photoperiod and temperature regimes adequately represented actual bud set timing in wild. Selection appears to favour genotypes that maximize growth rate during a growing period within the seasonal limits of each environment. Differentiation among populations is thus driven on one hand by differential selection for competitive growth rates and, on the other hand, for growth rhythm synchronization with the available frost-free period.

Local adaptation at range peripheries

Numerous factors may influence adaptation in peripheral populations, including phylogeographical constraints (Pujol & Pannell, 2008), metapopulation dynamics (Holt *et al.*, 2005), adaptive trade-offs (Angert *et al.*, 2008), dispersal ability (Geber, 2008; Darling *et al.*, 2008) and interspecific interactions (Case & Taper, 2000; Case *et al.*, 2005). A high dispersal rate can also hamper local adaptive divergence (reviewed in Kawecki & Ebert, 2004). High pollen flow among populations is frequently observed in wind-pollinated forest tree species. Such high gene flow can hamper the adaptive divergence among populations in different environments; however, steep clines in growth and phenological traits are often associated with climate or geographical gradients (e.g. Cambell, 1979; Morgenstern, 1996; Howe *et al.*, 2003; Savolainen *et al.*, 2007). Thus, natural selection appears to be fairly strong to overcome the negative effects of gene flow among populations. In this study, we also showed strong evidence of clinal variation in adaptive growth and phenological traits in Sitka spruce, throughout the species' range.

Sitka spruce is thought to have expanded its range from southern refugia in coastal California after the last glacial maximum. Additive genetic variances in leading edge peripheral populations can be depleted during range expansion or migration, and reduce the ability of populations to respond to selection (Pujol & Pannell, 2008). Our results, however, showed adaptive gradients among populations across the entire species range, including geographically peripheral populations. Somewhat paradoxically, the species studied here exhibited clinal variation of adaptive traits to the range limits, not only in populations with high gene flow but also in populations in areas of recent post-glacial population expansion. The northern isolated population (KI) is thought to have arrived on Kodiak Island only 400–600 years ago, corresponding to approximately 3–5 generations. This estimate is based on observations that many living trees on the island are ~300 years old, along with few fallen and decayed logs (Griggs, 1937; J. Alden, personal communication). In addition, in our previous study, we found a higher selfing rate (S) in the peripheral populations, especially in peripheral and disjunct populations, both at

the northern (KI; $S = 35\%$) and at the southern limits (FB; $S = 15\%$; Mimura & Aitken, 2007b). This observation was at the seed stage, and subsequent early selection on inbred germinants or seedlings may offset or reduce the effects of high selfing rate at the adult stage. Our tested individuals in this study were from the same seed collections used by Mimura & Aitken (2007b) to study mating systems. The life history characteristics of Sitka spruce, including high genetic variation within populations, mixed mating system, high fecundity and great longevity, may increase the effective population size and response to natural selection, even with severe inbreeding depression as a feature of this species. Holliday *et al.* (2008) also found that many genes are involved in determining the degree of cold hardiness in Sitka spruce. High genetic diversity in the genes responsible for local adaptation may also contribute to the rapid development of adaptive gradients. Nonetheless, Sitka spruce clearly demonstrates the ability to develop local adaptation and plastic responses to current climatic conditions, at least partially, despite high gene flow between neighbouring populations (Gapare *et al.*, 2006; Mimura & Aitken, 2007a) and an apparently rapid expansion after deglaciation (Mimura & Aitken, 2007b).

Similar patterns of putatively adaptive clinal variation in bud phenology and growth have often been observed in tree species along latitudinal (e.g. Collignon *et al.*, 2002; Hall *et al.*, 2007) and elevational (e.g. Oleksyn *et al.*, 1998) gradients, including our study species (Mimura & Aitken, 2007a). The molecular basis of clinal variation in phenology is now being investigated in our Sitka spruce populations (Holliday *et al.*, 2008; and J.A. Holliday and S.N. Aitken, unpublished data) as well as other species (e.g. Hall *et al.*, 2007). However, few studies have analysed adaptation across entire tree species ranges and within the context of range limits, as provenance trials usually include only populations and sites within a limited portion of species ranges as the focus is often limited to geopolitical units in which species are actively planted and managed for forestry. Our study provides a clearer picture of how populations exhibit a range of responses to extreme environments at range peripheries, and how local adaptation resulting from a combination of traits plays an important role in population growth at range limits, including both the leading and rear edge of post-glacial migration.

Although an adaptive gradient was evident, epigenetic effects should also be considered as the seeds we used in this study were collected from wild stands. Epigenetic differences in phenology and cold hardiness, because of differences in maternal parent environments (Kvaalen & Johnson, 2008), may increase the phenotypic clines if the seeds for common garden experiments are collected from wild stands (Aitken & Hannerz, 2000). Moreover, phenotypic gradients with environmental conditions do not necessarily suggest that the populations are *optimally* adapted to local conditions. Population colonization

history may also influence the degree of local adaptation since a longer history of population colonization would be expected to have more generations to adapt to local environments (Fischer, 1960). The optimum cline could be steeper than what we see now. Further investigation including epigenetic effects and adaptation lag, in combination with distribution of candidate genes for local adaptation at the range limits, especially at the northern limits, will elucidate the abilities of plant species to both shift their range and adapt in response to new climates.

Acknowledgments

The authors thank MC Whitlock, PE Arcese, J Whitton and DJ Schoen for their useful comments and suggestions on the earlier version of this manuscript; and C Chourmouzis and J Tuytel for field and editorial assistance. The authors also show our gratitude to J Alden for providing biological information for Sitka spruce in Alaska and permitting us to access his collections of Sitka spruce seeds from a wide range of Alaska, and to J King, BC Ministry of Forests and Range, for providing seeds from many other populations. This study was supported by an NSERC Discovery Grant and funding from the Forest Investment Account through the Forest Genetics Council of BC to SNA. Funding for the Global Center of Excellence for Ecological Risk Management, and Yokohama National University also supported MM in preparing this manuscript.

References

- Aitken, S.N. & Hannerz, M. 2000. Genecology and gene resource management strategies for conifer cold hardiness. In: *Conifer Cold Hardiness* (F. Bigras & S. Colombo, eds), pp. 23–54. Kluwer Academic Press, NY.
- Alleaume-Benharira, M., Pen, I.R. & Ronce, O. 2006. Geographical patterns of adaptation within a species' range: interactions between drift and gene flow. *J. Evol. Biol.* **19**: 203–215.
- Angert, A.L. 2006. Demography of central and marginal populations of monkeyflowers (*Mimulus cardinalis* and *M. lewisii*). *Ecology* **87**: 2014–2025.
- Angert, A.L., Bradshaw, H.D. Jr & Schemske, D.W. 2008. Using experimental evolution to investigate geographic range limits in monkeyflowers. *Evolution* **62**: 2660–2675.
- Barton, N.H. 2001. Adaptation at the edge of a species' range. In: *Integrating Ecology and Evolution in a Spatial Context* (J. Antonovics, ed.), pp. 365–392. Blackwell Scientific, Oxford.
- Beaulieu, J., Perron, M. & Bousquet, J. 2004. Multivariate patterns of adaptive genetic variation and seed source transfer in black spruce. *Can. J. For. Res.* **34**: 531–545.
- Bennuah, S.Y., Wang, T.L. & Aitken, S.N. 2004. Genetic analysis of the *Picea sitchensis* x *glauca* introgression zone in British Columbia. *For. Ecol. Manag.* **197**: 65–77.
- Brown, J.H. 1984. On the relationship between abundance and distribution of species. *Am. Nat.* **124**: 255–279.
- Brown, J.S. & Pavlovic, N.B. 1992. Evolution in heterogeneous environments: effects of migration on habitat specialization. *Evol. Ecol.* **6**: 360–382.
- Brown, J.H., Stevens, G.C. & Kaufman, D.M. 1996. The geographic range: size, shape, boundaries, and internal structure. *Annu. Rev. Ecol. Syst.* **27**: 597–623.
- Cambell, R.K. 1979. Genecology of Douglas-fir in a watershed in the Oregon cascades. *Ecology* **60**: 1036–1050.
- Case, T.J. & Taper, M.L. 2000. Interspecific competition, gene flow, environmental gradients, and the coevolution of species borders. *Am. Nat.* **155**: 583–605.
- Case, T.J., Holt, R.D., McPeck, M.A. & Keitt, T.H. 2005. The community context of species' borders: ecological and evolutionary perspectives. *Oikos* **108**: 28–46.
- Chuine, I., Aitken, S.N. & Ying, C.C. 2001. Temperature thresholds of shoot elongation in provenances of *Pinus contorta*. *Can. J. For. Res.* **31**: 1444–1455.
- Collignon, A.H., van de Syde, H. & Favre, J.M. 2002. Geographical variation in random amplified polymorphic DNA and quantitative traits in Norway spruce. *Can. J. For. Res.* **32**: 266–282.
- Darling, E., Samis, K.E. & Eckert, C.G. 2008. Increased seed dispersal potential towards geographic range limits in a Pacific coast dune plant. *New Phytol.* **178**: 424–435.
- Eckert, C.G., Samis, K.E. & Loughheed, S.C. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Mol. Ecol.* **17**: 1170–1188.
- Fischer, A.G. 1960. Latitudinal variations in organic diversity. *Evolution*, **14**: 64–81.
- Gapare, W.J. & Aitken, S.N. 2006. Strong spatial genetic structure in peripheral but not core populations of Sitka spruce [*Picea sitchensis* (Bong.) Carr.]. *Mol. Ecol.* **14**: 2659–2667.
- Gapare, W.J., Aitken, S.N. & Ritland, C.E. 2006. Genetic diversity of core and peripheral Sitka spruce (*Picea sitchensis* (Bong. Carr.) populations: implications for conservation of widespread species. *Biol. Conserv.* **123**: 113–123.
- Geber, M.A. 2008. To the edge: studies of species' range limits. *New Phytol.* **178**: 228–230.
- Griggs, R.F. 1937. Timberlines as indicators of climate trends. *Science* **85**: 251–255.
- Haldane, J.B. 1956. The relation between density regulation and natural selection. *Proc. R. Soc. Lond. B Biol. Sci.* **145**: 306–308.
- Hall, D., Luquez, V., Garcia, V.M., St Onge, K.R., Jansson, D. & Ingvarsson, P.K. 2007. Adaptive population differentiation in phenology across a latitudinal gradient in European Aspen (*Populus tremula*, L.): a comparison of neutral markers, candidate genes and phenotypic traits. *Evolution* **61**: 2849–2860.
- Hampe, A. & Petit, R.J. 2005. Conserving biodiversity under climate change: the rear edge matters. *Ecol. Lett.* **5**: 461–467.
- Hamrick, J.L., Godt, M.J.W. & Sherman-Broyles, S.L. 1992. Factors influencing levels of genetic diversity in woody plant species. *New For.* **6**: 95–124.
- Holliday, J.A., Ralph, S.G., White, R., Bohlmann, J. & Aitken, S.N. 2008. Global monitoring of autumn gene expression within and among phenotypically divergent populations of Sitka spruce (*Picea sitchensis*). *New Phytol.* **178**: 103–122.
- Holt, R.D. 1996. Demographic constraints in evolution: towards unifying the evolutionary theories of senescence and niche conservation. *Evol. Ecol.* **6**: 433–447.
- Holt, R.D. & Gomulkiewicz, R. 1997. How does immigration influence local adaptation? A reexamination of a familiar paradigm. *Am. Nat.* **149**: 563–572.
- Holt, R.D., Keitt, T.H., Lewis, M.A., Maurer, B.A. & Taper, M.L. 2005. Theoretical models of species' borders: single species approaches. *Oikos* **108**: 18–27.

- Howe, G.T., Aitken, S.N., Neale, D.B., Jermstad, K.D., Wheeler, N.C. & Chen, T.H.H. 2003. From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. *Can. J. Bot.* **81**: 1247–1266.
- Ingvarsson, P.K., Garcia, M.V., Hall, D., Luquez, V. & Jansson, S. 2006. Clinal variation in *phyB2*, a candidate gene for day-length-induced growth cessation and bud set, across a latitudinal gradient in European Aspen (*Populus tremula*). *Genetics* **172**: 1845–1853.
- Kawecki, T.J. 2008. Adaptation to marginal habitats. *Annu. Rev. Ecol. Syst.* **39**: 321–342.
- Kawecki, T.J. & Ebert, D. 2004. Conceptual issues in local adaptation. *Ecol. Lett.* **7**: 1225–1241.
- Kisdi, E. 2002. Dispersal: risk spreading versus local adaptation. *Am. Nat.* **159**: 579–596.
- Kirkpatrick, M. & Barton, N.H. 1997. Evolution of a species' range. *Am. Nat.* **150**: 1–23.
- Kvaalen, H. & Johnson, Ø. 2008. Timing of bud set in *Picea abies* is regulated by a memory of temperature during zygotic and somatic embryogenesis. *New Phytol.* **177**: 49–59.
- Lande, R. 1972. Rapid origin of sexual isolation and character divergence in a cline. *Evolution* **36**: 213–223.
- Lenormand, T. 2002. Gene flow and the limits to natural selection. *Trends Ecol. Evol.* **17**: 183–189.
- Lesica, P. & Allendorf, F.W. 1995. When are peripheral populations valuable for conservation? *Conserv. Biol.* **9**: 753–760.
- Manier, M.K. & Arnold, S.J. 2005. Population genetic analysis identifies source-sink dynamics for two sympatric garter snake species (*Thamnophis elegans* and *Thamnophis sirtalis*). *Mol. Ecol.* **14**: 3965–3976.
- Mayr, E. 1963. *Animal Species and Evolution*. Harvard Univ. Press, Cambridge.
- Mimura, M. & Aitken, S.N. 2007a. Adaptive gradients and isolation by distance with postglacial migration in *Picea sitchensis*. *Heredity* **99**: 224–232.
- Mimura, M. & Aitken, S.N. 2007b. Increased selfing and decreased effective pollen donor number in peripheral relative to central populations in *Picea sitchensis* (Pinaceae). *Am. J. Bot.* **94**: 991–998.
- Morgenstern, E.K. 1996. *Geographic Variation in Forest Trees*. UBC Press, Vancouver.
- Nienstaedt, H. 1967. Chilling requirements in seven *Picea* species. *Silvae. Genet.* **16**: 65–68.
- Oleksyn, J., Modrzyński, J., Tjoelker, N.G., Zytkowski, R., Reich, P.B. & Karolewski, P. 1998. Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. *Func. Ecol.* **12**: 573–590.
- Parmesan, C. & Yohe, G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**: 37–42.
- Pujol, B. & Pannell, J.R. 2008. Reduced responses to selection after species range expansion. *Science* **321**: 96.
- R Development Core Team 2007. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. URL <http://www.R-project.org>.
- Rehfeldt, G.E. 1989. Ecological adaptations in Douglas-fir (*Pseudotsuga menziesii* var. *glauca*): a synthesis. *For. Ecol. Manage.* **28**: 203–215.
- Sagarin, R.D., Gaines, S.D. & Gaylord, B. 2006. Moving beyond assumptions to understand abundance distributions across the ranges of species. *Trends Ecol. Evol.* **21**: 524–530.
- Savolainen, O., Pyhäjärvi, T. & Knürr, T. 2007. Gene flow and local adaptation in trees. *Annu. Rev. Ecol. Syst.* **38**: 595–619.
- Søgaard, G., Johnsen, Ø., Nilsen, J. & Junntila, O. 2008. Climatic control of bud burst in young seedlings of nine provenances of Norway spruce. *Tree Physiol.* **29**: 311–320.
- Spichtig, M. & Kawecki, T.J. 2004. The maintenance (or not) of polygenic variation by soft selection in heterogeneous environments. *Am. Nat.* **164**: 70–84.
- Worrall, J. & Mergen, F. 1967. Environmental and genetic control of dormancy in *Picea abies*. *Physiol. Plant* **20**: 733–745.

Received 23 April 2009; revised 2 November 2009; accepted 4 November 2009