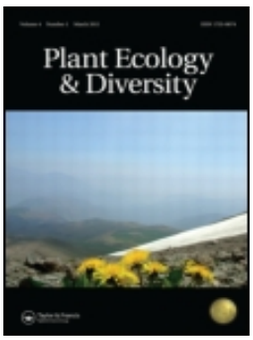

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Spring phenology shows genetic variation among and within populations in seedlings of Scots pine (*Pinus sylvestris* L.) in the Scottish Highlands

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Background: Genetic differentiation in phenotypic traits is often observed among forest tree populations, but less is known about patterns of adaptive variation within populations. Such variation is expected to enhance the survival likelihood of extant populations under climate change.
Aims: Scots pine (*Pinus sylvestris*) occurs over a spatially and temporally heterogeneous landscape in Scotland. Our goal was to examine whether populations had differentiated genetically in timing of bud flush in response to spatial heterogeneity and whether variation was also maintained within populations.
Methods: Two common-garden studies, involving maternal families of seedlings from 21 native pinewoods, were established and variation in the trait was measured at the beginning of the second growing season.
Results: Populations showed genetic differences in the trait correlated with the length of growing season at their site of origin, but the majority of variation was observed within populations. Populations also differed in their levels of variation in the trait; a pattern that may be influenced by spatial variation in the extent of temporal climate variability.
Conclusions: Our findings suggest that populations have adapted to their home environments and that they also have substantial ability to adapt in situ to changes in growing season length.

Keywords: adaptation; adaptive potential; genetic differentiation; spatial heterogeneity; temporal heterogeneity; variation within populations

Introduction

When a species is distributed across a spatially heterogeneous landscape, natural selection is expected to favour different trait optima in divergent environments. For example, growth in plants may continue furthest into the autumn in populations that experience the longest growing seasons (e.g. Mikola 1982). This may lead to genetic differentiation in selected phenotypic traits among populations and each population surviving and growing best at its home site, i.e. local adaptation (Kawecki and Ebert 2004). Adaptations to local environments have been described in many plant species (Linhart and Grant 1996), and in trees, numerous common-garden studies have demonstrated that, for example, growth phenology and timing of cold hardiness are optimised to local climates (Howe et al. 2003; Savolainen et al. 2007). Local adaptation has also been demonstrated in transfer trials in which populations have been grown at home and foreign sites (e.g. Persson and Ståhl 1990); a possible reason for poorer survival and growth at foreign sites is a mismatch between annual climatic variation and growth phenology (Eriksson et al. 1980).

Ongoing rapid climate change is affecting ecosystems globally, which is evident as range shifts and changes in timing of growth and reproductive events in several species (Parmesan 2006). Due to the commercial importance of many species and conservation issues, predicting evolutionary responses to a changing climate has become an active area of research (Hendry et al. 2011; Hoffmann and Sgrò 2011). For example, ecological modelling often considers associations between species distributions and environmental factors: following environmental change, ranges may expand into new areas that become suitable for a species, while extinction may take place at sites that become unfavourable (Elith and Leathwick 2009). In trees, common-garden experiments replicated in multiple environments have been used to estimate how environmental changes will affect contemporary populations and to demonstrate that in the future, some populations might be exposed to non-optimal conditions which might result in poorer growth (Rehfeldt et al. 2002; Reich and Oleksyn 2008). However, such approaches can only be used to examine how existing populations will respond to environmental changes as they neglect an important feature of natural populations: their capacity to adapt to environmental changes in situ (Hoffmann and Sgrò 2011). This capability means that, as well as range shifts, responses to environmental change should also involve genetic changes between generations exposed to different environments, allowing

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populations to adapt to new conditions. Indeed, taking adaptation and phenotypic plasticity into account in models can greatly enhance the survival likelihood of current populations (Aitken et al. 2008; Benito Garzón et al. 2011). Adaptation to novel environments has certainly taken place in many species when they expanded their ranges following the last ice age (Davis and Shaw 2001), but it is possible that future changes will occur too rapidly for populations to track them (Savolainen et al. 2004; Aitken et al. 2008) or that intensively managed contemporary landscapes will be impermeable to migration.

In situ adaptive responses to a changing environment are possible when phenotypes vary within populations and when such variation is due to genetic factors (Hoffmann and Sgrò 2011). However, research on adaptation has largely focused on examining how populations differ from each other in terms of trait means and how environmental differences among the sites occupied by different populations contribute to such divergence (reviewed for example in Savolainen et al. 2007). Thus, we currently have only a limited understanding of patterns of adaptive trait variation within populations (Kramer and Havens 2009). When populations adapt to their home environments, variation in traits affected by selection is expected to be lost as individuals that differ too much from the local optimum have poorer chances of survival (e.g. Falconer and Mackay 1996). Still, it is commonly found across different kinds of organisms that significant genetic variation can be preserved even in traits under the strongest type of selection (Houle 1992; Merilä and Sheldon 1999; Barton and Keightley 2002). Also in trees, within-population variation in traits under selection has been widely found in common-garden studies (Howe et al. 2003). A similar pattern can also be seen in trees observed in their natural habitats: in a stand of *Betula pendula* Roth growing in south-eastern Finland, trees flushed at different times, among-tree differences being the smallest during warm springs (Rousi and Heinonen 2007). The reasons for the persistence of such variation in nature despite natural selection remain poorly understood, but a number of factors might contribute to the maintenance of adaptive genetic diversity in forest trees. Due to their longevity, trees are likely to experience a wide range of environmental conditions during their lifespan (Petit and Hampe 2006), and mechanisms enabling individuals to modify their phenotype according to the environment are expected to evolve under such conditions (Bull 1987). Indeed, phenotypic plasticity in initiation of growth allows trees to survive in environments where temperature conditions in spring vary among years (Rousi and Heinonen 2007; Chmura et al. 2011). It is also possible that a population is found over a spatially variable area so that selection pressure also varies across short distances (i.e. there is no single trait optimum in a population; Campbell 1979), or that the environment varies between years so that different age groups within populations may have experienced differing selection pressure. This may maintain diversity if selection acts only on specific age groups (e.g. young seedlings) while having less effect on others (Ellner and Hairston 1994). Variation may also be introduced by gene flow via pollen from environmentally different sites (Yeaman and Jarvis 2006). What the relative contributions of these factors in nature are remains largely an unexplored topic.

Adaptive potential can be compromised especially in small and fragmented populations in which random factors such as sudden population size changes may have shaped patterns of genetic variation more than natural selection (e.g. Willi et al. 2006). Scots pine (*Pinus sylvestris* L.) is the only pine native to northern Europe and has an extensive distribution across Eurasia (Critchfield and Little 1966). The Scottish populations of the species are geographically separated on the north-western edge of this range and have been subjected to heavy human interference in the past. Currently, 84 discrete native pinewood sites of variable size are recognised by the Forestry Commission of Great Britain, which cover only about 1% of their original post-glacial maximum areal cover (Mason et al. 2004). Scots pine is a foundation species upon which the persistence of many of the species in Scottish forests depends. The native pinewoods are found over a geographically small but spatially highly heterogeneous landscape, with steep gradients in temperature and precipitation between the oceanic west coast and the more continental east (Salmela et al. 2010). Despite a significant decrease in abundance which has led to fragmentation, most of the populations are as diverse at selectively neutral molecular markers as more continuous continental populations, and show very little differentiation for these neutral markers (Kinloch et al. 1986; Wachowiak et al. 2010). This suggests that, at least historically, these populations have been connected by gene flow. However, little is currently known about the patterns of adaptive trait variation in this part of the species' distribution. In a recent experiment under natural climate conditions in south-eastern Scotland, plants from eight populations were found to differ in their response to winter and spring temperatures, which suggested environment-driven genetic differentiation among some of the populations despite the small geographic scale (Salmela et al. 2011). Similar differentiation was found for timing of growth initiation in spring, which was earlier in populations from cooler, high-altitude locations.

In common with other parts of the world, increases in summer and winter temperatures and changes in rainfall patterns are expected in Scotland in the coming decades (Ray 2008), possibly leading to changes in selection pressures for traits related to timing of growth in Scots pine. For current populations, these changes in climate have been predicted to be detrimental (Ray 2008). However, the possibility of adaptation within populations has not been considered in these predictions, which might result in their conclusions being too conservative and potentially in management actions detrimental to the genetic integrity of current populations. Considering how allowing for adaptation influences model predictions on the effects of climate change in tree populations (Aitken et al. 2008), it is important for the conservation of the remaining native pinewood resources that the patterns of adaptive trait variation among

and within populations are investigated (Salmela et al. 2010).

Due to the highly variable climate conditions that they are found in, adaptive genetic differentiation is expected to have taken place among pine populations from different parts of Scotland. In addition to spatial environmental variation among and within populations, the Scottish climate is also characterised by temporal (among-year) fluctuations, for instance in the length of the growing season (Perry and Hollis 2005) and winter severity (Harrison 1997). Such fluctuations probably account for phenomena such as the observed temporal variation in timing of bud flush under natural climate conditions in two birch species, *Betula pubescens* Ehrh. and *B. pendula* (Billington and Pelham 1991). The effects of climate fluctuations have also been recognised in animals: sheep mortality on the island of St. Kilda has been found to be higher in wet and warm winters which often coincide with positive phases of North Atlantic Oscillation (NAO) (Milner et al. 1999), a climatic phenomenon linked to the strength of westerly winds across the northern Atlantic (Stenseth et al. 2002). Although temporally variable selection has been suggested as one factor that may contribute to the maintenance of adaptive diversity in long-lived trees (Howe et al. 2003; Westfall and Millar 2004; Yeaman and Jarvis 2006), its potential role is yet to be studied in more detail. If populations are exposed to different levels of spatial and temporal environmental heterogeneity, they might also differ in the level of genetic diversity in adaptive traits and consequently their adaptive capacity. In this study, we used Scots pine as a model system to examine how a phenological trait varied among and within populations sampled across a spatially and temporally heterogeneous landscape. More specifically, we collected families from 21 environmentally diverse sites in Scotland and grew their progeny in two separate glasshouse experiments to address the following questions:

(1) When grown under common-garden conditions, are 21 native Scots pine populations differentiated for timing of growth initiation in spring at the beginning of their second growing season?
(2) Are observed population differences associated with environmental variation among their home sites?
(3) Is there significant variation within populations? If so, what is the pattern of this variation?
(4) Could the relative amounts of within-population variation be accounted for by within-site spatial and/or temporal variation in environment?

Materials and methods

Measuring genetic diversity in timing of bud flush

Phenotypes of adaptive traits are determined by both genetic factors and the environment (Falconer and Mackay 1996). Therefore, to reveal differences due to the genetic component, samples from different populations must be raised in a common-garden environment. To estimate the levels of variation within populations, a family-structured design is needed so that total variation in phenotype can be partitioned into among- and within-population components. In tree populations, this can be accomplished by sampling multiple open-pollinated seed from a number of mother trees in each population (e.g. White et al. 2007). Due to high outcrossing rates (mother trees are generally pollinated by a large number of pollen donors), such progeny are often assumed to consist mostly of half-siblings (i.e. family members share only the maternal parent).

Study populations

A total of 21 native populations were sampled for this study, representing all parts of the species' range in Scotland (Figure 1, Table 1). Cones were collected from 10 maternal trees in each population in March 2007. Open-pollinated seed was extracted from cones and stored by family.

Provenance/progeny trials

Sampled seed was used to establish two glasshouse-based common-garden trials located in Edinburgh and Aberdeen (Figure 1) in late spring 2007. The two trials were set up by independent investigators and they consequently had rather different germination conditions and layout designs. In the Edinburgh trial located at the Centre for Ecology and Hydrology (55.86° N, 3.21° W), seed were sampled from four mother trees per population (i.e. 84 families in total) and sown on trays (75:25 compost type John Innes 1: sand) in June 2007 under common-garden glasshouse conditions. After germination, seedlings were transferred to pots of size 0.62 l (diameter 11 cm, depth 9.6 cm) and kept under natural light conditions (glasshouse was shaded to avoid excess light) with watering applied two or three times per week during the growing season. No heating was applied during winter. Each family consisted of 40 progeny (∼3360 seedlings in total). The trial was divided into 40 blocks, each having one member from each of 84 families, and the order of the families within blocks was randomised.

The trial in Aberdeen was located at the James Hutton Institute (57.13° N, 2.16° W). Seed were sampled from 10 mother trees per population (i.e. 210 families in total). Cones were placed in a warm room (30 °C) for 2 weeks so that they opened and seed could be extracted for germination. Seed from the individual trees were kept separate and were soaked in water for 3 h, then laid between sheets of damp paper towel placed in a cool room (3 °C) for several weeks to break dormancy. Seed were taken out of the cool room and left (wrapped in damp paper) in the laboratory at room temperature until they germinated. Germination took approximately 7 days and seed from all the sampled families germinated at this time. On germination they were transplanted into potting medium in the glasshouse into 8 × 8 × 9 cm (0.4 l) pots. Each family

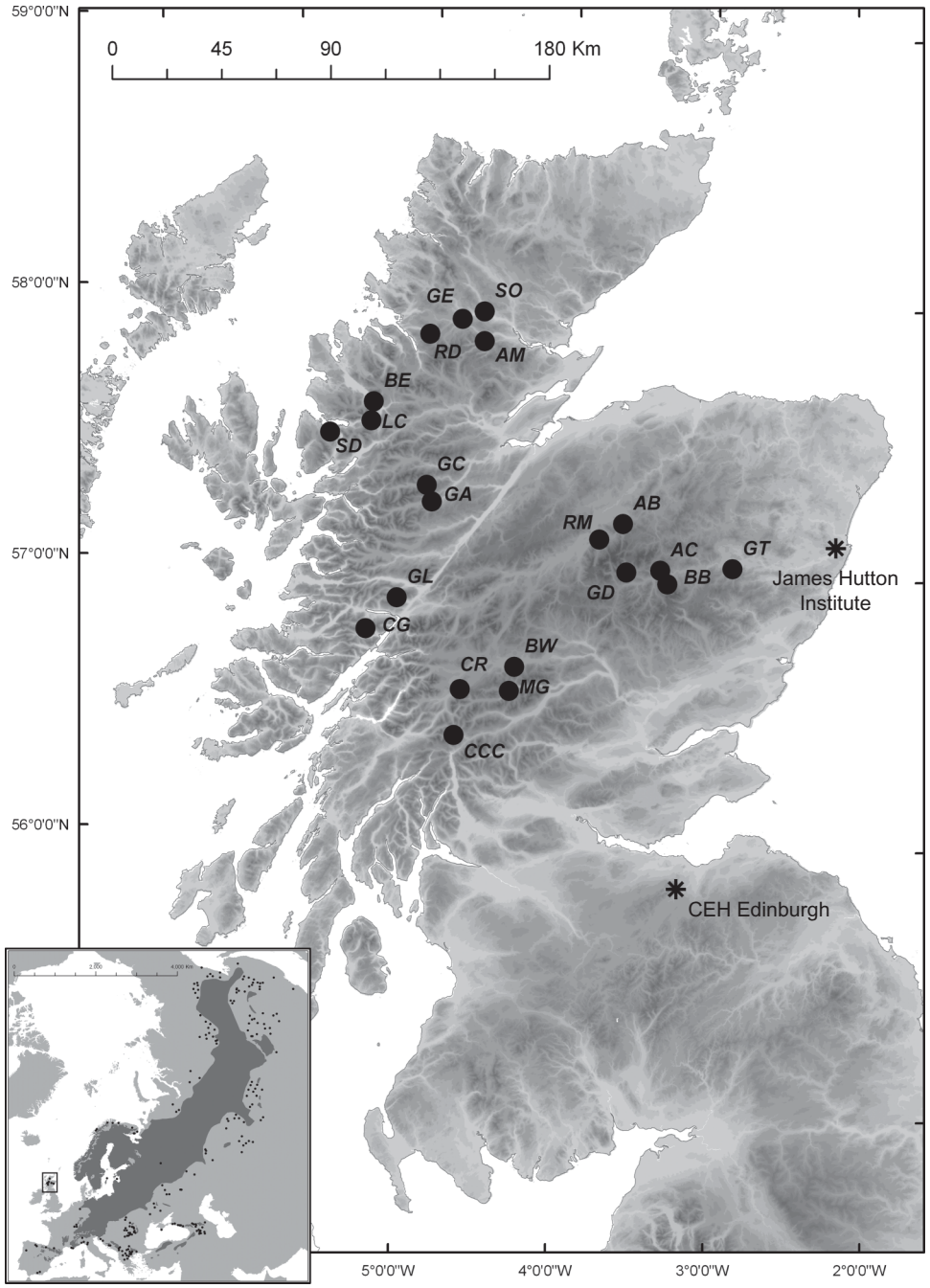

Figure 1. Map of the sampled native *Pinus sylvestris* populations and locations of the two trial sites in Scotland. Climatic features of the sites are given in Table 1. Inset: full distribution of *P. sylvestris*, with study area highlighted by box. (Distribution data from EUFORGEN.)

consisted of eight progeny (~1680 seedlings in total). The trial was divided into 40 blocks with 42 plants per block, each block containing two plants from a different mother from each population. The 84 mother trees sampled in the Edinburgh trial were a subset of those included in the Aberdeen trial. Watering was applied automatically and no artificial light was used.

In both trials, growth initiation at the beginning of the second growing season was considered to have taken place when new green needle tips started to emerge from the apical bud (bud or needle flush), and this was measured as the number of days since the first scoring date. Bud flush was scored twice weekly in Edinburgh between March 23 and May 9 2008 and once weekly in Aberdeen between March 31 and May 27 2008.

Testing for genetic differentiation among populations and families

Data from the Edinburgh trial were analysed using nested analysis of variance (ANOVA), with populations considered as fixed and families within them and blocks as random factors. Unbalanced nested ANOVA was applied to the data from the Aberdeen trial. To examine the relative

Table 1. Populations of *Pinus sylvestris* in Scotland included in the study, their latitude (Lat.), longitude (Long.), altitudinal range sampled (Alt.), core pinewood area according to Mason et al. (2004), and mean (1961–2000) calculated climate features: growing season length (GSL; days), growing degree days (GDD: day degrees), and February and July mean temperatures (FMT and JMT).

Population	Lat.	Long.	Alt. (m)	Area (ha)	GSL	GDD	FMT (°C)	JMT (°C)
Abernethy (AB)	57.21	3.61	311–370	2452	211	990	1.15	12.73
Allt Cul (AC)	57.04	3.35	435–512	13	145	513	−1.01	10.41
Amat (AM)	57.87	4.60	39–201	181	214	892	1.22	12.29
Ballochbuie (BB)	56.98	3.30	421–531	775	116	446	−1.69	9.46
Beinn Eighe (BE)	57.63	5.40	17–91	182	283	1329	3.68	14.16
Black Wood of Rannoch (BW)	56.68	4.37	250–321	1011	254	1138	2.12	13.55
Coille Coire Chuilc (CCC)	56.42	4.71	222–311	67	226	928	1.64	12.32
Conaglen (CG)	56.79	5.33	89–193	189	246	887	2.20	11.73
Crannach (CR)	56.58	4.68	258–338	70	231	1019	1.81	12.62
Glen Affric (GA)	57.26	4.92	205–293	1532	210	769	0.88	11.62
Glen Cannich (GC)	57.35	4.95	182–381	301	212	778	0.96	11.71
Glen Derry (GD)	57.03	3.58	426–493	235	168	593	−0.46	11.34
Glen Einig (GE)	57.96	4.76	45–92	27	242	1089	2.19	13.15
Glen Loy (GL)	56.91	5.13	136–219	74	191	541	0.49	9.80
Glen Tanar (GT)	57.02	2.86	289–422	1564	235	1105	2.21	13.63
Loch Clair (LC)	57.56	5.36	98–166	126	277	1253	3.44	13.68
Meggernie (MG)	56.58	4.35	254–385	277	223	916	1.07	12.04
Rhidorroch (RD)	57.89	4.98	138–220	103	221	840	1.51	11.62
Rothiemurchus (RM)	57.15	3.77	295–329	1744	224	1087	1.39	13.15
Shieldaig (SD)	57.50	5.63	44–132	103	273	1093	3.21	12.83
Strath Oykel (SO)	57.98	4.61	35–160	14	257	1276	2.69	14.05

contributions of different factors to total variation in the trait, variance components due to populations, families, and blocks were estimated using the restricted maximum likelihood (REML) approach. Correlation analysis was used to test whether similar trends were observed in the two trials. All statistical analyses in this study were carried out using GenStat Ver. 13.1.0.4470.

Measuring the level of variation within populations

To examine the variability of the trait within each population in more detail, standard deviations (SD_{POP}) were examined separately in the two trials. *SD*s were calculated using raw values because of their strong correlation ($r = 0.94$–0.99) with values adjusted for the effects of individual blocks. Because timing of bud flush does not have fixed means (i.e. means vary depending on the date from which the timing is calculated), we used *SD*s instead of normalised measures of dispersion (see for example Garcia-Gonzalez et al. 2012). Correlation analysis was used to test whether similar patterns of variation among SD_{POP}s were observed in the two trials.

In a study design consisting of families grouped within populations, within-population variation can arise from two components: among families and within families (residual variation). Variation among families is considered to reflect the level of heritable (additive) genetic variation (Falconer and Mackay 1996), and populations with higher levels of heritable variation are expected to have better adaptive potential (Houle 1992). Because the amount of additive genetic variation is directly proportional to the amount of variation among families (Falconer and Mackay 1996), we only used estimates of among-family variation in the analyses presented. In order to examine whether the level of among- and within-family variation differed among populations, the REML approach was used to calculate variance components due to families (V_{AF}) and individuals within families (V_{WF}) separately within each trial and population (i.e. variation within each population was divided into components due to families, blocks, and residual variation). Variation within families may include a component due to the genetic diversity of pollen donors sampled by mother trees. *SD*s were also calculated for individual families (SD_{WF}).

Climate data

Long-term means at the sampled sites. To investigate how the sampled sites varied in terms of temperature conditions, UK Meteorological Office 40-year mean (1961–2000) climate data (Perry and Hollis 2005) were used to create climatic profiles of the populations' origins. Data were extracted for the length of the growing season (GSL), number of growing degree days (GDD), and mean February and July temperatures (FMT and JMT; these represent, on average, the coldest and warmest months, respectively). GSL is defined as the period bounded by daily mean temperature above 5 °C for more than five consecutive days and daily mean temperature below 5 °C for more than five consecutive days (after 1 July), while GDD expresses the sum of daily heat sum accumulation above 5.5 °C. Exact details on how the climate data were generated are given in Perry and Hollis (2005). Climate data are available in 5 × 5 km grids and are based on interpolation of

observations from the nearest weather stations. It is possible that due to within-grid variation in the landscape, actual climate conditions experienced at our study sites differ from the estimates; therefore, the climatic variables should only be considered as proxies. The range of altitude sampled at each site was used as a proxy for fine-scale (within-population) environmental variation.

Temporal variation in the Scottish climate. To investigate patterns of temporal variability in temperature in Scotland and at the sampled sites, annual estimates of GSL, GDD, February and July temperatures (FT and JT) for the 5 × 5 km grids in the period 1961–2000 were used. Using these 40-year data, mean absolute deviations (*MAD*) of FT and JT, and coefficients of variation (*CV*) of GSL and GDD (CV_{GSL}, CV_{GDD}) were calculated separately for each site. A combined estimate of monthly temperature variability was calculated as the average of the *MAD*s of FT and JT. Annual means were also calculated over all 21 sites. To test whether winter temperature in Scotland was associated with the NAO, linear regression was also used to test for an association between annual FTs and February NAO indices provided by the Climate Analysis Section, NCAR, Boulder, USA, at NAO Index http://www.cgd.ucar.edu/cas/jhurrell/indices.html. Linear regression was used to explore whether the level of temporal variation in climate varied along latitudinal, longitudinal, or altitudinal gradients.

Associations between population means of timing of bud flush and climate at home site. To investigate associations between variation in timing of bud flush, the locations of the populations and their climate, population means in the two trials were regressed against their longitude, latitude, altitude, and long-term mean temperature estimates (GSL, GDD, FMT, JMT) at origin.

Associations between the level of variation within populations and latitude, longitude, and altitude. Linear regression was used to explore whether the level of variation within populations (SD_{POP}) was linked to the range of altitude sampled at each site, or whether it varied along any spatial gradients (longitude, latitude, altitude). We also tested for associations between the locations of the sampled families and among and within-family trait variation (V_{AF}, V_{WF}, SD_{WF}) in each trial.

Results

Genetic differentiation in timing of bud flush among populations and families

In the Edinburgh trial, population means ranged between 11 days after March 23 for AC, BB, GA, GD, and GT, and 18 days after March 23 for AB, BE, SD, and RD (see Table 1 for population name abbreviations). ANOVA provided some evidence of differences among populations ($P = 0.058$), and differences among families within populations, and among blocks were significant (Table 2(a)). The variance component due to differences among families within populations (15.39; 22% of total variation) was approximately five times larger than that among populations (2.98; 4% of total variation).

In the Aberdeen trial, the range of population means was from 16 days after March 31 for AC and GL to 22 days after March 31 for AB. Significant differences were observed among populations, families within populations, and blocks (Table 2(b)). The variance component due to families (5.88; 11% of total variation) was approximately four times larger than that of populations (1.45; 3% of total variation). Population means between the two trials were significantly and moderately correlated ($r = 0.48$, $P < 0.05$). In both trials, the great majority of the variation was residual, i.e. within families (Table 2(a), (b); 72% in the Edinburgh trial, 77% in the Aberdeen trial).

Associations between population means and climate at home site

Temperature conditions varied greatly within Scotland: for instance, mean GSL ranged from 116 days in BB to 283 days in BE, and mean GDD from 446 to 1329 dd at the same sites (Table 1). Spatial variation was also found

Table 2. Variation in timing of bud flush in populations of *Pinus sylvestris* in Scotland. Results of the nested ANOVA testing the effects of population (fixed factor), families within populations (random factor), and blocks (random factor) in the (a) Edinburgh and (b) Aberdeen trials.

Source of variation	df	MS	*F*-ratio	*P*-value	Variance component
(a) Edinburgh trial					
Population	20	1153.35	1.70	0.058	2.98
Families within populations	63	679.52	13.50	<0.001	15.39
Block	39	176.66	3.51	<0.001	1.50
Residual	3120	50.35			50.40
(b) Aberdeen trial					
Population	20	183.26	2.18	<0.01	1.45
Families within populations	188	83.96	2.01	<0.001	5.88
Block	39	221.37	5.29	<0.001	5.09
Residual	1216	41.87			42.26

in timing of bud flush, and results from the two trials showed similar trends although there were differences in the absolute values. In the Edinburgh trial, when examining associations between population means and geographical surrogates of environmental variation (latitude, longitude, altitude), means were best associated with altitude at their site of origin. Low-altitude populations generally flushed later than those from higher locations, and altitude explained 20% of the variation among population means ($\beta_0 = 16.70$, $\beta_1 = -0.010$, $P < 0.05$). Altitude of the populations was negatively correlated with mean GSL ($r = -0.80$, $P < 0.001$) and GDD ($r = -0.65$, $P < 0.01$) at origin, and higher R^2s were obtained when using these climate variables instead of altitude. Earlier bud flush occurred in populations from areas with shorter GSL ($\beta_0 = 5.87$, $\beta_1 = 0.038$, $P < 0.01$, $R^2 = 31\%$), fewer GDD (Figure 2; $\beta_0 = 8.14$, $\beta_1 = 0.0066$, $P < 0.01$, $R^2 = 35\%$), lower FMT ($\beta_0 = 12.62$, $\beta_1 = 1.13$, $P < 0.01$, $R^2 = 29\%$), and lower JMT ($\beta_0 = 0.35$, $\beta_1 = 1.13$, $P < 0.010$, $R^2 = 27\%$).

A similar trend with altitude was also found in the Aberdeen trial ($\beta_0 = 20.25$, $\beta_1 = -0.0035$), but the association was not statistically significant ($P = 0.22$, $R^2 = 3\%$). However, significant associations were obtained when temperature estimates were used instead of altitude, and sites with shorter GSL ($\beta_0 = 14.14$, $\beta_1 = 0.024$, $P < 0.001$, $R^2 = 31\%$), fewer GDD (Figure 2; $\beta_0 = 15.70$, $\beta_1 = 0.0040$, $P < 0.01$, $R^2 = 32\%$), lower FMT ($\beta_0 = 18.38$, $\beta_1 = 0.69$, $P < 0.01$, $R^2 = 28\%$), and lower JMT ($\beta_0 = 9.68$, $\beta_1 = 0.79$, $P < 0.01$, $R^2 = 35\%$) had earlier bud flush.

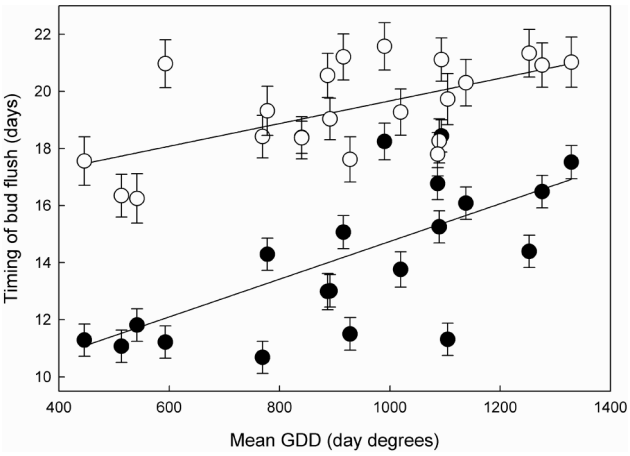

Figure 2. Relationship between mean growing degree days (GDD) at origin and population means of timing of bud flush in the two trials with *Pinus sylvestris* seedlings in Scotland. In the Edinburgh trial (filled circles): $\beta_0 = 8.14$, $\beta_1 = 0.0066$, $P < 0.01$, $R^2 = 35\%$; in the Aberdeen trial (hollow circles): $\beta_0 = 15.70$, $\beta_1 = 0.0040$, $P < 0.001$, $R^2 = 32\%$. Error bars indicate standard errors of the means.

Differences in the level of variation within populations

Variation among SD_{POP}s suggested that populations might have differed in the level of variation in timing of bud flush (Table 3). In the Edinburgh trial, SD_{POP}s varied between 6.25 in GT and 9.53 in AB, while in the Aberdeen trial with a larger number of families within each population, SD_{POP}s ranged from 4.29 in CG to 11.10 in GD. SD_{POP}s across the two trials were not significantly correlated ($P = 0.79$).

Table 3. The amount of within-population variation in timing of bud flush in Scottish populations of *Pinus sylvestris* seedlings. Standard deviations (SD_{POP}), variance components due to families (V_{AF}), residual variation (V_{WF}), and blocks (V_{Block}), and the range among family means in each population within the two trials.

	Edinburgh					Aberdeen				
Population	SD_{POP}	V_{AF}	Mean range	V_{WF}	V_{Block}	SD_{POP}	V_{AF}	Mean range	V_{WF}	V_{Block}
Abernethy (AB)	9.53	43.95	15.63	70.12	0.00	8.48	0.00	14.09	76.92	0.00
Allt Cul (AC)	7.76	8.90	6.20	57.18	0.00	8.98	2.39	12.19	62.75	16.32
Amat (AM)	7.32	15.41	8.90	40.15	1.80	8.68	3.43	12.09	70.03	2.11
Ballochbuie (BB)	8.86	27.93	12.38	62.28	0.00	8.30	0.00	10.30	52.46	22.99
Beinn Eighe (BE)	7.62	9.56	6.72	48.00	2.84	6.29	0.00	6.09	34.88	6.64
Black Wood of Rannoch (BW)	8.00	11.79	8.24	55.55	0.00	7.92	0.00	9.62	27.07	35.22
Coille Coire Chuilc (CCC)	7.25	6.26	5.73	42.20	5.65	7.92	13.16	12.15	43.73	6.08
Conaglen (CG)	7.97	4.56	5.69	54.22	5.81	4.29	0.01	7.08	20.36	0.00
Crannach (CR)	9.19	55.18	16.34	51.34	0.80	5.52	11.40	10.02	23.81	0.00
Glen Affric (GA)	6.79	5.14	4.78	43.25	0.00	5.29	0.00	3.15	36.67	0.00
Glen Cannich (GC)	8.17	23.58	12.02	45.50	3.70	6.18	12.00	14.13	24.92	3.02
Glen Derry (GD)	7.95	30.24	11.85	39.48	1.07	11.10	29.05	24.03	94.57	5.24
Glen Einig (GE)	7.93	2.95	5.06	66.41	0.00	5.81	3.79	8.79	19.06	10.44
Glen Loy (GL)	6.78	7.31	6.75	37.61	2.75	6.71	7.03	10.19	41.43	0.00
Glen Tanar (GT)	6.25	8.09	6.22	32.43	0.44	8.17	11.87	16.91	53.83	2.84
Loch Clair (LC)	8.51	0.00	1.98	64.00	9.16	7.76	0.00	11.99	76.21	0.00
Meggernie (MG)	7.10	0.72	3.48	47.31	2.61	8.51	4.76	13.92	71.13	0.00
Rhidorroch (RD)	8.10	8.00	6.53	58.24	0.41	5.42	2.88	12.46	26.72	0.06
Rothiemurchus (RM)	9.02	22.73	10.55	61.92	2.35	6.17	11.53	12.84	18.53	6.97
Shieldaig (SD)	8.49	26.01	10.84	53.52	0.00	5.42	0.00	6.28	37.54	0.00
Strath Oykel (SO)	7.27	14.68	6.94	42.00	0.00	5.55	0.00	6.61	27.00	4.22

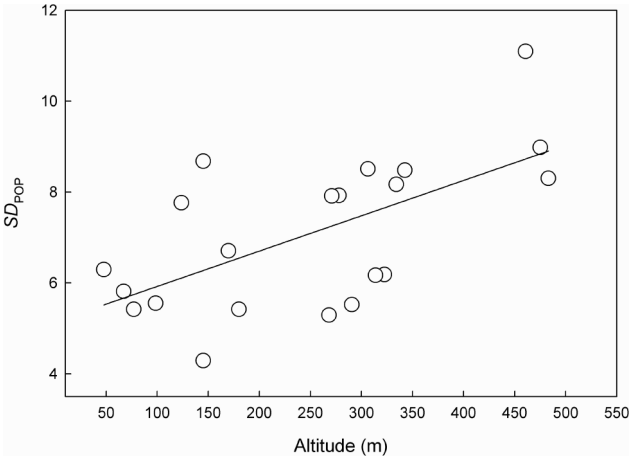

Figure 3. Relationship between altitude at origin and the amount of variation (SD_{POP}) in timing of bud flush within *Pinus sylvestris* populations in the Aberdeen, Scotland trial ($\beta_0 = 5.14$, $\beta_1 = 0.0080$, $P < 0.01$, $R^2 = 35\%$).

In the Edinburgh trial, SD_{POP}s were not associated with latitude, longitude, or altitude. However, in the Aberdeen trial, the pattern of variation among SD_{POP}s was related to the geographic location of populations and individual mother trees: higher amounts of variation were observed at higher-altitude sites (Figure 3; $\beta_0 = 5.14$, $\beta_1 = 0.0080$, $P < 0.01$, $R^2 = 35\%$). The regression was strongly influenced by the three high-altitude sites with large SD_{POP}s. When excluding these, the linear regression remained positive but became statistically non-significant ($\beta_0 = 5.59$, $\beta_1 = 0.0050$, $P = 0.112$, $R^2 = 10\%$).

In the Aberdeen trial, differences among families were generally larger at higher altitudes, and altitude explained 16% of the variation among V_{AF}s ($\beta_0 = -0.65$, $\beta_1 = 0.024$, $P < 0.05$); however, eight populations had V_{AF} estimates of 0. A positive correlation was observed between values of V_{AF} and the range of family means within each population ($r = 0.77$, $P < 0.001$). R^2 increased to 31% when the range of family means within each population was used instead of V_{AF} ($\beta_0 = 6.36$, $\beta_1 = 0.019$, $P < 0.01$). In the Edinburgh trial, higher SD_{POP}s were associated with higher V_{AF}s ($r = 0.69$, $P < 0.001$). The association between altitude and V_{AF}s was only suggestive of higher levels of among-family variation at higher altitudes ($\beta_0 = 6.074$, $\beta_1 = 0.040$, $P = 0.11$, $R^2 = 9\%$).

Similarly to among-family differences, there was some evidence of larger V_{WF}s at higher-altitude sites in the Aberdeen trial ($\beta_0 = 27.39$, $\beta_1 = 0.070$, $R^2 = 13\%$, $P = 0.062$). This pattern was also reflected in variation among SD_{WF}s which ranged between 0 in nine families from seven populations and 17.92 in a family from AC. Altitude explained 4% of the variation among SD_{WF}s ($\beta_0 = 4.76$, $\beta_1 = 0.0053$, $P < 0.01$), but the association was non-significant ($P = 0.53$) when the three highest-altitude sites were excluded. In the Edinburgh trial, there was no association between altitude and V_{WF}s, but they were positively and significantly correlated with SD_{POP}s ($r = 0.78$, $P < 0.001$). SD_{WF}s varied between 4.16 in a family from GT and 11.04 in a family from CR, but they were not associated with altitude ($P = 0.48$).

Fine-scale environmental variation and the level of variation within populations

Mother trees within populations were sampled at different altitudes, and consequently the altitudinal range sampled at each site varied from 23 m at RM to 179 m at GC in the Edinburgh trial, and from 34 m at RM to 199 m at GC in the Aberdeen trial. However, increasing altitudinal range sampled within populations did not account for larger SD_{POP}s (the Edinburgh trial: $\beta_0 = 8.42$, $\beta_1 = -0.0060$, $R^2 = 4\%$, $P = 0.20$; the Aberdeen trial: $\beta_0 = 7.010$, $\beta_1 = 0.001$, $R^2 = 0\%$, $P = 0.95$).

Temporal climate variation

Historical climate differed markedly from year to year among sites. For example, annual GSL and GDD of the sites occupied by the 21 pinewoods showed extensive temporal fluctuation in the period 1961–2000 (Figure 4(a)). GSL varied between 174 days in 1968 and 271 days in 1989, while the lowest GDD (756 dd) was reached in 1974 and the highest (1167 dd) in 1995. Temporal variability was also found in monthly winter and summer temperatures. The range of JTs was 9.90 °C in 1965 to 14.88 °C in 1983, and annual JTs were significantly correlated with GSL ($r = 0.46$, $P < 0.01$) and GDD ($r = 0.64$, $P < 0.001$) in the same year. FTs varied between −2.38 °C in 1963 and 5.94 °C in 1998, and were found to be associated with the NAO, with colder temperatures coinciding with lower NAO indices (Figure 4(b); $\beta_0 = 1.29$, $\beta_1 = 0.58$, $P < 0.0001$, $R^2 = 33\%$).

Populations from different parts of Scotland experienced different levels of temporal variation in these climate features. The combined *MAD* of FT and JT increased with ascending altitudes (Figure 4(c); $\beta_0 = 1.032$, $\beta_1 = 0.00072$, $P < 0.0001$, $R^2 = 77\%$), while for GSL and GDD, temporal variability increased very little from altitudes of 48–343 m, but was higher at the three sites located above 450 m (Figure 4(d)).

Discussion

In this study, we combined phenotypic and climate data to examine the patterns of variation in a phenological trait among and within native Scottish populations of Scots pine. Under common-garden conditions, populations sampled across a spatially highly heterogeneous landscape were found to differ in timing of bud flush at the beginning of the second growing season, which generally was earlier in populations from cooler locations. This suggests environment-driven genetic differentiation. However, significant amounts of variation were also found within populations. In addition, the data suggested that populations may differ in their level of adaptive variation: in the

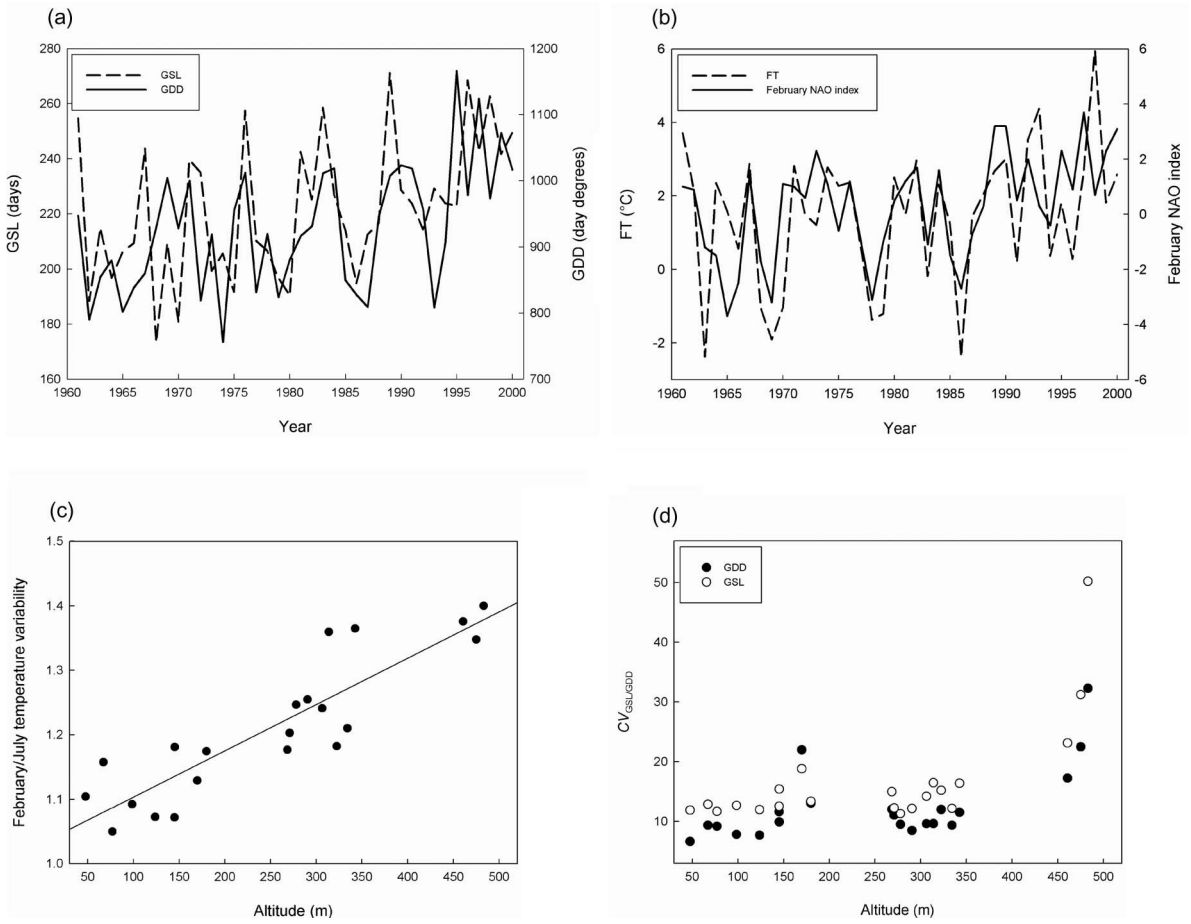

Figure 4. Among-year variation in the Scottish climate, 1961–2000. (a) Temporal variation in growing season length (GSL) and growing degree days (GDD); (b) temporal variation in February temperature (FT) and February North Atlantic Oscillation (NAO) indices; (c) relationship between the altitudes of the 21 native pinewood sites and among-year variability of winter and summer temperatures (combined mean absolute deviation of FT and JT); (d) temporal variation in GSL and GDD (coefficients of variation, *CV*) plotted against the altitudes of the 21 sampled sites. In (a) and (b), annual means were calculated over the 5 × 5 km grids within which the 21 pinewood sites are located.

Aberdeen trial, we found some evidence of higher levels of such variation in populations from high-altitude sites that experience the most among-year variation in temperature conditions.

Populations are differentiated in timing of bud flush

The annual cycle of temperate trees is divided into two phases: active growing period in summer, and winter dormancy (Howe et al. 2003). Due to differences in the length of the frost-free period in the northern hemisphere, phenological differences are common among tree populations (Howe et al. 2003; Savolainen et al. 2007). However, such patterns have mainly been examined across wide geographic areas, and less is known about genetic differences among populations separated by shorter distances. Despite the small geographic area (maximum distance between two native pinewoods is less than 200 km), spatial heterogeneity in climate within Scotland is extensive (Salmela et al. 2010). Thus, conditions are ideal for development of local adaptation. Indeed, population differences observed in our study suggest that adaptive differentiation in response to environmental variation has occurred. Under glasshouse conditions, bud flush generally took place earlier in populations from the coolest high-altitude locations in the eastern Highlands and later in those from the maritime west coast. However, possible home site advantage of these populations cannot be inferred without reciprocal transplant experiments (Kawecki and Ebert 2004). Also note that due to the format of the partially interpolated climate data (5 × 5 km grids) and spatially complex landscapes in Scotland, it is possible that the home site conditions of the populations differ from those described in Table 1. Weather station coverage in the UK is especially sparse in the Scottish Highlands, which is likely to result in inaccuracies in the climate variables (Perry and Holliss 2005).

In spring, growth is initiated from stem units formed in buds during the previous growing season after genetically determined chilling and heat sum requirements have been fulfilled (Aitken and Hannerz 2001; Howe et al. 2003). The patterns observed in our study could reflect longer chilling and higher heat sum requirements of populations

from warmer locations, so that growth initiation is prevented under mild winter conditions (e.g. Leinonen 1996). Due to the strong dependence of the trait on temperature, these patterns of variation may differ among years (e.g. Sagnard et al. 2002, but see Beuker 1994). However, corresponding spatial patterns of variation in this trait have been found in provenance studies on adult trees of the same species sampled across Eurasia (Steiner 1979) and along a latitudinal gradient in North Europe (Beuker 1994). Also, in an outdoor trial consisting of a small subset of seedlings from eight populations included in the Edinburgh trial, timing of bud flush at the beginning of the fourth growing season was slightly earlier in populations from cooler high-altitude locations (Salmela et al. 2011). Nonetheless, in accordance with earlier findings also in other species (Aitken and Hannerz 2001), population differences in growth initiation appeared small. Although similar overall trends were found in the two trials, there were differences in absolute values. This may be due to differences in winter and spring temperatures between the experimental sites.

It is possible that phenotypic variation is influenced not only by genetic variation due to segregating genes among seedlings, but also by differences in seed maturation conditions experienced by different mother trees in their home environments. These effects have been shown to be strong for instance in *Picea abies* (L.) H.Karst (Johnsen et al. 2005). Although we cannot exclude the possibility of such effects influencing the observed patterns of variation also among Scottish pine populations, earlier studies suggest that in Scots pine, such effects are not of the same magnitude as in *P. abies*. For example, Ruotsalainen et al. (1995) found that seed maturing conditions did not have major effect on variation in another phenological trait, timing of bud set, under common-garden conditions. Further, in addition to young seedlings, evidence of adaptive differentiation among populations in timing of bud flush has been observed when examining Scots pine trees aged over 10 (Steiner 1979) or approximately 60 years (Beuker 1994). Thus, in further discussion we assume that the differences observed in this study reflect mainly genetic variation among seedlings.

Populations consist of genetically and phenotypically diverse individuals

Despite the evidence of population differentiation, a much larger proportion of variation was due to differences among families and individual seedlings within populations. This observation is akin for instance to the one found by Sagnard et al. (2002) among *Abies alba* Mill. populations from the south-western Alps, and indicates that the trait is heritable and that populations maintain considerable internal potential to adapt to changing conditions (e.g. Kramer and Havens 2009; Hoffmann and Sgrò 2011), such as variable growing season length. Furthermore, our data suggested that populations differed in levels of internal variation: family differences were larger and families more variable at high-altitude locations. This pattern was found only in the Aberdeen trial, which may be due to the larger number of families sampled within each population. Evolutionary biology models predict the loss of genetic variation in adaptive traits due to selection favouring only optimal phenotypes in each population (Falconer and Mackay 1996), but significant levels of within-population variation are often documented in adaptive traits that are differentiated among populations (Howe et al. 2003; Alberto et al. 2011; Savolainen et al. 2011). Still, population differences in the amount of variation have been assessed only on few occasions (Savolainen et al. 2004; Notivol et al. 2007; Alberto et al. 2011).

Several factors could contribute to the population differences in the level of trait diversity along an altitudinal gradient observed in our study. Although the areas covered by the 21 pinewoods vary in size (Mason et al. 2004), differences in population size are an unlikely explanation, as earlier work using selectively neutral molecular markers in the nuclear genome has shown no significant differences in molecular diversity across Scotland (Kinloch et al. 1986; Wachowiak et al. 2010). Gene flow among heterogeneous sites may increase variation within populations (Howe et al. 2003) and, at least historically, Scottish populations have been linked by gene flow (Kinloch et al. 1986). Whether population differences in the extent of long-range gene flow contribute to the patterns observed here requires further exploration. However, studies of pollen flow suggest that the great majority of the fertilising pollen usually comes from local trees (Smouse and Sork 2004), suggesting that a large proportion of within-population diversity can also arise from matings between local parents. Thus, in a common-garden study design sampling open-pollinated seed from natural stands, gene flow from other populations might contribute more to variation within than among families because offspring from matings between parents located in different populations might not be as well adapted to their home site as those with local parents, and might not become established in a population (Burczyk et al. 2004). The high levels of residual variation found in both our trials might have resulted from effective outcrossing, while population differences in the level of variation within families may reflect variation in the extent of long-distance pollen flow and/or the genetic diversity of local pollen donors. However, the current study design does not allow the separation of genetic effects from other possible causes of residual variation.

Expression of phenotypic variation is strongly influenced by the environment and consequently, artificial growing conditions in glasshouses may induce the expression of variation that would normally be ‘hidden’ in nature (e.g. Hoffmann and Merilä 1999). Although growth conditions are known to affect population means in trees (Oleksyn et al. 1998; Mimura and Aitken 2010), their effects on trait variances remain poorly characterised. It is possible that seedlings have expressed different levels of their total potential if the growing conditions in the glasshouses were more novel to some populations. This possibility

could be investigated further by experiments in additional growing environments. In addition to differences in within-population sampling, discrepancies between the spatial patterns of variation among SD_{POP}s between the trials could be due to different growing environments or scoring intervals.

Adaptive diversity may be increased in environments that are highly variable across space or time (Yeaman and Jarvis 2006). Assuming that the altitudinal range sampled at each site also reflects the level of fine-scale environmental variation within populations, there was no evidence of increased spatial heterogeneity at high altitudes. Finer-scale climate data are needed to explore how environmental conditions vary across short distances in complex landscapes like the Scottish Highlands. However, there is significant temporal heterogeneity in climate in Scotland and, for instance, mid-winter temperatures were found to be associated with the NAO phenomenon, which is known to influence a variety of biological events in both plants and animals (Stenseth et al. 2002). The effect of the NAO in Europe is known to be particularly strong in winter, but positive phases of the NAO during spring (February–April) have also been demonstrated to be associated with elevated temperatures and earlier phenological events in plants (Chmielewski and Rötzer 2001). Such variation may also partly explain the observed temporal variation in the length of the growing seasons and the number of growing degree days in Scotland.

We also found that the extent of temporal variation varied spatially within Scotland, and higher-altitude locations were characterised by more variable climates. Interestingly, our common-garden data suggest that genetic diversity, at least in timing of bud flush, may be higher in populations found at the environmentally most variable sites. The association of temporal climate variability with altitude most likely arises from the fact that in our sampling sites, the highest-altitude sites are located at the most continental sites in the eastern Highlands, while the lowest-altitude sites are found on the maritime west coast (see Vasseur and Yodzis 2004). Although the climate data were based on interpolation across a temporally and spatially variable number of weather stations and the precision of estimates varies depending on the variable being estimated (Perry and Hollis 2005), patterns of variation in latewood density chronologies among five Scottish pinewoods provide indirect but corresponding evidence of site differences in temporal variability, at least in summer temperatures (Hughes et al. 1984).

Temporal variation in temperatures across the whole year suggests that phenotypic optima in phenological traits in populations vary from year to year. Furthermore, among-year variation in trait optima within Scotland may be more pronounced at sites with more among-year variation in climate. Large-scale climate fluctuations are known to have shaped the distributions of many tree species (Westfall and Millar 2004), and among-year variation, for instance in summer temperatures, has been documented to decrease the likelihood of good seed years especially in harsh conditions at high latitudes (e.g. Hilli et al. 2008), but so far the role of temporal variation in factors likely to drive adaptation in plant populations has received only little attention. The contribution of temporal heterogeneity in maintaining adaptive diversity has often been considered weak (Hedrick 1986), but on the other hand, variation in environmental factors has not been studied in such detail as variation in phenotype. The role of temporally variable environment might be important especially in long-lived trees with overlapping generations and low climate-related mortality in adults (see Persson and Ståhl 1990; Ellner and Hairston 1994). Trees aged over 400 years have been found in Scottish pinewoods (Fish et al. 2010), and considering the evidence for substantial temporal heterogeneity in the European climate since 1500 (Luterbacher et al. 2004) and the age structure of populations, it is possible that different age cohorts have experienced differing selection pressure during their sensitive early life stages and that the current patterns of diversity reflect adaptations to a range of past environments. Accordingly, a more variable environment might also support higher levels of genetic variation and more diverse phenotypes. This possibility could be explored further by examining genetic differences among age groups from sites that differ in temporal heterogeneity: larger differences among age groups in more variable environments would provide stronger evidence for a positive association between the levels of environmental and genetic diversity. Phenotypic plasticity is also expected to evolve in heterogeneous environments (Valladares et al. 2007); whether differences in plasticity contribute to our observations could be investigated in more detail by examining the range of phenotypic variation expressed across multiple growing environments.

Potential to adapt in situ is found in natural populations occupying heterogeneous environments

Genetic variation in adaptive traits has important biological implications. When populations are assumed to have no capacity to evolve, changing environmental conditions may result in the reduced survival or extinction of current populations in some areas. When the internal capacity of populations to adapt is accounted for, the chance of survival is increased (Aitken et al. 2008). Thus, natural populations inhabiting heterogeneous habitats should not be treated as fixed and independent entities whose responses to changes will be determined solely by the environment. Also, studies on adaption in tree and other plant populations would benefit from more thorough examinations of environmental factors likely to drive adaptation processes both within and among populations. Despite the evidence of temporal heterogeneity being a general feature of natural environments (Vasseur and Yodzis 2004), local climates in evolutionary studies have generally been considered to be rather static and non-overlapping, and the potential role of environmental heterogeneity in maintaining genetic diversity has not been extensively explored.

In this study, we have shown that the environmental variability natural populations are exposed to may differ even across short geographic distances and that this may influence the amount of adaptive diversity found within populations. Predicting the future of natural populations is complicated by possible correlations between adaptive traits, complex effects of the environment on the expression of phenotypes, and many non-genetic factors, but clearly, the fluctuating behaviour of environmental factors and the ubiquitous finding of adaptive potential at least in some key traits needs to be taken into account in further studies which aim to predict the effects of global change on natural populations. More family-structured trials grown across a range of sites are also needed to characterise within-population variation and its causes in more detail. Thanks to the long history of common-garden studies with appropriate study designs in forest trees, existing data from a large number of completed studies can be used to test for similar patterns in other species.

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