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Recent invasion of the mountain birch *Betula pubescens* ssp. *tortuosa* above the treeline due to climate change: genetic and ecological study in northern Sweden

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Abstract

Mountain birch, *Betula pubescens* ssp. *tortuosa*, forms the treeline in northern Sweden. A recent shift in the range of the species associated with an elevation of the treeline is commonly attributed to climate warming. Using microsatellite markers, we explored the genetic structure of populations along an altitudinal gradient close to the treeline. Low genetic differentiation was found between populations, whereas high genetic diversity was maintained within populations. High level of gene flow compensated for possible losses of genetic diversity at higher elevations and dissipated the founding effect of newly established populations above the treeline. Spatial autocorrelation analysis showed low spatial genetic structure within populations because of extensive gene flow. At the treeline, significant genetic structure within the juvenile age class at small distances did not persist in the adult age class, indicating recent expansion of young recruits due to the warming of the climate. Finally, seedling performance above the treeline was positively correlated with parameters related to temperature. These data confirm the high migration potential of the species in response to fluctuating environmental conditions and indicate that it is now invading higher altitudes due to the recent warming of the climate.

Keywords: climate change; microsatellites; redundancy analysis; spatial autocorrelation; treeline dynamics.

Introduction

Climate models predict increases in global mean temperatures of 1.4–5.8 °C by the end of the century, in response to increasing concentrations of atmospheric greenhouse gases resulting from human activities (Houghton *et al.*, 2001). This increase is expected to be greatest at high latitudes. Northern Europe has become markedly warmer since the beginning of the 19th century, particularly in Lapland, North Fennoscandia (Holmgren & Tjus, 1996). The position of the treeline, the upper elevational or latitudinal limit of trees, is widely recognized to be correlated with climatic parameters, primarily air temperature, which influence tree growth and survival (Tranquillini, 1979; Slatyer & Noble, 1992). Therefore, monitoring the position of treelines has long been seen as an indicator of global climatic change. Many studies have recorded a rise in the treeline, both altitudinal and latitudinal, associated with recent climate warming throughout the world (e.g. LescopSinclair & Payette, 1995; Meshinev *et al.*, 2000).

In the Swedish part of the Scandes mountains, tree limit rising of a majority of species, primarily mountain birch (*Betula pubescens* ssp. *tortuosa*), Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*), has been confirmed in many studies by Kullman (2001, 2002). This phenomenon of unique magnitude and rate is commonly attributed to anomalous human-induced climate warming during the past decade (Kullman, 2003). However, responses of treeline dynamics to climate change remain complex. Trees often respond to climate warming by an increase in the density of individuals rather than real movement of the treeline (LescopSinclair & Payette, 1995). Massive establishment of seedlings does not

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necessarily lead to a change in the position of the treeline, as warming may not be of sufficient magnitude or duration to promote sexual reproduction. Slatyer & Noble (1992) suggested that the treeline may not represent an absolute limit on growth, but rather a narrow zone in which the establishment of seedlings above the adult treeline was relatively common but balanced by periods of catastrophic mortality caused by disturbance or severe climate. Therefore, climatic control of the position of the treeline may result more from limitations on the recruitment of trees, rather than from a physiological barrier to survival and growth. Climate change may therefore induce profound changes in the structure of populations, both demographic and genetic.

Adaptation to major environmental alterations, like change in climate, is normally achieved through a combination of two processes: migration and evolution (Crawford & Abbott, 1994). The potential for survival of a species depends on its physiological and ecological range of tolerance, and also on its intrinsic genetic variability, within and among populations, on which selection can work. Nowadays, climate change may occur at unprecedented rates, outpacing natural rates of adaptation. The responses of organisms will largely be a function of the genetic variation present within the species and their ability to migrate into more hospitable areas. Woody species, like forest trees, tend to have high genetic diversity within populations, thus they are usually able to respond quickly to environmental changes (Kremer, 1994; Hamrick & Godt, 1996). In accordance with this, Kullman (2002) observed that seed-regenerating tree and shrub species tracked recent climate change in the Scandes mountains more rapidly and sensitively than vegetatively propagating field-layer species. However, adaptation occurs through variation at genes affecting ecologically important traits and not through neutral genetic variation. Unfortunately the latter does not seem to be a very good predictor of the former (Reed & Frankham, 2001) and simulation studies indicate that with high levels of gene flow, neutral markers can give quite different estimates of population diversity than those found by quantitative trait loci (QTL) or by measuring the heritability of the traits (Le Corre & Kremer, 2003). Neutral genetic diversity should therefore not be directly interpreted as adaptive potential but rather, as Reed & Frankham (2001) suggest, quantitative genetic variation should be measured if we want to evaluate the short-term adaptive potential of a population. On the other hand, genetic diversity at neutral markers is expected to be correlated with parameters such as effective population size and inbreeding (Hartl & Clark, 1989; Frankham, 1996), which can have long- and short-term effects on the survival of a species or population. Reed & Frankham (2003) for example, studied 34 datasets and found a significant correlation between neutral genetic diversity and population fitness.

Dispersal ability, through pollen and seeds, is of great importance in the response to climate change because it enables migration to new, more suitable habitats. It also contributes to the maintenance of a high level of genetic diversity within populations and makes it possible for genes important for adaptation to move quickly between populations. In this way, a northern population could receive genes from a southern population, which could be significant for the survival of the population in the event of climate warming. Other factors such as the history of the species and its populations (Boileau *et al.*, 1992; Ibrahim *et al.*, 1996), life history characteristics (Hamrick & Godt, 1996; Austerlitz *et al.*, 2000) and past and ongoing selection (Le Corre & Kremer, 2003) can all affect the current genetic structure, which can in turn potentially have an impact on species survival. *Betula pubescens* is a self-incompatible, widely distributed tree species with both its seeds and pollen dispersed by wind, which leads us to expect a high level of diversity but low level of differentiation among populations (Kremer, 1994; Hamrick & Godt, 1996; Austerlitz *et al.*, 2000). It is also a cold-tolerant species which quickly colonized Europe as the climate improved after the last glacial maximum (Paus, 1995; MacDonald *et al.*, 2000), suggesting that not much genetic variation would have been lost during glaciation.

Mountain birch forms the treeline at about 700 m a.s.l in the Torneträsk area in northern Sweden. Thickening of the woods and expansion of shrubs above the treeline have been noticed early by Sandberg (1965), in parallel with the retreat of the glaciers. Altitudinal expansion of the treeline was confirmed later, when Sonesson & Hoogesteger (1983) found that there were no birches older than 40 years old at the present treeline and up to 50 m below, indicating that great reproductive effort started in the area at the end of the 1930s, just after the peak of a warm period. Recently increasing mean annual temperatures have been recorded at the meteorological observatory of the Abisko Scientific Research Station, associated with an increase in the length of the growing period and snow-free season. Young recruits of mountain birch have been found above the treeline as high as 1200 m a.s.l. around Lake Latnjajaure in the mid-alpine belt, presumably due to the recent warming of the climate.

In this work, we used microsatellites (Truong *et al.*, 2005) to explore the genetic structure of the mountain birch along an altitudinal gradient close to the treeline in the Torneträsk area and also investigated seedling performance above the treeline. We wanted to address the following questions: Does the genetic data indicate high dispersal among populations? Does it indicate a high number of founding individuals in the newly established population above the treeline? Both these patterns would indicate that *B. pubescens* ssp. *tortuosa* has a high migration potential and could therefore respond readily to climate change. We also performed spatial autocorrelation analysis to explore the spatial genetic structure of the populations and age classes and to investigate the history of establishment of the species in the area. Finally, we wanted to determine if seedling performance above the treeline was positively correlated with temperature, which would suggest that an increase in temperature would be beneficial to the establishment of *B. pubescens* ssp. *tortuosa* in this area.

Materials and methods

Study species

In Europe, the tetraploid birch *B. pubescens* is commonly subdivided into two different subspecies (Walters, 1964): ssp. *pubescens*, found in the lowlands, with the same distribution as *B. pendula*, and ssp. *tortuosa*, the mountain birch, with a distribution restricted to upland areas, overlapping with *B. nana*.

Betula pubescens Ehrh. ssp. *tortuosa* (Ledeb.) Nyman is a monoecious, strongly self-incompatible, wind-pollinated, low tree or shrub, with small seeds easily dispersed by wind (Atkinson, 1992). Its morphology is close to dwarf birch *B. nana*, with a curved, polycormic trunk, procumbent growth form and a tendency towards vegetative propagation. Sensitive to shade, birch establishes most efficiently on bare soil due to the low ability of the seedlings to penetrate the canopy (Atkinson, 1992). It is a pioneer that can quickly colonize open areas and is well adapted to marginal habitats. In Europe, the mountain birch forms stable natural forests in the sub-artic zone and defines the altitudinal and latitudinal treeline in Fennoscandia.

The taxonomy of the genus *Betula* is very complex and remains unclear, partly due to the high level of hybridization between the different species. Hybrids have been largely described between the three European birches, the diploid *B. pendula* and *B. nana* and the tetraploid *B. pubescens* (Walters, 1964; Jonsell, 2000). Palmé *et al.* (2004) found extensive sharing of chloroplast haplotypes among the three species, most likely caused by hybridization. High morphological variation, especially in the leaf, has been frequently recorded in *B. pubescens*, presumably as a result of hybridization with close relatives. Consequently, the origin of polyploidy in *B. pubescens* remains unclear. Howland *et al.* (1995) suggested that it was an allotetraploid of *B. pendula* and *B. humilis* or another extinct species, or alternatively that it might be an autotetraploid of *B. pendula*. The mountain birch is believed to result from the hybridization between *B. pubescens* and *B. nana* and therefore to inherit its characteristic traits from *B. nana* via introgression (Elkington, 1968; Jonsell, 2000). Introgressive hybridization and subsequent gene flow among the two species have been identified through morphological and cytological evidence (Thorsson *et al.*, 2001).

Study area and sampling

Three areas of study (which will be referred to as populations) were delimited along an altitudinal gradient in the Torneträsk area (Fig. 1 and Table 1): Abisko (ABI), Karsavagge (KAR) and Latnjajaure (LAT).

The ABI population was delimited as a 270 × 270 m study plot, in a typical polycormic forest stand at the bottom of the Abisko valley (about 450 m a.s.l.), on the south shore of Lake Torneträsk close to the Abisko Scientific Research Station. The general climate of the area is oceanic, but there is a strong oceanic-continental gradient West to East, with a minimum annual precipitation of about 300–400 mm in the sheltered Abisko valley. At the lake, the average annual temperature is about −1.0 °C and July is the warmest month (mean + 11 °C). Partial sampling was performed along a grid every 30 m for adults and every 25 m for juveniles. Samplings of the adults and juveniles covered the same area but were performed independently, in order to avoid exclusive sampling of juveniles close to their selected parents. Leaves were collected and dried with silica gel and metric coordinates of all individuals were measured along the grid, with GPS values taken for plants at the corners. A total of 81 adults and 99 juveniles were sampled.

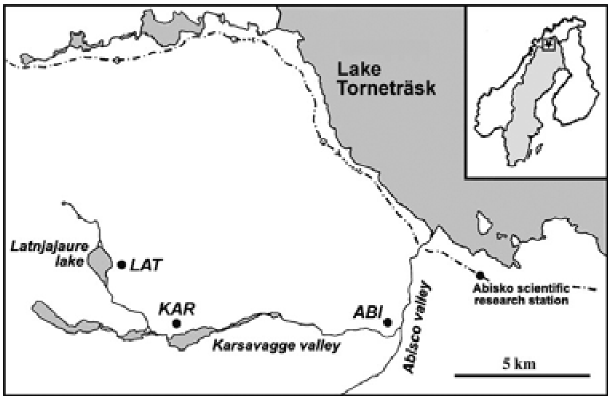

Fig. 1 Torneträsk area in northern Sweden. Black circles indicate the location of each population, Abisko (ABI), Karsavagge (KAR) and Latnjajaure (LAT), on the map.

Table 1 Populations of *Betula pubescens* ssp. *tortuosa* along an altitudinal gradient in the Torneträsk area, northern Sweden.

Population	Location	Elevation (m a.s.l.)	No. of adults	No. of juveniles	Total
Abisko	Forest	450	81	99	180
Karsavagge	Treeline	700–750	71	116	187
Latnjajaure	Above treeline	990–1200	–	87	87
				Overall	454

At the nearby treeline along the slopes of the Karsavagge valley (700–750 m a.s.l.), a study plot of the same size but delimited as a rectangle of 180 × 400 m, formed the KAR population. It was situated on the south-facing side of the valley, in a sparse stand very close to the uppermost trees. We recorded coordinates and collected leaves from one adult every 30 m. Due to the greater amount of regeneration, one juvenile was sampled every 20 m. Again, adults and juveniles were sampled independently. A total of 71 adults and 116 juveniles were sampled.

Above the treeline, the LAT population was delimited as the east side of the catchment area of Lake Latnjajaure (990–1200 m a.s.l.) in the mid-alpine belt, in the middle of a large U-shaped glacial valley, home of the Latnjajaure Field Station. The total surface of the area was about 5 km^2. Average annual temperature at the lake is about −2.7 °C and July is the warmest month (mean + 7.4 °C). The annual precipitation ranges from 600 to 900 mm, which is much higher than at Abisko, because of the altitude and proximity to the Atlantic Ocean. The valley floor is dominated by acidic glacial moraines, sparsely covered by poor heath. As the surrounding mountains are calcareous, there is a rapid shift from acidic to basic-rich soil in the transition zone at the flanks of the valley (Björk, 2000). The valley slopes are characterized by cliff terraces covered by dry-mesic meadow vegetation. The surrounding gravel plateaus (1300–1500 m a.s.l.) exhibit a discontinuous plant cover characteristic of the high-alpine belt. Leaves of all individuals found during two consecutive summers, 2001 and 2002, were collected and spatial coordinates of the individuals were measured using both GPS and a metric grid for distances closer than 10 m. A total of 85 juveniles were sampled and no fertile adult was found within the whole investigation area.

Microsatellite analysis

Microsatellites are commonly known to be highly variable and therefore are a powerful tool for detecting genetic variation within species. We developed nine microsatellite markers in *B. pubescens* ssp. *tortuosa* and tested the 452 individuals from the three populations for polymorphism. All loci were highly polymorphic and showed similar F expectations (see Truong *et al.*, 2005 for details). As it is a tetraploid genome, one to four bands can be detected at each locus. Most samples were run twice and allelic dosage was resolved manually then compared with threshold values of the relative intensity peak of the band, measured in the computer program GENE IMAGIR 4.03 (Scanalytics, Fairfax, VA, USA). All diverging results were left as missing data.

Data analysis

Genetic diversity and Hardy–Weinberg equilibrium

Polymorphism (P), the percentage of the loci exhibiting more than one allele, the average number of alleles per polymorphic locus (AP) and allelic frequencies were calculated and compared for each population. We calculated heterozygosities and inbreeding coefficient F for each population, using the software AUTOTET (Thrall & Young, 2000). The existence of multiple heterozygous states in polyploids makes the estimation of observed heterozygosity (H_o) from the proportion of full homozygotes excessively conservative, because this class is considerably less sensitive to inbreeding than it is in diploids (Bever & Felber, 1992). We therefore used a more rigorous calculation of H_o, called path analysis, where the five possible classes of genotypes are weighted inversely to the probability of any of their alleles being identical by descent. Furthermore, in autopolyploids, segregation at a given locus varies between two extremes – random chromosome segregation (RC_eS), and random chromatid segregation (RC_dS). Under RC_dS, sister chromatids can sort into the same gamete resulting in double reduction, causing an increase in the production of homozygous gametes over what is expected under random mating (Bever & Felber, 1992). The probability of double reduction occurring at a locus (α) ranges from 0 under RC_eS to a theoretical maximum of 1/7 (Wricke & Weber, 1986), according to the proximity of the locus to the centromere, as it is dependent on the type and number of cross-over. F will vary depending both on the selfing rate and on the proportion of double reduction (α), which itself varies among loci (Ronfort *et al.*, 1998). Expected heterozygosity (H_E) was therefore computed separately under either RC_eS or RC_dS assuming maximum double reduction ($\alpha = 1/7$). F was calculated as $1 - (H_o/H_E)$. Departure from $F = 0$ was assessed with a Wilcoxon signed-rank test (nonparametric), in the software S-PLUS 6.0. (Insightfull, Seattle, WA, USA)

Departure from Hardy–Weinberg (HW) equilibrium was tested for each population with χ^2 goodness of fit tests for observed-to-expected genotype frequencies, under either RC_eS or RC_dS assuming maximum double reduction ($\alpha = 1/7$), using the software AUTOTET (Thrall & Young, 2000). χ^2 is known to give doubtful results when expected frequencies of some genotypic classes are low. Consequently, we pooled all alleles except the most common one into the same class, according to Swofford & Selander (1989).

Differentiation among populations

In autopolyploids, double reduction tends to reduce the effective subpopulation size and promote differentiation among subpopulations, increasing F_{ST} as would do selfing. Therefore, Ronfort *et al.* (1998) defined a new parameter related to F_{ST}, ρ, which is independent of both the selfing rate and the proportion of double reduction. It is therefore identical for all loci, independently of their distance to the centromere. We estimated both global and pairwise ρ among populations using an ANOVA framework in the program SPAGEDI (Hardy & Vekemans, 2002). Significance was assessed by permutation tests, with 999 random permutations of locations, individuals and genes.

To test for a pattern of isolation by distance (IBD) (Wright, 1943), the coefficient of relationship r_{ij} (Hardy & Vekemans, 1999, see below) among all individuals, independently of their affiliation to a population, were regressed on pairwise spatial distances using the program SPAGEDI (Hardy & Vekemans, 2002). Significance of the fit of the model was assessed with 999 permutations of locations, individuals and genes. As LAT contains no adults, this 'deme' can only receive migrants and the gene flow is therefore unidirectional. The analysis was run again, excluding the individuals from LAT and the results compared with the global analysis.

Gene flow

Under the island model of population structure (Wright, 1951) and assuming no mutation rate, ρ can be used to estimate the number of migrants from the product N_m (Ronfort *et al.*, 1998):

$$\rho \approx \frac{1}{1 + 2N_m\theta}$$

for tetraploids, where θ = number of subpopulations $(n)/n - 1$. We calculated indirect estimates of gene flow among populations (N_m, the number of migrants per generation) using both ρ and the private allele method of Slatkin (1985). Assuming the populations to be at equilibrium between gene flow and drift, the logarithm of $\bar{p}(1)$, the average frequency of private alleles, is an approximate linear function of the logarithm of N_m:

$$\log[\bar{p}(1)] = a\log(N_m) + b$$

where $\bar{p}(1)$ is relatively independent of both selection and mutation rate, but varies slightly according to the sample size per deme. Simulations for 10 demes of $n = 25$ individuals indicate a slope of $a = -0.576$ and an intercept of $b = -1.11$ that can be used to estimate N_m from $\bar{p}(1)$. Correction for sample size was made by multiplying the uncorrected estimate of N_m by the ratio of the average number of individuals per deme (N_{sam}) to 25 (Barton & Slatkin, 1986):

$$N_{m(COR)} = N_{m(EST)}\frac{25}{N_{sam}}.$$

There is still some error compared with simulation models, but the estimates are of the correct order of magnitude. Slatkin's private allele method is assumed to be independent of the degree of ploidy of the study species (Kjellsson *et al.*, 1997).

Both methods assume that populations are in equilibrium and this is not necessarily the case, especially not in the younger population. The genetic structure of a newly established population depends largely on the founding individuals and high similarity with other populations might not be because of current gene flow but population history. This should be kept in mind while interpreting the N_m estimates.

Spatial autocorrelation analysis

We performed spatial autocorrelation analysis to describe the spatial pattern of the distribution of genetic diversity within each population. We used the coefficient of relationship r_{ij} among individuals (Hardy & Vekemans, 1999), which is computed as the correlation between individual allele frequencies. Averaging this estimator over distance classes gives mean values of Moran's I statistics. At constant gene flow parameters, r_{ij} is not influenced by the selfing rate or the ploidy level, which makes it useful to study polyploid organisms.

Spatial coordinates of all individuals were converted into the same metric grid using the software CIRCE 2000 (Institut Géographique National, Paris, France). Within each population, r_{ij} was calculated for all pairs of individuals and averaged over five distance classes using the software SPAGEDI (Hardy & Vekemans, 2002). The upper limits of the distance classes were chosen as 25, 50, 100, 200 and 400 m. Detailed fine-scale genetic structure, which only represent direct seed fall at the bottom of the mother tree, was voluntary not taken into account here as sampling was partial only. Significance was assessed by permutation tests, with 999 random permutations of locations among individuals. As autocorrelation analysis involves multiple testing, we used the progressive Bonferroni correction (Hewitt *et al.*, 1997), which assumes the process generating the autocorrelation to be stronger at small distances. That is, the autocorrelation coefficient of the first distance class is tested against the 0.05 significance level (α) and the coefficients of the next k distance classes are tested against the Bonferroni-corrected levels α/k. Global genetic structure within each population was estimated with the slope of the regression of r_{ij} over ln(distance). The advantages of such single statistic are that it avoids arbitrarily set distance intervals and the conservativeness of multiple testing (Vekemans & Hardy, 2004). For both ABI and KAR, spatial autocorrelation analysis was also performed for each generation (adults and juveniles) separately, in order to examine how the genetic diversity is distributed among the age classes.

Seedling performance above the treeline

Above the treeline, we investigated the performance of the 85 individuals of LAT in relation to their habitat. We measured morphological variables related to the fitness of individuals: size, number of branches, stems and leaves of each individual. At each station we recorded the environmental conditions, such as exposure, slope and vegetation coverage. We described the vegetation in the 0.5 m^2 surrounding each plant: we estimated vascular plant diversity, lichen and moss abundance, and recorded the dominant plant species. Plant species were grouped according to life-form (Table 2) and the presence or absence of each life-form was inferred for each station.

Table 2 Species list of vascular plants dominating the vegetation above the treeline (LAT). The species are grouped according to life-form.

Deciduous dwarf shrubs	Herbs
Betula nana	*Antennaria alpina*
Salix herbacea	*Bartsia alpina*
Salix polaris	*Polygonum viviparum*
Salix reticulata	*Potentilla crantzii*
Vaccinium myrtillus	*Solidago virgaurea*
Vaccinium uliginosum	*Thalictrum alpinum*
	Viola biflora
Evergreen dwarf shrubs	Graminoids
Andromeda polifolia	*Anthoxanthum alpinum*
Arctostaphylos alpina	*Calamagrostis lapponica*
Calluna vulgaris	*Carex bigelowii*
Cassiope hypnoides	*Carex rupestris*
Cassiope tetragona	*Deschampsia flexuosa*
Diapensia lapponica	*Festuca ovina*
Empetrum hermaphroditum	*Juncus trifidus*
Phyllodoce coerulea	*Luzula arcuata*
Vaccinium vitis-idaea	*Poa alpina*

We explored the distribution of individuals for each environmental variable, in order to detect general trends linking the performances of individuals to their habitat. Using the software CANOCO 3.1 (Microcomputer Power, Ithaca, NY, USA), we first performed a principal component analysis on the morphological dataset (size, number of branches, stems and leaves) to assess how it describes the distribution of individuals. We then performed a redundancy analysis (RDA) to test for correlations between the environmental variables and the morphological variables related to the fitness of individuals. All variables were previously centred-reduced to give them the same weight and scores were attributed for nonquantitative variables. We used Monte-Carlo permutation tests (999 permutations) to assess for significance. Finally, we constructed a tri-plot graph with the distribution of individuals as a function of their morphological variables and constrained by the environmental variables.

Results

Allelic dosage showed the same results when resolved manually or using the relative intensity peak of the band, as well as among both run of the samples. Less than 2% of the results were divergent and were left as missing data. Because of hybridization with related diploid species, diploid and triploid individuals may occur within *B. pubescens* populations. However, in our case, 94.3% of individuals showed four bands at one or more loci and only 2.85% of individuals were not proved to be tetraploid, i.e. a 4th allele could not be allocated at least once. This was considered rare enough to regard all individuals as tetraploid while analysing the data.

Genetic diversity and Hardy–Weinberg equilibrium

The three populations had all loci polymorphic and a high level of genetic diversity, with an average number of alleles (*AP*) of 20 to 23 per locus and an expected heterozygosity (H_{E}) of 0.73 under $RC_{\mathrm{e}}S$ and 0.68 under $RC_{\mathrm{d}}S$ (Table 3). Overall, 46% of alleles were rare (frequency <1%) and 33% of rare alleles were private to one of the three populations. Genetic diversity was slightly lower in LAT, in which a smaller number of rare and private alleles were found, but the difference from the other populations was nonsignificant (standard errors overlapping). The three populations showed H_{o} substantially lower than H_{E} under both $RC_{\mathrm{e}}S$ and $RC_{\mathrm{d}}S$, with F slightly above 0. Exact Wilcoxon signed-rank tests indicated that F was significantly different from 0 under $RC_{\mathrm{e}}S$ ($P < 0.05$), but not under $RC_{\mathrm{d}}S$.

Departure from HW equilibrium was not significant ($P > 0.05$) for most loci except *L021* and *L2.3*, under both $RC_{\mathrm{e}}S$ and $RC_{\mathrm{d}}S$, indicating that the three populations were following HW expectations.

Population differentiation and gene flow

Estimates of among-population variation were very low with a significant global value of ρ of 0.0069 ($P < 0.001$). Pairwise estimates of ρ among populations stayed

Table 3 Genetic diversity parameters along an altitudinal gradient: forest, treeline and above treeline.

				Average overall loci (%)						
Populations	*N*	*P* (%)	*AP*	Alleles <1% (%)	Private alleles[†]	H_{o}	H_{E} (C_{e})	F (C_{e})	H_{E} (C_{d})	F (C_{d})
ABI (forest)	180	100	23.11 (9.08)	38.41 (14.77)	17.14 (11.31)	0.66 (0.26)	0.73 (0.24)	0.092* (0.048)	0.68 (0.22)	0.027 (0.055)
KAR (treeline)	187	100	23.67 (8.79)	42.55 (16.60)	16.40 (10.52)	0.67 (0.26)	0.73 (0.26)	0.083* (0.056)	0.68 (0.24)	0.017 (0.057)
LAT (above treeline)	87	100	20.00 (7.16)	36.16 (13.27)	11.51 (17.58)	0.67 (0.24)	0.73 (0.24)	0.075* (0.052)	0.68 (0.23)	0.009 (0.053)
Overall	454	100	26.33 (9.47)	45.64 (14.09)	32.93 (13.08)	0.67 (0.25)	0.73 (0.25)	0.087* (0.049)	0.68 (0.23)	0.022 (0.053)

Values in parentheses are standard errors.

N, sample size; *P*, % of the loci polymorphic; *AP*, average number of alleles per polymorphic locus; H_{o}, observed heterozygosity; H_{E}, expected heterozygosity; *F*, inbreeding coefficient; C_{e}, chromosome segregation; C_{d}, chromatid segregation.

[†] Percentage of rare alleles that are private.

Significance for departure from $F = 0$ (Wilcoxon signed-rank test): $^{*}P < 0.05$.

nonsignificant. The slope of the regression of the coefficient of relationship r_{ij} among all individuals plotted against the logarithm of their geographical distance was very low (−0.0022) but significant ($P < 0.001$), indicating a weak pattern of IBD among individuals. Excluding LAT did not improve the model, except that the slope was even weaker (−0.0019).

Due to the low values of global ρ, the estimate of gene flow among populations was very high, with a number of migrants per generation (N_m) of 47.98. The private allele method of Slatkin independently gave similar results, although they were slightly higher ($N_m = 56.57$).

Table 4 Regression of the relationship coefficient r_{ij} as a function of ln(distance) in the three populations at different elevations.

Population	Slope
Abisko (forest)	−0.0073*
Karsavagge (treeline)	−0.0093*
Latnjajaure (above treeline)	−0.0014
Abisko (adults)	−0.0061
Absiko (juveniles)	−0.0054
Karsavagge (adults)	−0.0110*
Karsavagge (juveniles)	−0.0073*

*Significance after 999 permutations ($P < 0.05$).

Spatial autocorrelation analysis

The correlograms based on r_{ij} indicated weak but significant positive autocorrelation for distances up to 200 m in the populations of ABI and KAR (Fig. 2 and Table 4). The slope of the regression over ln(distance) was slightly higher in KAR than in ABI. LAT did not show any pattern of spatial structure at any distance.

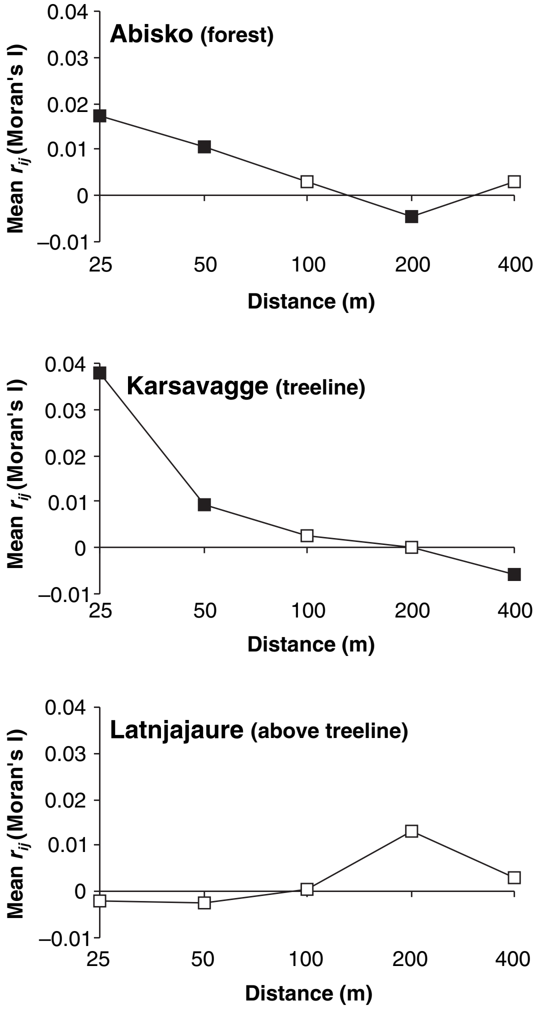

Fig. 2 Moran's correlograms of the relationship coefficient r_{ij} as a function of distance in the three populations at different elevations. Black symbols indicate significance ($P < 0.05$) after progressive Bonferroni correction.

Most of the spatial structure observed in ABI occurred among adults and juveniles, as both age classes did not show a significant autocorrelation (Table 4). On the other hand, the slope of the regression over ln(distance) was significant in both age classes in KAR, along with a significant positive autocorrelation between juveniles at small distances, which however did not persist in the adult age class (Fig. 3).

Seedling performance above the treeline

The individuals were found homogeneously in contrasting habitats, with a slight tendency for a southern exposure, mid to low vegetation recovery and plant diversity and mid to high lichen abundance. The presence of birch was not found to be associated with any particular type of plant species according to life-form.

Principal component analysis based on the morphological data set showed that these variables were valuable to describe the distribution of individuals, with 63.7% of the variance explained within the first axis and 14.9% within the second axis. RDA associating the morphological and environmental variables showed that 23.8% of the variance was explained within the first axis and 3.2% within the second axis. The correlation coefficient between the two data sets was 0.62 for the first axis and 0.48 for the second axis. The overall analysis and the first axis significantly explained the variation ($P = 0.001$ for both after 999 permutations), but the second axis was not significant. Three environmental variables significantly explained the morphological variation among individuals: exposure ($P = 0.001$), lichen abundance ($P = 0.018$) and moss abundance ($P = 0.005$). The tri-plot graph (Fig. 4) showed that a north–south exposure and lichen abundance were positively correlated with the morphological variables, whereas moss abundance was negatively correlated.

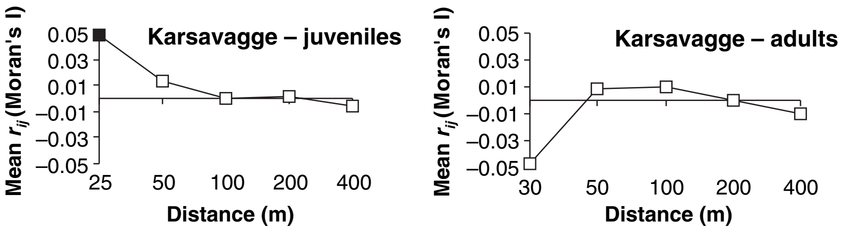

Fig. 3 Moran's correlograms of the relationship coefficient r_{ij} as a function of distance for each age class in KAR. Black symbols indicate significance ($P < 0.05$) after progressive Bonferroni correction.

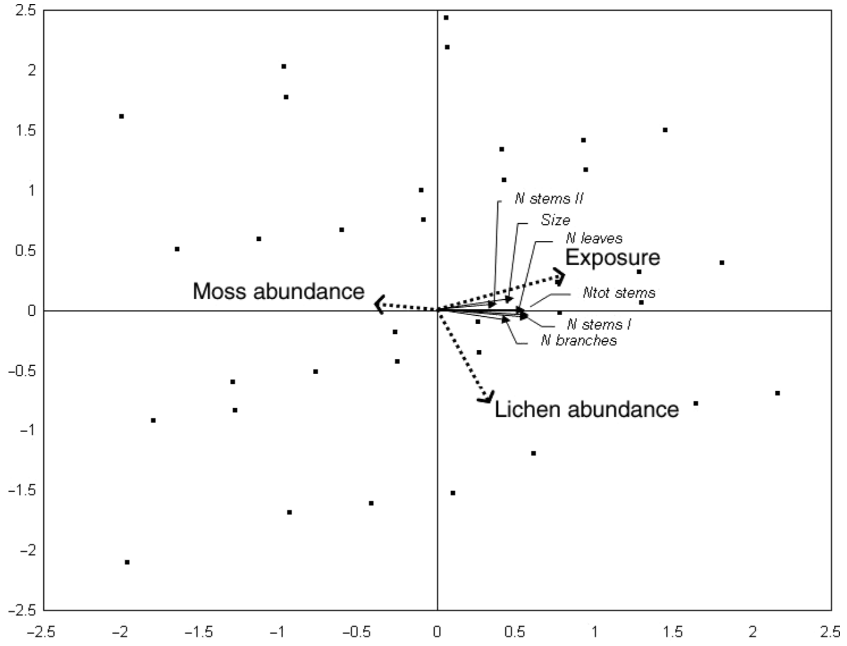

Fig. 4 Tri-plot graph of the distribution of individuals of LAT (black squares) as a function of their morphological variables (small arrows) and constrained by the environmental variables (big arrows). Only significant environmental variables are shown here. Arrow trends show that exposure (from North to South) and lichen abundance are positively correlated with most of the morphological variables (size, number of branches, number of stems, number of leaves), whereas moss abundance is negatively correlated.

Discussion

Origin of polyploidy in the species

The difficulties associated with the taxonomy of the genus *Betula* are, in part, due to the high level of hybridization between the different species (Walters, 1964; Jonsell, 2000) and the question of the origin of polyploidy in *B. pubescens* is not yet solved. The only safe way to determine the mode of inheritance in a tetraploid is by analysing offspring of experimental crosses. Here, the HW equilibrium observed in the three populations for most loci, under both $RC_{e}S$ and $RC_{d}S$, is compatible with a model of random segregation of alleles at meiosis. Although disomic inheritance cannot be excluded, we found that considering the species as autopolyploid was the most suitable way to analyse the data.

Genetic diversity within and among populations at different elevations

The three populations exhibited high levels of genetic diversity within populations and low level of variation among populations. This is a general pattern observed in the majority of tree species, presumably because of their great dispersal abilities of pollen and seeds (Kremer, 1994; Hamrick & Godt, 1996). Subsequent high gene flow will not only tend to homogenize the genetic variation among populations, but also to increase the genetic diversity within populations, as it makes them less likely to lose novel or rare alleles through drift or bottlenecks. Mountain birch is a strongly self-incompatible, long-lived perennial woody species, with wind-dispersed pollen and seeds, such characteristics all presuming a potential for high genetic diversity. Myking (2002), while evaluating the genetic resources of forest trees by means of life-history traits, considered *B. pubescens* as viable in response to future climate change. Furthermore, species with a wide continuous geographical range, like mountain birch, are more likely to exhibit high level of genetic diversity than patchily distributed species in which the influence of population size on genetic diversity is much greater (Hamrick & Godt, 1996). It is also a cold-tolerant species and there are indications that *Betula* was present in central Europe during the last glacial maximum (Huntley & Birks, 1983; Willis *et al.*, 2000) and quickly colonized Europe as the climate improved (Paus, 1995; MacDonald *et al.*, 2000), suggesting that not much genetic variation would have been lost during glaciation. Selection seems to have successfully acted to adapt birch to different local climates after the glaciation as there are gradients for several important traits (Myking, 1999; Welling *et al.*, 2004). Steep selection gradients and high levels of migration can lead to different patterns in neutral markers, QTLs and traits, resulting in population differentiation in traits but not in neutral markers (Le Corre & Kremer, 2003).

Gene flow as a source of genetic diversity at higher elevations

The spatial distribution of individuals becomes fragmented with increasing elevation, especially in LAT. Mountain birch reproductive success has been shown to diminish with increasing elevation in the Torneträsk area (Sveinbjörnsson *et al.*, 1996). Increasing elevation also leads to an increased mortality rate, especially in the early stages of life, due to the harsher climatic conditions (Kullman, 1986; Sveinbjörnsson *et al.*, 1996). Marginal populations, often isolated and in less suitable environments, are usually expected to have reduced genetic diversity compared with more central populations because of drift and natural selection (Lesica & Allendorf, 1995). In our case, however, we did not observe any loss of genetic diversity with increasing elevation as heterozygosities were similar in the three populations. Departure from $F = 0$ was significant under RC_eS because double reduction was excluded. It remained nonsignificant, however, when assuming RC_dS. The absence of departure from HW equilibrium under both RC_eS and RC_dS, in the three populations and over most of the loci corroborates these results and indicates that there is no effect of inbreeding within population. The large number of migrants per generation (N_m) found using both ρ and Slatkin's method, as well as the very low slope of the regression of IBD over all individuals, both indicate that high level of gene flow largely overbalances genetic drift (Slatkin, 1993).

Extensive gene flow between the three populations is most likely due to the high pollen and seed dispersal rates of the species. Assuming that migration potential through seeds can be inferred from post-glacial migration rates (Myking, 2002), birch, as an early colonist, showed great dispersal abilities in the past. Long distance pollen dispersal has been recorded over distances of up to 600 km in northern Sweden (Wallin *et al.*, 1991) and dispersal of the tiny winged seeds over many kilometers (Holm, 1994). Birch seeds are easily dispersed upwards, from lower elevations with a more regular production of seeds, and may survive in the seed bank for several years (Kullman, 1986). The presence of mountain birch has been recorded in the seed bank in the Latnjajaure area more than 300 m above the uppermost fertile trees at the treeline (Molau & Larsson, 2000). This attests to the occurrence of long distance seed dispersal in the Torneträsk area. Continued immigration of seeds from lower elevations seems to play a decisive role in the maintenance of genetic diversity at the treeline and therefore greatly contributes to the viability of populations at higher elevations. Furthermore, the long juvenile stage of the life-cycle of trees induces a delay in reproduction. This allows genetic diversity to accumulate by way of a larger number of initial founders and contributes to the dissipation of the founding effect of newly established populations above the treeline (Mariette *et al.*, 1997).

Spatial autocorrelation analysis within populations

Genetic differentiation within a continuous population like a forest is usually ascribed to IBD caused by restricted gene flow through seed and pollen (Streiff *et al.*, 1998). In such cases, mating is no longer random in the whole population but instead determined by the distance between individuals. Spatial autocorrelation analysis showed a weak but significant genetic structure up to 200 m apart within the ABI and KAR populations (Fig. 2 and Table 4). These results are in accordance with the majority of studies on forest tree species, with varying reproductive systems and histories (Leonardi & Menozzi, 1996; Streiff *et al.*, 1998). It may be the result of common life-history traits: longevity and extensive gene flow, in comparison with other plants. In our case, gene flow is shown to be very high, which contributes to a decrease in the spatial genetic structuring within populations.

The lack of any pattern of genetic structure above the treeline shows that LAT is a young population that has recently colonized the area. As no individual has yet reached fertility, the spatial structure only reflects the pattern of the immigration of seeds. Emanuelsson (1987) put forward the hypothesis that recent treeline rising and thickening of the woods may result from the recovery of a former upper treeline. Changes in reindeer husbandry practices by the Saami people at the beginning of the 20th century, associated with climate warming, enabled the regeneration of the woods. In our case, if LAT was the relict of a former forest population, the pattern of distribution of seeds in the seed bank would be representative of the seed shadows of the former trees and would still exhibit the remains of their past genetic structure. Furthermore, when considering LAT alone, we failed to detect any pattern of IBD between individuals (results not shown). Associated with a large value of N_{m}, this suggests that the population is young and has recently colonized the area (Slatkin, 1993).

Spatial autocorrelation analysis within and among age classes at different elevations

Detailed fine-scale genetic structure within the stands, i.e. direct seed fall at the bottom of the mother tree, was voluntarily not taken into account here, as sampling was partial only. Still, the fact that most of the spatial structure is found between adults and juveniles shows the limitation of the dispersal of most seeds at the foot of the trees. Furthermore, the slope of the regression indicated that ABI exhibited weaker positive autocorrelation than KAR (Table 4). This difference is strengthened in juveniles, when measuring spatial autocorrelation within age classes, as no significant autocorrelation was observed in ABI, contrary to KAR. This may result from differences in adult densities. The higher density of adults of the forest population (ABI) may result in overlapping seed shadows of the trees, inducing competition for space among the seedlings (Chung *et al.*, 2003). Moreover, the dense heath canopy of the forest floor may interfere strongly with the establishment of the seedlings, due to their weak ability to penetrate the canopy (Atkinson, 1992). At the treeline (KAR), a more open structure enables the establishment of a higher density of regeneration around the trees. Indeed, the amount of regeneration found at the treeline was much higher than inside the forest.

The significant positive autocorrelation between juveniles at small distances did not, however, persist in the adult age class in KAR (Fig. 3). This loss of structure can be explained by strong local mortality of the seedlings, due to the harsh environmental conditions prevailing at the treeline, which may severely limit the survival of the early stages of life (Gonzalez-Martinez *et al.*, 2002). Competition for space among juveniles while they grow can also erode the spatial genetic structure. However, both factors have more influence on the fine-scale structure which was not taken into account here. An alternative possibility is that the difference in spatial genetic structure among age classes may be attributable to different histories of establishment between generations (Chung *et al.*, 2003). Recent improvement of the climatic conditions in the Tornträsk area may have contributed to the expansion of clusters of young recruits at the treeline, inducing spatial genetic structuring within this age class. During past establishment of the adult age class, harsher conditions prevailing at that time may have caused the establishment of fewer recruits, inducing a lack of spatial genetic structure in this class.

Performance of seedlings in contrasting habitats above the treeline

Above the treeline (LAT), young recruits of mountain birch were found in diverse habitats, from poor heath to rich meadow and rocky places. We found no correlation between seedling performance and the life-form of dominant species in the vegetation, indicating that seedling performance was not limited within any particular plant community.

The positive correlation found between morphological variables related to the fitness of individuals and a southern exposure can be easily associated with temperature, which is a major factor affecting growth. Kullman (1986) suggested that mortality in the early stages of life was mainly a winter process due to frost-heave. Therefore, seedling survival was directly proportional to the length of the growth period during the first summer, which determines the potential for the accumulation of reserves. Thick snow cover prevents drought above the treeline and melting will start earlier in places which receive more sun. This is a great advantage at high latitudes, where the growing season is very short. Assuming that seedling performance is positively correlated with temperature, climate warming, i.e. earlier onset of the snowmelt, will be favourable for the survival and growth of the species above the treeline.

Differential response of seedling performance in correlation to lichen and moss abundance can be directly linked to the exposure. Indeed, temperature enhancement has been shown to increase lichen cover while decreasing moss cover (Molau & Alatalo, 1998). Therefore, lichen abundance is likely to be enhanced in southern places, which receive more sun, to the detriment of mosses. Differential influence of lichen and moss abundance on seedling performance may also be the result of differences in ground cover. Lichens are usually sparse whereas moss cover can be thick and continuous and may interfere with the establishment of seedlings, due to their low ability to penetrate the canopy (Atkinson, 1992). While looking at vegetation cover, we did observe a general trend for the occurrence of seedlings in places with low ground cover, but it did not seem to affect performance, as the correlation remained nonsignificant.

Finally, these results indicate that temperature enhancement may have a positive effect on the growth performance of mountain birch seedlings, resulting in the expansion of young recruits above the treeline in relation to climate warming.

Our results demonstrate that recent rising of the treeline in the Torneträsk area is most likely because of climate warming and that mountain birch has the potential to respond positively to changes in climate. High level of genetic diversity is maintained within populations because of high level of gene flow through pollen and seed dispersal. It compensates for possible losses of genetic diversity at higher elevations and presumes to the high migration potential of the species. Gene flow greatly contributes to homogenize the gene pool and to dissipate the founding effect of newly established populations above the treeline. This leads to very low genetic differentiation among populations, despite the contrasting environmental conditions, fertility and density parameters prevailing at different elevations. Changes in spatial genetic structuring among age classes at the treeline may indicate recent expansion of young individuals due to the warming of the climate. Furthermore, seedling performance above the treeline was positively correlated with parameters related to temperature, indicating that climate warming would have positive effects on birch performance above the treeline.

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