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RESEARCH PAPER

Past and future range shifts and loss of diversity in dwarf willow (*Salix herbacea* L.) inferred from genetics, fossils and modelling

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ABSTRACT

Aim Climate change may cause loss of genetic diversity. Here we explore how a multidisciplinary approach can be used to infer effects of past climate change on species distribution and genetic diversity and also to predict loss of diversity due to future climate change. We use the arctic-alpine plant *Salix herbacea* L. as a model.

Location Europe, Greenland and eastern North America.

Methods We analysed 399 samples from 41 populations for amplified fragment length polymorphism (AFLP) to identify current patterns of genetic structure and diversity and likely historical dispersal routes. Macrofossil records were compiled to infer past distribution, and species distribution models were used to predict the Last Glacial Maximum (LGM) and future distribution of climatically suitable areas.

Results We found strong genetic differentiation between the populations from Europe/East Greenland and those from Canada/West Greenland, indicating a split probably predating the LGM. Much less differentiation was observed among the four genetic groups identified in Europe, and diversity was high in the Scandinavian as well as in southern alpine populations. Continuous distribution in Central Europe during the last glaciation was inferred based on the fossil records and distribution modelling. A 46–57% reduction in suitable areas was predicted in 2080 compared to present. However, mainly southern alpine populations may go extinct, causing a loss of about 5% of the genetic diversity in the species.

Main conclusions From a continuous range in Central Europe during the last glaciation, northward colonization probably occurred as a broad front maintaining diversity as the climate warmed. This explains why potential extinction of southern populations by 2080 will cause a comparatively low loss of the genetic diversity in *S. herbacea*. For other species with different glacial histories, however, the expected climate-change induced regional extinction may cause a more severe loss of genetic diversity. We conclude that our multidisciplinary approach may be a useful tool for assessing impact of climate change on loss of genetic diversity.

Keywords
Amphi-Atlantic, arctic-alpine, climate change, colonization, conservation genetics, glacial refugia, ice age, phylogeography, range shifts.

INTRODUCTION

There is now an increased awareness of the importance of assessing the impact of ongoing and future climate change on genetic diversity as well as on geographic ranges of species (Hampe & Petit, 2005; Petit *et al.*, 2005; Willis & Birks, 2006). For arctic-alpine species, there is a high risk of losing southern alpine populations with a warmer climate (Thuiller *et al.*, 2005). How this will affect the total genetic diversity in each species and thus their evolutionary and colonization potential may, to a large degree, depend on their glacial refugial and postglacial colonization history, which have shaped their current genetic structure and diversity.

Arctic and alpine plants typically survived glaciations in peripheral refugia surrounding the ice caps, or perhaps even on nunataks (Brochmann *et al.*, 2003; Stehlik, 2003; Schönswetter *et al.*, 2005). Some cold-adapted species may simply have shifted

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their ranges in accordance with the climate, and thus not experience any contraction into glacial or interglacial refugia. Fossil records from the Last Glacial Maximum (LGM) indicate a dry, steppe tundra vegetation in Central European lowlands (north of the Alps to south of Fennoscandia), suggesting that this region may have acted as a corridor between or as a source for colonization of both the Arctic and southern alpine regions (Huntley & Webb, 1988; Frenzel *et al.*, 1992; Lang, 1994). However, only the north-western part of this region was comparable to the present-day tundra; most of the remaining area may have been too dry for many arctic-alpine species. In the arctic-alpine plant *Arabis alpina*, a periglacial population extending from the northern edge of the Alps to the southern margin of the northern European glaciers probably existed in locally humid microhabitats (Ehrich *et al.*, 2007). A common origin of populations from Northern Europe and the Alps has also been suggested for *Dryas octopetala* (Skrede *et al.*, 2006). However, in two arctic-alpine buttercups, *Ranunculus glacialis* and *R. pygmaeus*, colonization of arctic areas may have been from the eastern Alps and the Urals, respectively, rather than from Central European lowland refugia, resulting in different genetic structures in these two species (Schönswetter *et al.*, 2003; Schönswetter *et al.*, 2006a). The importance of the Central European glacial tundra for persistence and postglacial expansion, and thus for the current genetic structuring in arctic-alpine species, is still not clear and may vary significantly among species.

In North America, the fossil record indicates a much narrower periglacial belt where arctic and alpine species may have persisted throughout glacial periods (Ritchie, 1987). With no transverse mountain ridges to cross, species may rather easily have retreated southwards during periods of cooling, followed by northward colonization in warm periods (Tremblay & Schoen, 1999; Eidesen *et al.*, 2007a,b).

The genetic effects of climate change-induced range shifts differ markedly among arctic-alpine species. Recolonization of previously glaciated areas in the north was probably extensive and broad-fronted in some taxa, maintaining high levels of genetic diversity (Alsos *et al.*, 2007; Eidesen *et al.*, 2007a). In other taxa, however, genetic diversity decreases from the refugia regions to the recolonized areas, for example in *Saxifraga oppositifolia* (Abbott *et al.*, 2000) and *Cassiope tetragona* (Eidesen *et al.*, 2007b). In the most extreme cases, colonization involved severe genetic bottlenecks leading to populations with virtually no variation over vast northern areas, while alpine populations remained genetically diverse (Schönswetter *et al.*, 2006a; Ehrich *et al.*, 2007). Such species will certainly loose most of their genetic diversity if their alpine populations go extinct.

All approaches currently used to infer glacial refugia and postglacial colonization routes have advantages and limitations. Molecular data may indicate both areas of glacial persistence (areas with high genetic diversity and/or distinct genetic markers) and the direction of colonization. Different molecular techniques have been applied, for example sequencing of organelle markers, which in some cases also can be used to calculate approximate ages of lineage splits within species (Alsos *et al.*, 2005; Koch *et al.*, 2006), and various fingerprinting techniques. Recent studies have shown that fingerprinting gives better resolution and may be more representative for species phylogenies than organelle markers (Eidesen *et al.*, 2007a; Eidesen, 2007). One disadvantage of fingerprinting data is that they do not allow for age estimates. Fossil records, on the other hand, can be quite reliably dated, and macrofossils especially give firm proof of occurrence of a species at a certain place and time. However, the availability of fossil data is often limited temporarily or geographically. Thus, areas where no fossils have been recorded may nevertheless have been important for persistence during the glaciations.

Species distribution models can, as a new approach, provide insights into past ranges and help to locate additional, potential refugial areas (Cheddadi *et al.*, 2006; Hijmans & Graham, 2006; Martínez-Meyer & Peterson, 2006). Several studies have shown that dual approaches, i.e. combining molecular and fossil data (Lascoux *et al.*, 2004; Magri *et al.*, 2006; Ehrich *et al.*, 2007), molecular data and species distribution modelling (Weaver *et al.*, 2006; Carstens & Richards, 2007; Jakob *et al.*, 2007), or fossil data and species distribution modelling (Martínez-Meyer & Peterson, 2006; Pearman *et al.*, 2008) provide advantages as compared to a single-method approach. Only one study has so far combined all three approaches to show how isolation in refugia and the Holocene colonization pattern shape modern genetic diversity (Cheddadi *et al.*, 2006).

Here, we take such a multidisciplinary approach one step further by exploring the impact of past climate changes on current genetic diversity as well as exploring the impact of future climate change on species distribution and genetic diversity. As a model we selected the arctic-alpine plant *Salix herbacea* L., which has well-known climatic requirements and a quite extensive fossil record (Tralau, 1963; Conolly & Dahl, 1970; Beerling, 1998). We aimed (1) to identify its late glacial distribution and postglacial colonization routes, (2) to assess the impact of the glacial periods on its modern patterns of genetic diversity, and (3) to predict the impact of future climate change on its distribution and potential loss of genetic diversity.

MATERIALS AND METHODS

Study species: *Salix herbacea* L.

Salix herbacea is found in alpine and northern regions of Europe and north-eastern North America as well as on Greenland (Fig. 1a). Except for some scattered populations in Central Europe and Russia, its entire present distribution is restricted to previously glaciated regions. The species grows in wind-exposed fell-fields, screes with little protection from snow cover, and snow beds. It is a poor competitor and requires habitats with some disturbance to persist (Beerling, 1998). *Salix herbacea* is dioecious and thus strictly outcrossing, and possibly insect-pollinated (Beerling, 1998). It requires a snow-free period of more than three months to flower and fruit (Ellenberg, 1988), and the seed set may amount to over 4000 seeds per square metre (Nyléhn *et al.*, 2000). The plumed seeds are dispersed by convection currents and wind, and only able to germinate if they land on a well-oxygenated substrate shortly after ripening (Ellenberg,

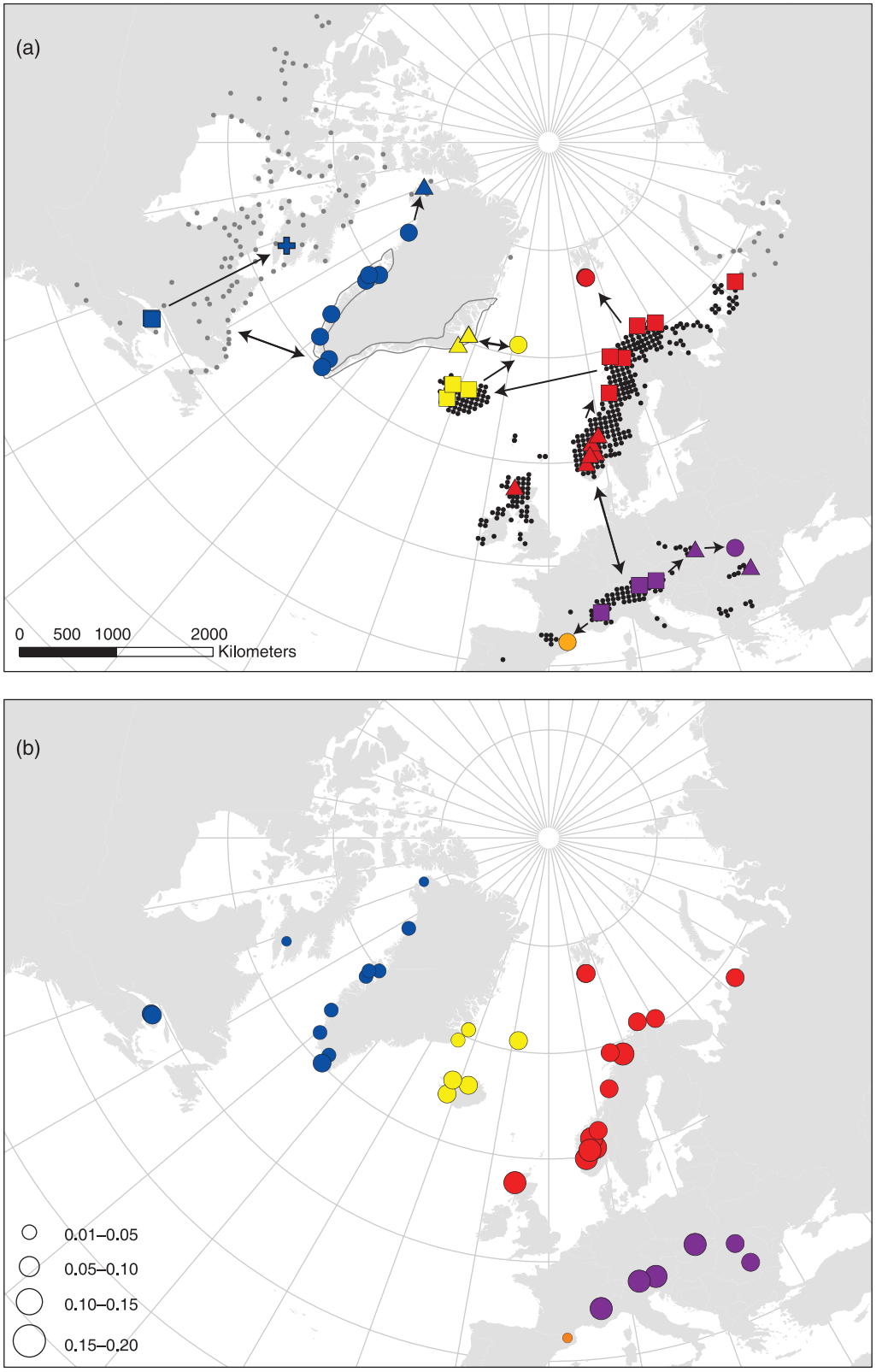

Figure 1 (a) Geographic origin and genetic structuring of the 41 *Salix herbacea* populations analysed for amplified fragment length polymorphism (AFLPs). Colours identify the five main genetic groups according to Bayesian clustering analyses run with STRUCTURE and BAPS (see text); symbols identify subgroups within main groups. The entire present distribution of the species is given according to *Atlas Florae Europaeae* (Jalas & Suominen, 1976; black dots) and Hultén & Fries (1986; grey dots/outline). Arrows represent dispersal routes inferred from assignment to STRUCTURE/BAPS groups (Tables 2 and 3) and fossil records (see Appendix S2). (b) Intrapopulation diversity in *S. herbacea* based on 250 polymorphic AFLP markers (average proportion of pairwise differences).

1988). The viability of the seeds decreases rapidly, but observation of seedlings just after snowmelt suggests that some seeds remain viable over winter (Söyrinki, 1938; Beerling, 1998). Reproduction by seeds is, nonetheless, generally rare and the majority of stands spread vegetatively (Jeník & Kubíkóva, 1962).

Salix herbacea can colonize deglaciated terrain within 20 years (Nyléhn *et al.*, 2000). Extensive fossil records exist from late glacial deposits in Europe (Huntley & Webb, 1988; Hultén & Fries, 1986). The leaves are readily preserved due to their thick cuticles, and are often found in excellent condition. They can be distinguished from those of *S. polaris* by their denticulate (versus entire) margin and nerves reaching the marginal teeth. Although some authors distinguish pollen of *S. herbacea* from those of other *Salix* species (Straka, 1952; Funder, 1978), this distinction may be uncertain (Beerling, 1998).

Interspecific hybridization may influence genetic patterns within species and thus bias inferences of phylogeographic histories (Palmé *et al.*, 2003, 2004; Eidesen, 2007). Hybrids between the diploid *S. herbacea* ($2n = 38$) and other diploid as well as some hexaploid *Salix* species, such as *S. polaris*, have been reported (Beerling, 1998; Elven, 2005). However, by including related species in an analysis, possible impacts of hybridization on the history of a species can be detected (Eidesen, 2007).

Molecular data: material, methods and data analyses

Material

Leaf samples were collected in 2002–2004 from 5–11 individuals from each of 41 populations and dried in silica gel. If possible, the samples were collected at 25-m distances along a transect to ensure that the sampling distance exceeded the clone size known for this species (1 m^2, Reisch *et al.*, 2007; up to 7-m distance, Stamati *et al*, 2007). The sampling covered most of the range of the species, except for the westernmost part in North America (Fig. 1, see also Appendix S1 in Supporting Information). We also included one population of *S. herbacea* × *polaris* hybrids (identified based on morphology) and one population of each of the closely related species *S. berberifolia* Pallas ($2n = 38$), *S. nummularia* Anderss. ($2n = 38$) and *S. polaris* Wahlenb. ($2n = 114$) (see Appendix S1, ploidy levels according to Löve & Löve, 1975; Lomonosova *et al.*, 1992). One sample per population was duplicated in the field or during extraction and used as a control and to calculate genotyping errors (Bonin *et al.*, 2004). The leaf samples/DNA extracts as well as voucher specimens from all populations were deposited in the DNAbank of the National Centre for Biosystematics at the Natural History Museum in Oslo (O).

AFLP methods

A subset of the data presented here was previously published by Alsos *et al.* (2007) as part of a multi-species dataset, and most details of the DNA extraction and AFLP methods for *S. herbacea* were provided in that paper. A few additional specifications are given here: for restriction ligation, 1.5 U T4 DNA ligase (Roche, Mannheim, Germany) was used, and the incubation time was 3 h. The preselective PCR product was diluted 15 times. Initially, 27 primer combinations were tested on four samples from different geographical regions. The six best primers were further tested on 24 samples, and the three best primer combinations were chosen (6-FAM *Eco*RI-ACT – *Mse*I-CTA, VIC *Eco*RI-ACA – *Mse*I-CAG, and NED *Eco*RI-AGC – *Mse*I-CTC) and run on the total set of samples (see Appendix S1). The three primers were co-loaded and run on a capillary sequencer (ABI 3100, Applied Biosystems).

Raw data were collected and aligned with the internal size standard using the ABI Prism GeneScan® ver. 3.7. analysis software (Applied Biosystems, 1989–2001). Fragments in the size range 100–480 bp were scored with Genographer version 1.6 (http://hordeum.oscs.montana.edu/genographer). The data were exported as a presence/absence matrix.

Data analyses

The genotyping error rate was calculated according to Bonin *et al.* (2004), based on 33 samples that were replicated in the field or during extraction and on 17 samples that were replicated from restriction legation, totalling 50 replicated samples. Potential resampling of clones was checked for with AFLPdat R-script (Ehrich, 2006), but was of insignificant importance and thus not corrected for.

To find the main genetic structure within *S. herbacea* as well as how it relates to the other species, different analyses were conducted and the results compared. A neighbour joining tree using Nei & Li's (1979) distance measure was constructed with TREECON 1.3b (van de Peer & de Wachter, 1994). The tree was midpoint rooted and branch support was estimated with 1000 bootstrap replicates. A principal coordinate analysis (PCO) was used to compare pairwise similarity among the AFLP multilocus phenotypes as implemented in NTSYS-pc 2.02 h (Rohlf, 1990) using simple matching. Two model-based clustering approaches were used, as follows. (1) a Bayesian clustering approach implemented in STRUCTURE 2.0 (Pritchard *et al.*, 2000) was run with the no-admixture model and assuming allele frequencies to be independent. The number of groups (K) was estimated based on 10^6 iterations, with a burn-in period of 10^5 iterations. Ten replicates were run for each $K = 1$–10 on the Bioportal at the University of Oslo (http://www.bioportal.uio.no). The K value with the highest likelihood, with a high similarity coefficient (> 0.85, Nordborg *et al.*, 2005) between runs, and with most individuals clearly grouped was chosen as the number of main genetic groups in the dataset. The same analyses were also run for each main group to search for further structure. (2) Another Bayesian clustering algorithm was run with the program BAPS 3.2 (Corander & Marttinen, 2006), which gives only one optimal grouping. To assess the geographic origin of all *S. herbacea* populations, we performed assignment tests based on the multilocus genetic data using AFLPOP v1.0 program (Duchesne & Bernatchez, 2002) with the following settings: marker frequencies of zero were replaced by (1/number of sample size + 1); the minimal log likelihood difference to assign an individual was set to 1 or 2, i.e. it was only assigned if the allocation to a certain

population was 10 or 100 times more probable than to another population.

Average gene diversity over loci *D* (estimated as average proportion of pairwise differences) and analyses of molecular variance (AMOVAs) were computed (Excoffier *et al.*, 1992). AMOVA was used to test for significant divergence among groups (run in Arlequin 2.000, Schneider *et al.*, 2000). Data format conversion, estimation of diversity indices and estimation of similarity coefficients between STRUCTURE runs were carried out with AFLPdat R-script (Ehrich, 2006). The numbers of polymorphic and private markers were counted for each main group.

Fossil data

Fossil data were compiled from the literature. As noted above, only macrofossils (leaves) of *S. herbacea* can be positively identified to species. Contrary to pollen, which is easily transported over long distances, macrofossils may be regarded as a robust proof of local presence. Tralau (1963) mapped macrofossil records of *Salix herbacea* in Europe as known by the early 1960s (although many of the studies included predate the advent of radiocarbon dating). These records were later updated by Lang (1994), but are only dated to time intervals (pre-Weichselian and glacial/late glacial). Here we provide a compilation of macrofossil records from studies with generally more precise datings (including a number of AMS-datings of the actual macrofossils, and less reliance on bulk datings of sediments). Pollen data have only been included in the case of east Greenland, where few macrofossil studies are available. For this area, Funder (1978) provides a detailed discussion of pollen identification. Dates were recalculated to calibrated before present (BP) dates using the program OxCAl 4.0 and the calibration curve IntCal04 for terrestrial fossils (https://c14.arch.ox.ac.uk/oxcal/OxCalPlot.html).

Species distribution modelling

Data on species occurrences and climate

Information on the European distribution of *Salix herbacea* was based on the geographical distribution of the populations included in this study combined with data extracted from the Global Biodiversity Facility (GBIF, http://www.gbif.org; provided by Haeupler & Schönfelder, 1989, Benkert *et al.*, 1996; Biologiezentrum Linz (ZOBODAT), Bundesamt für Naturschutz, Botanical Society of the British Isles, Environment and Heritage Service, Scottish Borders Biological Records Centre, and the herbaria at the Natural History Museum in Oslo (O), Biological Museum, Oskarshamn (OHN), Universität Wien (WU), Icelandic Institute of Natural History (ICEL) and Icelandic Institute of Natural History, Akureyri Division (AMNH)). The geographical coordinates were checked for uncertainty and removed if they had an accuracy > 5 km, resulting in a dataset of $n = 154$. This was done to ensure high precision in linking species occurrences and climate (see below). Data extracted from GBIF do not necessarily represent the full geographical distribution of a species. Therefore we additionally used the distribution of *Salix herbacea* extracted from *Atlas Florae Europaeae* (AFE; Jalas & Suominen, 1976) to validate how well the models predict the present distribution of *Salix herbacea* in Europe. AFE uses an equal area grid of 50×50 km^2 (AFE cell).

Data for monthly values of mean temperature and precipitation were obtained for the time periods Last Glacial Maximum (LGM; 21,000 BP, Oxygen Isotope Stage 3 Project, Barron & Pollard, 2002, http://www.esc.cam.ac.uk/oistage3/Secure/OIS-3 k.html), present (period 1961–1990; CRU CL 2.0 dataset, New *et al.*, 2002, http://www.cru.uea.ac.uk/cru/data/hrg.htm) and future (year 2080, averages 2070–2099; TYN SC 1.0 dataset, Mitchell *et al.*, 2004, http://www.cru.uea.ac.uk/~timm/index.html). We used two scenarios to represent the possible climates in year 2080, namely the A2 and B2 scenarios from the Intergovernmental Panel on Climate Change (IPCC, 2001). Data for present and future climate conditions were obtained at 10′ resolution, while data for past climatic conditions were obtained at *c.* 60 km^2 resolution. To improve representation of smaller-scale climatic variation, notably that caused by topography, high-resolution LGM climate estimates were obtained by calculating the difference between the current and past climate simulated by the Stage 3 Project, interpolating these to a 10′ resolution and subtracting them from the current climate according to the CRU dataset (cf. Hijmans & Graham (2006) for a similar approach). During LGM the eustatic sea level was lowered *c.* 110 m (Ruddiman, 2000). The LGM coastline was obtained by lowering the sea level of the Earth Topography-5 minute (ETOPO5; http://www.ngdc.noaa.gov/mgg/bathymetry/relief.html) elevation-bathymetry raster by 110 m.

Maximum summer temperature determines the southern distribution limit of *S. herbacea*, coinciding with the 23–25 °C maximum summer isotherm on the British Isles and the 26 °C isotherm in Scandinavia (Beerling, 1998). This variation may result from the modifying influence of continentality (Dahl, 1998). In addition, Huntley (in Beerling, 1998) has shown that European populations of *S. herbacea* occur in areas with minimum temperatures down to –20 °C, between 150–2400 growing days-degrees above 5 °C, and a rather narrow range of actual to potential evapotranspiration rates (1.0–0.95). This narrow range suggests that *S. herbacea* is intolerant to prolonged soil drying, which also has been suggested to limit its distribution in Svalbard (Crawford & Balfour, 1983). Finally, prolonged snow cover promotes the occurrence of *S. herbacea* (Beerling, 1998). Based on the above prior knowledge and the monthly values of mean temperature and precipitation, we calculated six bioclimatic variables of presumed importance for *S. herbacea*: (1) maximum summer temperature, (2) growing degree days (computed following Zimmermann & Kienast, (1999) using a 5 °C base temperature), (3) mean summer water balance (water balance was computed as the yearly sum of the monthly differences between precipitation and potential evapotranspiration following Skov & Svenning (2004)), (4) winter precipitation, a simple estimate of snowfall (computed as the summed precipitation in winter months with temperatures below 0 °C), (5) annual temperature range (maximum difference across the year – a

simple estimate of continentality), and (6) absolute minimum temperature of the coldest month (estimated following Prentice *et al.*, 1992). Correlations between all climatic variables were equal to or below 0.89.

Modelling approach

To model its ecological niche and predict the geographical distribution of *S. herbacea* at different times, we used Maxent version 2.3 (Phillips *et al.*, 2006), a method appropriate for presence-only data and recently shown to perform well in comparison with other methods (Elith *et al.*, 2006) and to be appropriate for predicting species past and future distribution (Hijmans & Graham, 2006).

The models were calibrated using the GBIF data, and the potential distributions obtained by projecting the estimated ecological niche onto LGM, present and future climate data. The potential distribution was only modelled for Europe, thus the area for which past and future climate data were available. Validation of the models' abilities to predict the present distribution of *Salix herbacea* was done in two ways: (1) by the Area Under the receiver operation characteristics Curve (AUC) computed with Maxent's internal validation procedure for random partitions of the GBIF data (75% for calibration, 25% for testing, Phillips *et al.*, 2006), and (2) by Kappa values (Fielding & Bell, 1997) estimating the ability of models calibrated on all GBIF occurrences to predict the distribution of *Salix herbacea* given by AFE. Background samples used 20,000 pixels taken as a random sample in the study area. Recommended default values were used for the convergence threshold (10^{-5}) and maximum number of iterations (500). Selection of 'features' (environmental variables or functions hereof) was done automatically.

RESULTS

Genetic structure and diversity

In total, 436 samples were successfully analysed and 283 AFLP markers were scored (error rate = 1.15%). The proportion of polymorphic markers was 97.88% (277 markers). The dataset for *S. herbacea* (excluding other species and hybrids) consisted of 399 individuals from 41 populations and 250 polymorphic AFLP markers (96.53% polymorphic, error rate = 1.99%, see Appendix S1).

The neighbour joining analyses separated *S. berberifolia* and *S. nummularia* from *S. herbacea* (Fig. 2). Also, the two *S. polaris* populations and the hybrid *S. herbacea* × *polaris* were in a separate lineage but nested within *S. herbacea.* As these species and the hybrid also were in separate groups in initial BAPS and STRUCTURE analysis (not shown), little impact from hybridization with the closest relatives was assumed and they were omitted from all further analysis.

For the *S. herbacea* dataset, BAPS yielded nine groups which were identical to the STRUCTURE groups at the same *K* value. However, the point of deflection in the STRUCTURE analysis was at $K = 5$, which also gave high similarity between runs (similarity coefficient = 0.97 ± 0.04). The assignment of individuals to five STRUCTURE groups was in accordance with BAPS with the same number of groups. Thus, we recognized five main genetic groups: the *W Atlantic group* (Canada, west and north-west Greenland), the *Mid-Atlantic group* (Iceland, Jan Mayen, and east Greenland), the *E Atlantic group* (Scotland, Scandinavia, north-west Russia and Svalbard), the *Alpine group* (the Alps and the Carpathians), and the *Pyrenean group* (Fig. 1a). Separate BAPS and STRUCTURE analysis within each of these main groups gave the same subgrouping for the Mid-Atlantic and the E Atlantic groups (Fig. 1a). For the W Atlantic group, the optimal subgrouping according to STRUCTURE was two subgroups (Canada and W Greenland, similarity coefficient = 1 ± 0.0), whereas BAPS divided these two subgroups into two further subgroups (Gaspé, Baffin Island, W Greenland and NW Greenland, identical with four STRUCTURE subgroups, similarity coefficient = 0.81 ± 0.18, Fig. 1a). For the Alpine group, BAPS gave two subgroups; E Carpathians and Alps/N-S Carpathians, identical to STRUCTURE with two subgroups. However, the optimal subgrouping found with STRUCTURE divided Alps/N-S Carpathians further into the Alps and N-S Carpathians (similarity coefficient = 0.99 ± 0.005, Fig. 1a). The W Atlantic and the Pyrenean main groups and the subgroups Baffin Island and E Carpathians had also more than 50% bootstrap support in the Neighbour Joining analyses (Fig. 2).

The PCO analysis reflected the main STRUCTURE groups with some intermingling among subgroups. The main division along the first axis in the PCO analysis (*c.* 17%) was between the W Atlantic group and the four European groups (Fig. 3). To a lesser extent, the first axis also separated the Alpine group from the Mid- and E Atlantic groups. The second axis (4.8%) separated the Pyrenean group and the third axis (3.9%) separated the Mid-Atlantic group from the other groups.

In hierarchical AMOVA analyses, the separation between the E Atlantic group and all the European groups explained 29.9% of the variation, whereas the separation among the four European groups only explained 17.3% of the variation (Table 1). Separate AMOVA analysis of each STRUCTURE main groups showed higher differentiation within the W Atlantic group (29.3%) than within the Mid-Atlantic group (22.1%), and especially within the E Atlantic group (10.0%) and the Alpine group (11.2%).

The pair-wise hierarchical AMOVA analysis showed that the W Atlantic group was more differentiated from the Mid- (42.2%) and E Atlantic group (37.7%) than from the Alpine group (26.3%, Table 2a). The mean level of diversity per population and total number of markers were higher in the E Atlantic and the Alpine groups than the other groups Table 2b). The numbers of markers private to all northern European populations including E Greenland and for all southern European Mountains were 22 and 21, respectively. There was a higher number of private markers in the Alpine group (15) than in the W Atlantic group (10) and the other groups (2–6, Table 2b).

The W Atlantic group allocated 100% to the Alpine group (Table 2c). Within this group, W Greenland and Gaspé allocated to each other (Table 3). In addition, populations in both areas had high levels of genetic diversity and few private markers (one

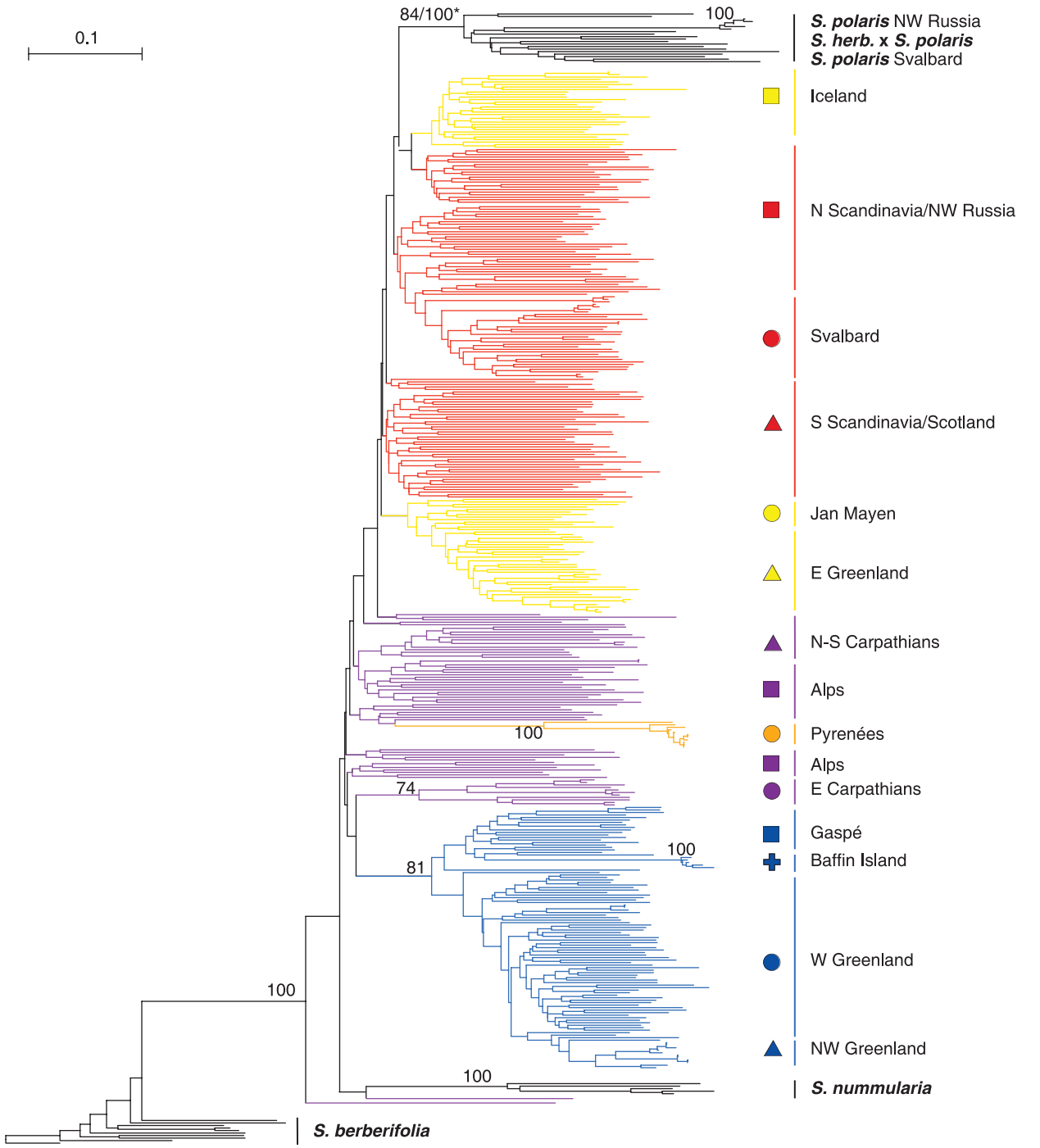

Figure 2 Neighbour joining tree of *Salix herbacea*, *S. nummularia*, *S. berberifolia*, *S. polaris* and *S. herbacea* × *polaris* based on 277 amplified fragment length polymorphic (AFLP) markers and Nei and Li's genetic distances. Bootstrap values below 50% or below population level are not shown. *Bootstrap value when hybrids are removed from the dataset.

and two, respectively). Thus, the direction of dispersal could not be determined between these areas (Fig. 1a). A high proportion (91%) of the Alpine group allocated to the E Atlantic group and visa versa (46%, Table 2c). There was high diversity in both groups ($D = 0.16$ and $D = 0.14$, respectively, Table 2b) and low differentiation among these groups (11.3%, Table 2a) suggesting gene flow in both directions (Fig. 1a). Within the Alpine and Pyrenean group, the allocation tests (Tables 2–3) indicate a spread from the Alps towards the Carpathians and to the Pyrenees (Fig. 1a). The Mid-Atlantic group allocated to the E Atlantic group (97%, Table 2c).

The level of genetic diversity within populations was generally higher in Europe and E Greenland (mean $D = 0.13$) than in W Greenland and E Canada (mean $D = 0.08$, see Appendix S1,

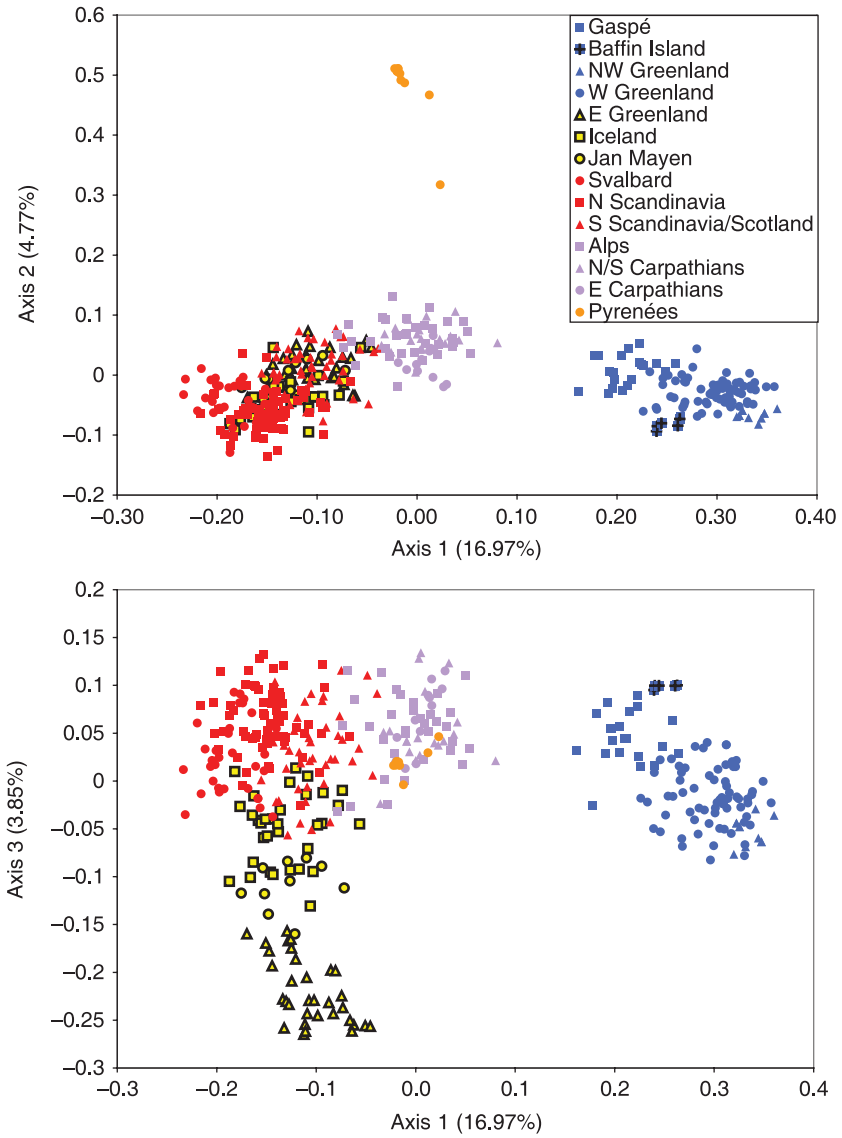

Figure 3 Principal coordinate analysis (PCoA) of *Salix herbacea* using simple matching similarities among 399 amplified fragment length polymorphic (AFLP) multilocus phenotypes. (a) PCoA axis 1 and 2, and (b) PCoA axis 1 and 3.

Fig. 1b). Populations with high levels of diversity were found in the Alps, N Carpathians, Scotland, Iceland and Scandinavia. Extremely low levels of diversity were found in Baffin Island, NW Greenland, and in the deviating population from the Pyrenees.

Fossil records

Fossil records dated to be older than the LGM were found south of the ice sheet both in Central Europe and in North America, for example, from earlier Weichselian/Würmian interstadials in Belgium (75,000–25,000 BP), during the penultimate (Saale/Riss) glaciation in the Netherlands (200,000–130,000 BP) and during a Middle Wisconsinan Interstadial in southwest Ontario (> 33,000 BP, Fig. 4, see Appendix S2), indicating that colonization across the Atlantic occurred before the LGM. *Salix herbacea* was widespread in the periglacial areas of Ireland, Britain, and the lowlands of Central and Eastern Europe during the final stages of the Weichselian glaciation (Fig. 4). Recent studies have also yielded macrofossils from some isolated outposts: Shetland, the Faroe Islands and Iceland. Taken together, the available data show an extensive distribution of *S. herbacea* in Europe south of the major Fennoscandian ice shield during the Weichselian glaciation, and a subsequent northwards colonization during the deglaciation. Isolated outposts like Shetland, the Faroe Islands and Iceland were colonized rapidly. In Greenland, all fossil data so far point to a Holocene recolonization of *Salix herbacea*, both of the west, south, east and north-east parts (presence during the Eemian interglacial has been recorded at Jameson Land in eastern Greenland). In eastern North America, macrofossil records from Canada and the USA demonstrate distribution in periglacial areas south of the Wisconsinan ice sheet. Neither here nor in Europe is there any fossil evidence of persistence in refugia situated within the area of the major ice sheets (Fig. 4, see Appendix S2).

Past, present and future distribution of *Salix herbacea*

Maximum summer temperature was the most important factor determining the present distribution of *S. herbacea* (AUC: 0.96,

Table 1 Analyses of molecular variance (AMOVA) based on 250 amplified fragment length polymorphic (AFLP) markers for 41 populations of *Salix herbacea*. All *P*-values were < 0.001. The first main grouping into two groups (W Atlantic group versus all other populations) is according to the principal coordinate analysis; other groups and subgroups are based on the STRUCTURE and BAPS analyses.

	Source of variation	d.f.	Total variance (%)	F_{ST}
Between the W Atlantic group and all other populations	Among groups	1	29.94	0.50
	Among populations	39	20.55	
	Within populations	358	49.51	
Among the five STRUCTURE groups	Among groups	4	28.64	0.44
	Among populations	36	15.11	
	Within populations	358	56.25	
Among the four European/E Greenlandic STRUCTURE groups	Among groups	3	17.32	0.34
	Among populations	25	16.41	
	Within populations	265	66.27	
Among subgroups within the W Atlantic group	Among groups	3	29.29	0.36
(N Greenland, W Greenland, Baffin Island, and Gaspé)	Among populations	8	6.84	
	Within populations	93	63.87	
Among subgroups within the Mid-Atlantic group	Among groups	2	22.12	0.29
(Iceland, E Greenlan, and Jan Mayen)	Among populations	4	6.94	
	Within populations	69	70.94	
Among subgroups within the E Atlantic group	Among groups	2	10.04	0.20
(N Scandinavia, S Scandinavia/Scotland, and Svalbard)	Among populations	12	10.38	
	Within populations	127	79.58	
Among subgroups within the Alpine group	Among groups	2	11.19	0.20
(Alps, N/S Carpathians, and E Carpathians)	Among populations	3	9.04	
	Within populations	59	79.78	

when included solely in the model). The importance of growing degree days was 0.94 (AUC), mean summer water balance 0.92, winter precipitation 0.86, annual temperature range 0.83 and minimum temperature of the coldest month 0.78. A model including only the first three variables best predicted the observed present distribution according to *Atlas Florae Europaeae* (Fig. 5a, Kappa 0.76).

The modelled distribution of climatically suitable areas during the LGM indicated a distribution across most of Central Europe and a total distribution area which was 41% larger than the present. (Fig. 5b). There was a general overlap between the modelled past distribution of *S. herbacea* and areas with extensive glacial or late glacial fossil records. For example, macrofossils have been recorded just south of the ice in Norfolk (UK) during *c.* 19,800–19,500 cal. yr BP (Fig. 5b, see Appendix S2). The past distribution model also indicated climatically suitable areas for *Salix herbacea* south and east of the Alps, where no fossils of this species have been recorded.

Based on the less severe B2 future scenario (Fig. 5c) we predicted a 46% reduction in suitable areas for *S. herbacea*, suggesting that it will persist mainly in mountainous regions including Scotland and the Alps, while the Pyrenees and Carpathians probably will become unsuitable. A larger range restriction (57%) was predicted by the A2 scenario (not shown).

A loss of all southern alpine populations would result in a loss of 21 of the AFLP markers, thus 8% of the polymorphic markers analysed in the species. The loss of the populations in the Pyrenees and Carpathians would result in a loss of 3% of the markers. The genetic diversity including all populations was $D = 0.19$ whereas the genetic diversity excluding all southern alpine populations was $D = 0.18$. Thus, 5.3% of the genetic diversity may disappear if all southern alpine populations are lost.

DISCUSSION

Our results demonstrated the power of a multi-faceted approach to infer past and present patterns and processes to better predict range shifts and potential loss of genetic diversity under a warmer climate. The genetic, macrofossil and modelling approaches all indicated an extensive distribution of *S. herbacea* in Central Europe during the last glacial maximum, followed by broad-fronted northward colonization. In Canada and western Greenland, the levels of genetic variation were lower overall than in Europe. However, the uniform distribution of genetic variation (except in the northernmost outpost populations), together with numerous fossil records, indicate broad-fronted colonization also there. The added value of three independent dataset showing complementary patterns allows for more confidence in the interpretation than based on a single dataset alone.

Hybridization has probably not been of major importance in shaping the pattern of AFLP variation in *S. herbacea*, as the other species and hybrids separated into well-supported groups in the neighbour joining tree and also were in separate STRUCTURE and BABS groups. Although the nesting of the hexaploid *S. polaris* within the diploid *S. herbacea* in the neighbour joining tree could be a result of hybridization, a more plausible explanation may be

Table 2 (a) Pairwise hierarchical analysis of molecular variance (AMOVA) between STRUCTURE groups presented as percentage of variation between groups/percentage of variation among populations within groups in *Salix herbacea*. All *P*-values were < 0.001. (b) Characteristics of the five main STRUCTURE groups. D = Nei's unbiased gene diversity averaged per population. (c) Percentage of assignments of individuals to the five STRUCTURE groups (see text). Assignment of individuals to its group of origin was not allowed. Values give percentage of assigned individuals if the allocation to a certain group was 10 (or 100) times more probable than to another group. Percentages above 20% are given in bold.

	(a) Pairwise hierarchical AMOVA between groups				(b) Characteristics and diversity of groups						(c) Proportion of assignment to groups					
Assign from group	W Atlantic	Mid-Atlantic	E Atlantic	Alpine	No. of individuals	No. of populations	D ± SD	Total no. of markers	Private markers	AFLP bands per individual	W Atlantic	Mid-Atlantic	E Atlantic	Alpine	Pyrenean	Not assigned
W Atlantic	–/27.2	–	–	–	105	12	0.08 ± 0.03	157	10	56 ± 3.6	–	–	–	**100 (100)**	–	
Mid-Atlantic	42.2/14.9	–/24.1	–	–	76	7	0.10 ± 0.02	146	3	54 ± 3.1	–	–	**97 (93)**	–	–	3 (7)
E Atlantic	37.7/13.0	10.3/17.8	–/17.8	–	142	15	0.14 ± 0.03	200	6	59 ± 4.4	–	**37 (30)**	–	**46 (39)**	–	16 (**31**)
Alpine	26.3/16.2	15.7/17.3	11.3/15.7	–/17.8	65	6	0.16 ± 0.02	214	15	57 ± 5.1	–	6 (2)	**91 (85)**	–	–	3 (14)
Pyrenean	60.4/11.4	53.1/12.4	44.8/10.6	37.8/12.7	11	1	0.03	78	2	66 ± 3.2	–	–	–	**100 (100)**	–	–

AFLP, amplified fragment length polymorphism; SD, standard deviation.

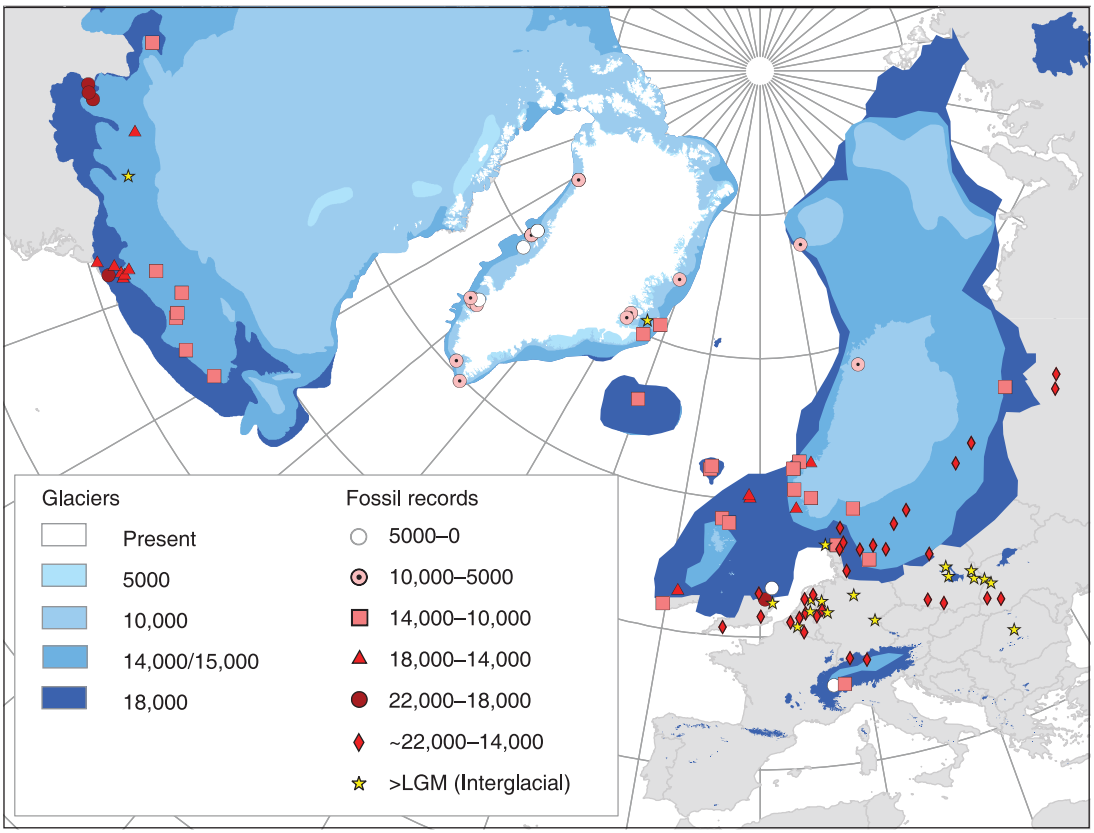

Figure 4 Fossil records of *Salix herbacea* according to our compilation (see Appendix S2 for exact dates and references) and previous reviews (Tralau, 1963; Lang, 1994). The fossil records dated as glacial/late glacial by Tralau (1963) and Lang (1994) are marked with a separate symbol (diamond) due to their more uncertain dates. The ice limits in North America, Greenland and Iceland are from Dyke *et al.* (2003). The ice limits in Europe are from Ehlers & Gibbard (2004; only Last Glacial Maximum (LGM) and Younger Dryas *c.* 10,000 BP available), and redrawn after Andersen & Borns (1997) for 15,000 BP. All main ice sheets in Europe had melted by 5000 BP.

that *S. herbacea* is one of the parental species of *S. polaris*. This is in contrast to the extensive hybridization found in other *Salix* species based on cpDNA markers (Palmé *et al.*, 2003). This may partly be explained by the type of markers, as chloroplast capture may lead to identical haplotypes in different lineages or species (Palmé *et al.*, 2003, 2004). Also, in *Betula* species, analyses of cpDNA showed extensive sharing of haplotypes (Palmé *et al.*, 2004; Maliouchenko *et al.*, 2007) whereas AFLP analyses show clear species boundaries (Eidesen, 2007). Thus, we assume that the observed AFLP-based genetic patterns in *S. herbacea* are likely to have been more influenced by climate-change induced range shifts than by interspecific hybridization.

Glacial distribution and postglacial colonization

The main genetic split observed in *S. herbacea* across the Greenlandic glacier probably reflects long-term separation into two lineages of the species. This view is also supported by the high number of private markers (10) compared to the relatively low number of markers in total (157) in the W Atlantic group. The occurrence of fossils from before the last glacial maximum as well as numerous late glacial fossil records in both north-east America and Central Europe (Fig. 2b) suggest that the species persisted during at least the last 33,000 years on both sides of the Atlantic. Glacial survival south of both the Weichselian and Wisconsinan ice sheets has also been inferred from molecular data in *Vaccinium uliginosum* (Alsos *et al.*, 2005; Eidesen *et al.*, 2007a) and *Deschampsia flexuosa* (I. G. Alsos *et al.*, unpublished data).

It was surprising that the W Atlantic group allocated exclusively to the Alpine group and was less differentiated from this group than the geographically closer Mid- and E Atlantic groups. This may indicate: (1) direct colonization of Canada and western Greenland from European alpine areas, or (2) an ancient colonization from European alpine areas via northern Europe and eastern Greenland, followed by extirpation and re-colonization of northern Europe and eastern Greenland after the last glacial period. The occurrence of fossils predating 33,000 BP in Canada and from the Eemian Interglacial in eastern Greenland, as well as the support for postglacial migration to Scandinavia, Iceland and eastern Greenland, support the second scenario. Colonization along a northern migration route most likely took place during one of the warmer periods of the Quaternary (Cheddadi *et al.*, 2005).

The data supported a glacial refugium south of the Wisconsinan ice sheet in North America followed by a spread northwards to Baffin Island and from western Greenland (9000 cal. yr BP) to

Table 3 Percentage of assignments of individuals of *Salix herbacea* to 14 geographically and genetically defined subgroups (see text). Assignment of individuals to its group of origin was not allowed. The values are percentages of assigned individuals given that the allocation to a certain group was 10 times more probable than to another group. Percentages above 20% are given in bold.

Assign to Assign from	Baffin Island	Gaspé	NW Greenland	W Greenland	E Greenland	Jan Mayen	Iceland	Svalbard	N Scandinavia	S Scandinavia/Scotland	Alps	N-S Carpathians	E Carpathians	Pyrenees	Not assign	No. of plants
Baffin Island		**60**													40	5
Gaspé				**90**											10	21
NW Greenland				**100**											0	11
W Greenland		**78**	15												7	68
E Greenland						**82**				6					12	33
Jan Mayen					**30**		10								60	10
Iceland					3	15			**30**	6					45	33
Svalbard									**88**	3					9	33
N Scandinavia							6	5		**70**					19	63
S Scandinavia/Scotland						2		2	**57**		**20**				20	46
Alps										**39**		**27**			33	33
N-S Carpathians											**71**				28	21
E Carpathians												**82**			18	11
Pyrenees											**100**				0	11

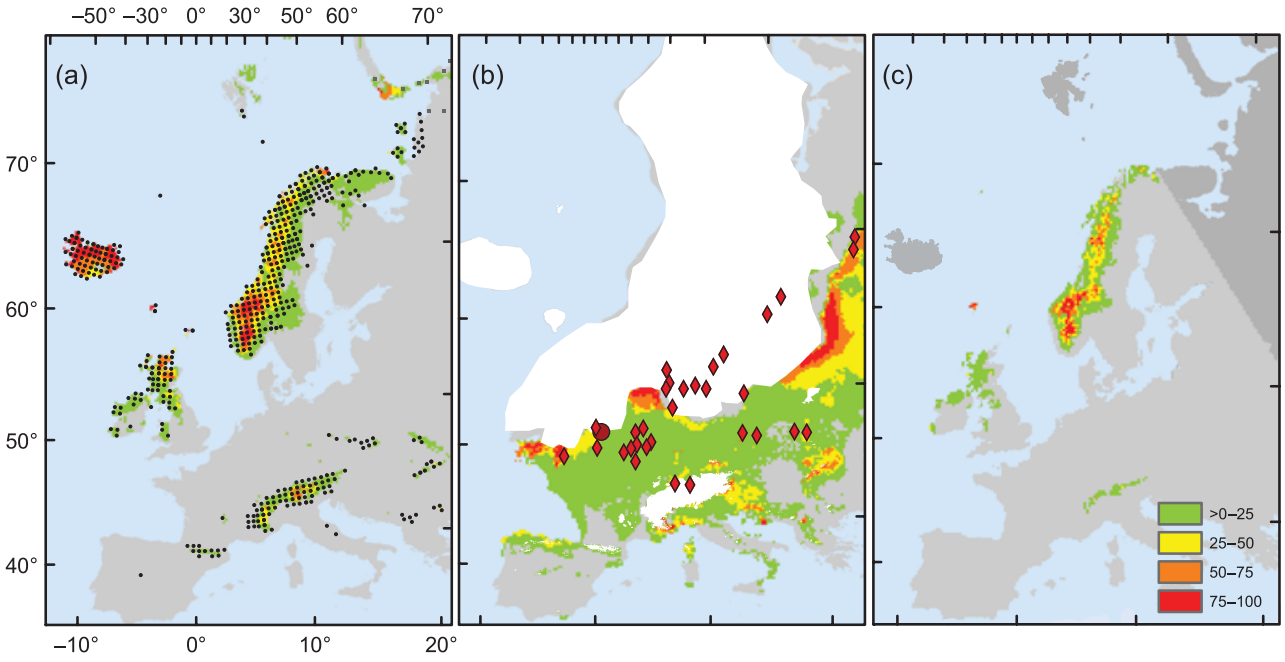

Figure 5 Distribution of *Salix herbacea* in Europe at different times. (a) Modelled present climatically suitable areas compared with the present distribution according to *Atlas Florae Europaeae* (dots). (b) Modelled past distribution of climatically suitable areas (Last Glacial Maximum (LGM), 21,000 BP). Diamonds represent glacial/late-glacial macrofossil records of *S. herbacea* (Tralau, 1963; Lang, 1994) and the circle represents a late glacial record (as in Fig. 4). Ice extent for LGM after Ehlers & Gibbard (2004). (c) Modelled future distribution (year 2080) of climatically suitable areas according to the B2 scenario (IPCC, 2001). Due to lack of climate data from other regions, only the regions shown in light grey on the map were modelled. The probability of distribution (%) is shown according to the colour key.

north-west Greenland (6700 cal. yr BP, Fig. 1 and 4, see Appendix S2). However, the AFLP data gave no indication of direction of dispersal between Gaspé and western Greenland. Thus, although ice free areas existed in western Greenland, our data did not provide any firm support for a glacial refugium also there.

The Central European lowland has probably acted as an important and large refugium as well as dispersal corridor for *S. herbacea* as indicated by the numerous macrofossils found there (Fig. 4), the suitable climate for *S. herbacea* there during the LGM (Fig. 5) and the AFLP data suggesting a common postglacial origin of the E Atlantic and Alpine groups. Thus, in contrast to temperate and boreal species that experienced considerable range contractions and survived in small Central European (Rendell & Ennos, 2002; Stewart & Lister, 2001; Cheddadi *et al*., 2006), Southern European (Lascoux *et al*., 2004; Taberlet *et al*., 1998) or eastern refugia (Taberlet *et al*., 1998), the glacial periods may have been favourable periods for some arctic-alpine species such as *S. herbacea*, allowing gene flow among regions that currently are separated. As shown by fossils records (see maps in Tralau, 1963), several other species, e.g. *Salix reticulata* and *Dryas octopetala*, had a much wider distribution in Europe during the Weichselian glaciation than at present, occurring across the periglacial areas of Central Europe. In the wind-dispersed *D. octopetala,* this region may have contributed to the relatively high levels of genetic diversity found in northern populations (Skrede *et al*., 2006). In contrast, limited gene-flow across this region has been found in other arctic-alpine species studied, e.g. *Ranunculus glacialis* and *R. pygmaeus* (Schönswetter *et al*., 2003; Schönswetter *et al*., 2006a) and *Arabis alpina* (Ehrich *et al*., 2007).

The high levels of genetic diversity in most *S. herbacea* populations as well as the appearance of fossil records immediately after the retraction of the glaciers, suggest that the northwards colonization was broad-fronted and rapid. Based on the assignment test and numerous fossil records, it spread from the glacial refugium in Central Europe northwest to Great Britain (15,600 cal. yr BP) and northwards to south-west Norway (17,000 cal. yr BP), west Norway (13,500 cal. yr BP), south-west Sweden (12,800 cal. yr BP), north Sweden (9000 cal. yr BP) and Svalbard (7900 cal. yr BP) (Fig. 1, see Appendix S2). So far, no macrofossils have been reported from north Norway but *Salix herbacea*-type pollen and development of suitable climatic conditions may, with some reservation, suggest its presence by 11,600 cal. yr BP (Alm, 1993).

The data indicated that *S. herbacea* spread westwards from north Scandinavia to Iceland (10,200 cal. yr BP), Jan Mayen and east Greenland (12,100 cal. yr BP) and thus that the spread across the Atlantic to Iceland was not along the shortest route from Scotland, where the Faroe and Shetland islands could have acted as stepping stones. Colonization of Iceland from Scandinavia rather than Scotland has also been indicated in *Dryas octopetala* (Skrede *et al*., 2006). However, neither of these studies included populations from the Faroe and Shetland islands, and hence the exact dispersal routes are uncertain.

Whereas the allocation test indicated that *S. herbacea* spread from the Alps (11,600 cal. yr BP) or possible periglacial areas surrounding the Alps (Würm Glacial) to the Carpathians, the modelled past distribution suggest that the species could have persisted in the Carpathians during the Last Glacial Maximum (Fig. 4b). According to the modelled past distribution, a large area with highly suitable climatic conditions existed south, north and east of the Alps as well as the Carpathians with their surroundings. There are seemingly no fossil records confirming refugia in these areas, nor in the suggested outpost in north-west Greece (A. Gerasmidis, pers. comm.). However, the low genetic differentiation among the Alps, N-S Carpathians and E Carpathians (Table 1, Fig. 3) suggest that extensive gene flow has occurred among these regions relatively recently. Recent gene flow among these regions has also been found in some other arctic-alpine species (e.g., *Vaccinium vitis-idaea* and *Loiseleuria procumbens*, Eidesen, 2007) whereas other show long-time isolation (e.g., *Ranunculus glacialis*, Schönswetter *et al*., 2003; *Carex atrofusca*, Schönswetter *et al*., 2006b; *Campanula alpina,* Ronikier *et al*., 2008).

The data suggested that the spread from the Alps to the Pyrenees took place a long time ago as the small population sampled in the Pyrenees was strongly differentiated from all other groups (Table 2). The low level of genetic variation, the low number of markers and that only two private markers were observed suggests that genetic drift has occurred within a fragmented population. This has also been observed in Pyreneen populations of other arctic-alpine species (Després *et al*., 2002; Schönswetter *et al*., 2003; Eidesen *et al*., 2007a). No macrofossils of *S. herbacea* have so far been found in the Pyrenees (G. Jalut, pers. comm.), where the modelled past distribution indicated a small area with 0–25% probability of occurrence of *S. herbacea*. However, a palaeoecological study by Jalut *et al*. (1982) also suggested that suitable conditions for boreal and arctic plant species occurred there during the Late Weichselian. Thus, *S. herbacea* may possibly have survived in the Pyrenees or its surrounding during the LGM.

The model past-distribution also suggests suitable climate for *S. herbacea* southeast of the Alps where no fossils of the species have been recorded. Pollen-analytical data, e.g. a long core sequence for Ioannina in north-west Greece, covering the last 420,000 years, suggest a dry, steppe-like climate during the cold (glacial) spells (Tzedakis, 1993; Grove & Rackham, 2000), thus a similar climate to northern Europe (Huntley & Webb, 1988; Frenzel *et al*., 1992; Lang, 1994) where many fossils of *S. herbacea* were found. In this case, the modelling approach may indicate an important glacial refugia area which has been overlooked by paloecological approaches. The scattered populations of *S. herbacea* in the Balkans may be relicts from this period.

Salix herbacea in the future

As predicted for many other arctic-alpine species (Thuiller *et al*., 2005), our models suggest a loss of most alpine populations by 2080, when suitable areas for *S. herbacea* will largely be confined to Scandinavia/Great Britain and to a lesser extent the Alps. The genetic differentiation between the E Atlantic and Alpine group was low and the Alpine group had only a slightly higher level of

genetic diversity (Table 2). The number of private markers was relatively low in both groups. Thus, compared to our predicted loss of about 50% of the climatically suitable areas, the proportion of genetic markers and overall genetic diversity that is at risk of being lost due to loss of southern alpine populations is low for this species. This is in strong contrast to what can be expected for other arctic-alpine species, such as *Ranunculus glacialis* (Schönswetter *et al.*, 2003) and *Arabis alpina* (Ehrich *et al.*, 2007), where most of the genetic diversity is found in southern alpine regions and the northern populations are genetically depauperated. Thus, although there is a risk of losing about 60% of the species in southern mountain ranges (Thuiller *et al.*, 2005), the genetic consequence of this loss depends dramatically on the patterns of genetic diversity and the history of each species.

CONCLUSIONS

We have shown that a combined approach as presented here can be an effective tool for evaluating the potential risk of future loss of genetic diversity, and thus to guide future conservation of species. For species with their main current distribution in northern regions, a more or less continuous distribution between the Fennoscandian and Alpine ice sheets during the LGM, and a broad-fronted, post-glacial colonization pattern as seen in *S. herbacea*, the predicted loss of alpine populations may have limited impact on the total genetic diversity within the species. However, for the majority of other arctic-alpine species, the future genetic loss due to climate-change induced loss of southern alpine populations may be substantial.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Collection data and gene diversity for 41 populations of *Salix herbacea*, four populations of three outgroup species, and one hybrid population.

Appendix S2 Sites with dated macrofossils of *Salix herbacea*, including some pollen records determined to or assumed to represent *Salix herbacea* for some regions in Greenland. A full reference list is provided.

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