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Genetic consequences of Pleistocene range shifts: contrast between the Arctic, the Alps and the East African mountains

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Abstract

In wide-ranging species, the genetic consequences of range shifts in response to climate change during the Pleistocene can be predicted to differ among different parts of the distribution area. We used amplified fragment length polymorphism data to compare the genetic structure of *Arabis alpina*, a widespread arctic-alpine and afro-alpine plant, in three distinct parts of its range: the North Atlantic region, which was recolonized after the last ice age, the European Alps, where range shifts were probably primarily altitudinal, and the high mountains of East Africa, where the contemporary mountain top populations result from range contraction. Genetic structure was inferred using clustering analyses and estimates of genetic diversity within and between populations. There was virtually no diversity in the vast North Atlantic region, which was probably recolonized from a single refugial population, possibly located between the Alps and the northern ice sheets. In the European mountains, genetic diversity was high and distinct genetic groups had a patchy and sometimes disjunct distribution. In the African mountains, genetic diversity was high, clearly structured and partially in accordance with a previous chloroplast phylogeography. The fragmented structure in the European and African mountains indicated that *A. alpina* disperses little among established populations. Occasional long-distance dispersal events were, however, suggested in all regions. The lack of genetic diversity in the north may be explained by leading-edge colonization by this pioneer plant in glacier forelands, closely following the retracting glaciers. Overall, the genetic structure observed corresponded to the expectations based on the environmental history of the different regions.

Keywords: AFLP, *Arabis alpina*, genetic diversity, leading-edge colonization, refugia

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Introduction

It is well known that the Pleistocene climate fluctuations caused major range shifts for many taxa (e.g. Hewitt 2004). While temperate species contracted their ranges to southern refugia (e.g. Taberlet *et al*. 1998; Hewitt 1999), the habitat of taxa adapted to cold and dry conditions was larger during cooler periods than today. For wide-ranging species, climate fluctuations may have induced range contraction in some parts of the range and expansion in others. The repeated cycles of range expansions and contractions, which followed distinct patterns in different species, formed the intraspecific genetic structure that we observe today (Avise 2000; Hewitt 2004).

Range shifts in response to climatic changes were both latitudinal (especially in northern areas) and altitudinal (in mountain regions). Whereas latitudinal recolonization

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occurred over large areas and involved long-distance dispersal, altitudinal range shifts were more localized. The genetic consequences of these types of range shift are likely to be different (Hewitt 1999). Rapid latitudinal recolonization most often results in loss of genetic diversity due to repeated bottlenecks when populations are founded by few individuals, and may create large areas of reduced diversity (leading-edge colonization; Hewitt 1996). This process is less likely in mountain areas, where colonization usually occurs over much shorter distances (Hewitt 1996). However, genetic diversity can be lost during recolonization in mountains as well (e.g. Tribsch *et al*. 2002). Once a new area has been colonized, it is more difficult for long-distance immigrants to establish and contribute to the gene pool of the new population (Hewitt 1999), allowing the persistence of areas characterized by low genetic diversity.

During periods of range contractions, populations are fragmented and isolated in separate refugia. Vicariance is expected to lead to genetic differentiation and result in distinct genetic lineages (Taberlet *et al*. 1998). At present, many taxa in temperate and northern regions are at a stage of range expansion, but this is not the case for the afro-alpine species. In the African high mountains, the afro-alpine habitat occupied a considerably larger area during the last glacial period, when the climate was cooler and drier, and expanded notably over large parts of the Ethiopian highlands (e.g. Flenley 1979; Gottelli *et al*. 2004). Nowadays, on the contrary, this habitat is highly fragmented and restricted to isolated high mountain refugia (Hedberg 1970).

Arabis alpina L. (Brassicaceae) is both an arctic-alpine and an afro-alpine pioneer plant, and it is more widely distributed than many other plants of both distribution types. In the north, its range extends from eastern Canada over the North Atlantic to the Ural Mountains. It grows in the European mountains and around the Mediterranean and reaches the Caucasus, Iran and Iraq in the east and the Arabian Peninsula in the southeast; in addition, it occurs in the mountain regions of Ethiopia and tropical East Africa (Koch *et al*. 2006). It is a perennial rosette plant with unbranched flowering stems and fairly small, usually white petals. Seeds are about 1 mm long and have a narrow wing all around, which may to some degree facilitate wind dispersal (Aiken *et al*. 1999). It has been reported to be self-compatible and partially selfing (Titz 1971; Brochmann & Steen 1999). In the north, *A. alpina* grows in moist habitats such as snow beds and river banks. In Central Europe it is found mostly in montane and subalpine to alpine habitats. It prefers moist, open, gravely or rocky sites and is often found in glacier foreland. In Africa, *A. alpina* occurs in the afro-alpine zone above 3500 m above sea level as well as in the ericaceous zone (3000–4000 m).

In a phylogeographical study based on sequences of chloroplast DNA (cpDNA) and the internal transcribed spacers (ITS) of nuclear ribosomal DNA, Koch *et al*. (2006) inferred that *A. alpina* most likely originated from the Middle East about half a million years ago. From there, one lineage spread westwards and northwards to give rise to the European and arctic populations, reaching as far as the Iberian Peninsula, Madeira and North Africa (the western lineage). Another lineage established in the Middle East and extended to the Caucasus in the east and through Yemen to the northernmost and easternmost Ethiopian mountains (the Asian lineage; Koch *et al*. 2006; Assefa *et al*. 2007). A third lineage spread to the eastern African mountains (the African lineage), where it was further subdivided in two phylogeographical groups. These two groups may descend from populations isolated on each side of the Great Rift Valley during a previous interglacial period (Assefa *et al*. 2007). According to the cpDNA data, the afro-alpine populations thus belong to two main phylogeographical lineages, of which one is subdivided. In the northern part of the distribution range, very little nucleotide variation was found, whereas there was variation, but no phylogeographical structure, in central Europe. There was, however, not enough resolution in the cpDNA and ITS data to infer where the glacial refugia for plants growing in Europe and the North Atlantic region were located (Kirchner 2002; Koch *et al*. 2006), and how the recolonization of these regions took place after the last glacial period.

The northern areas may have been recolonized from refugia in or around the European mountains, or from populations growing between the main mountain massifs and the edge of the northern European ice sheets (Fig. 1). The arctic-alpine *Ranunculus glacialis*, for example, colonized northern Europe from the eastern Alps, and genetic diversity is strongly reduced in the northern populations (Schönswetter *et al*. 2003). For such a widespread species as *A. alpina*, potential refugia in or close to mountains may be found from the Pyrenees over the Massif Central and the Alps to the Carpathians and the Balkans. Other species occurred north of the Alps during glaciations. Survival north of the classical southern European refugia was inferred, notably, for *Microtus oeconomus* (the root vole; Brunhoff *et al*. 2003). It has also been suggested for *Saxifraga paniculata* in Central Europe (Reisch *et al*. 2003) and *Cochlearia bavarica* in Germany (Koch 2002), and may have been the case for *A. alpina*. A few isolated populations of *A. alpina* occur today in Germany, north of the Alps. They could be relicts of this hypothetical ice age distribution, and may thus provide information about the genetic composition of past populations at the edge of the north European ice sheets. Such relict populations have notably been identified in *Cochlearia bavarica* (Koch 2002).

Here we used amplified fragment length polymorphism (AFLP) analysis (Vos *et al*. 1995), and an extensive sampling design to compare the genetic structure of *A. alpina* among three different parts of its range: the formerly glaciated areas surrounding the North Atlantic, the European mountain

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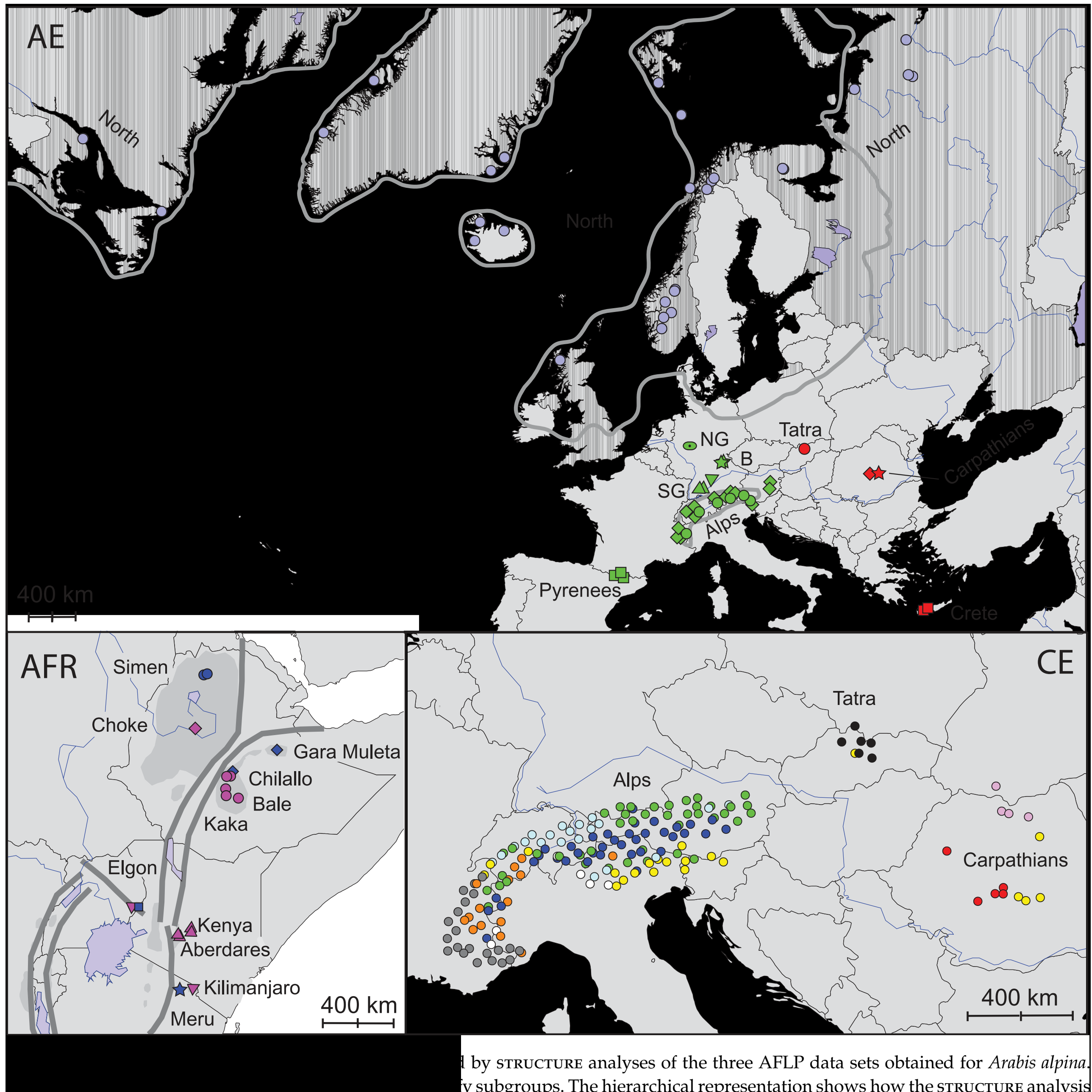

d by STRUCTURE analyses of the three AFLP data sets obtained for *Arabis alpina*. y subgroups. The hierarchical representation shows how the STRUCTURE analysis was carried out, but the distinctness between different groups should be evaluated based on the PCO plots on Fig. 2. AE refers to the amphi-Atlantic and European data set, CE to the central European data set, and AFR to the African data set. On the European map, NG designates the northernmost population in Germany, B the population from Bavaria in southeastern Germany, and SG the two sampling sites in southern Germany. The dark grey line indicates the approximate extent of the main ice sheets during the last glacial maximum (20 000 years BP in the Alps, Schönswetter *et al*. 2005 and references therein, and 20 000–15 000 years BP in the north, CAFF 2001; Svendsen *et al*. 2004). On the African map, the dark grey lines indicate the Great Rift Valley and the grey shaded areas represent highlands (rough contour of areas above 2000 m).

massifs (with emphasis on the Alps), and 11 high mountain regions of eastern Africa. Based on the climatic and glacial history of these areas, we assume that the populations in the three areas are at different stages of the range contraction/expansion cycle. In the North Atlantic region, the range must have expanded considerably after the last glaciation. In the central European mountains, the range shifts were probably mainly altitudinal and the present-day distribution is likely to be somewhat larger than during the ice age, when large glaciers covered most of the Alps and the tops of other mountain massifs. However, it is possible that the species was more widespread north of the Alps

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during glacial periods. In eastern Africa, the range of *A. alpina* is restricted to isolated mountain tops that represent the relict distribution (refugia) of a previously more extended vegetation zone.

The main objective of this study was to compare the genetic structure resulting from these contrasted range dynamics in different parts of the distribution area of *A. alpina* and to test the following predictions: In the North Atlantic region, reduced genetic diversity resulting from rapid postglacial recolonization and possibly several lineages descending from different refugia may be expected (Taberlet *et al*. 1998; Hewitt 1999, 2004). In the European mountains, several lineages descending from different refugia around the Alps, the Carpathians and the Pyrenees may be expected. Moreover, short-distance altitudinal migrations are likely to decrease the impact of founder effects, resulting in high within-population diversity. In Africa, it is likely that habitat fragmentation resulted in a high degree of genetic differentiation among populations from different mountain massifs, a pattern which would correspond to the expectation for separate refugia. Moreover, additional objectives were: (i) to examine whether multilocus AFLP data allow to identify the location of the refugia, from where *A. alpina* recolonized the northern part of its distribution range, and (ii) to investigate whether a genome-wide genetic analysis confirms the phylogeographical pattern obtained from the cpDNA analysis in Africa (Koch *et al*. 2006).

Materials and methods

Sampling and genetic analysis

For this study, three different AFLP data sets were combined. All three were produced within larger projects, addressing plant migration in a changing climate in the Arctic (amphi-Atlantic and European data set, AE), biodiversity in the Alps (IntraBioDiv; central European data set, CE) and comparative phylogeography of African mountain plants (African data set, AFR). For the AE data set, samples were collected throughout the northern amphi-Atlantic part of the range of the species (these will be referred to as northern populations) and further south in Europe (Fig. 1, Appendix II). As far as possible, 11 individuals were collected at 25 m distance along a straight line within each site. From some sites, only one or a few individuals were available (Appendix II). The material for the CE data set was collected throughout the Alps, the Tatra and the Carpathians according to a grid design (Fig. 1). The area was divided into squares of 25 × 25 km, and when the species was present, three individuals were collected in every other square at a distance of 10 m from each other. The samples for the AFR data set were collected in 11 different mountain massifs in Ethiopia and tropical East Africa (Fig. 1). In each mountain massif, plants were collected at three different localities separated by more than 1 km whenever possible (Appendix II). A population sample consisted of five to 21 individuals, collected at intervals of 10 m to a few hundred metres. In order to be able to use both the AE and CE data sets to investigate the origin of the northern populations, the two data sets were linked. Nineteen individuals from the northern populations were rerun and scored with the primers of the CE data set and 21 individuals from the CE data set were rerun and incorporated into the AE data set (Table 1). The AFLP data sets were produced and scored independently in two different laboratories (AE and AFR in Oslo by D.E., CE in Grenoble by M.G.).

Fresh plant material was collected in the field between 1998 and 2004, and dried on silica gel. Genomic DNA was extracted using the DNeasy plant extraction kit (QIAGEN) according to the manufacturer's instructions. The quality of the extracted DNA was checked on 1.5% agarose gels. After preliminary tests, three primer combinations which resulted in clear bands with sufficient variability were chosen: VIC-*Eco*RI-AAG/*Mse*I-CA, NED-*Eco*RI-AGC/*Mse*I-CT and 6FAM-*Eco*RI-AGT/*Mse*I-CAA for the AE and AFR data sets; 6FAM-*Eco*RI-AAT/*Mse*I-CAC, 6FAM-*Eco*RI-AGC/*Mse*I-CAC, 6FAM-*Eco*RI-ATC/*Mse*I-CAC for the CE data set. For the AE and AFR data sets the AFLP procedure followed Gaudeul *et al*. (2000) with a few modifications (Schönswetter *et al*. 2006). Selective polymerase chain reaction (PCR) products (2 μL 6-FAM, 2 μL VIC and 4 μL NED labelled products) were mixed and blended with 11.7 μL HiDi formamide and 0.3 μL GENESCAN ROX

Table 1 Overview of the three AFLP data sets obtained for *Arabis alpina*. CE refers to the central European data set, AE to the amphi-Atlantic and European data set, and AFR to the African data set. For each data set the number of sampling localities, individuals, polymorphic markers and number of replicates used to estimate reproducibility (including repeated DNA extraction/using the same DNA extraction) are given. For the CE data set, the number of localities does not include the samples from the northern regions

Data set	Localities	Individuals	Polymorphic markers	Replicates	Reproducibility
AE	64	402 (total)			
First scoring		305 (232 northern region)	242	29/18	> 99%
Second scoring		208 (37 northern region; 21 CE data set)	254	10/22	> 99%
CE	150	476 (19 AE data set from northern region)	151	39/0	> 98%
AFR	22	224	254	30/4	> 99%

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500 size standard. Electrophoresis was carried out on an ABI PRISM 3100 capillary sequencer (Applied Biosystems). For the CE data set, digestion of genomic DNA was performed for 2 h in a 20 μL mix using 2 U of *Mse*I and 5 U of *Eco*RI (New England Biolabs). Following this, double-stranded adaptors were ligated to DNA in a 40 μL volume for 2 h using 1 U of T4 DNA Ligase (New England Biolabs). Digested–ligated products were diluted 1:10 and preselective PCR amplification was carried out in a 25 μL volume containing 1.5 mM $MgCl_2$, 200 μM of each dNTP, 1.25 μM of each primer (sequences and PCR program as in Gaudeul *et al*. 2000), and 0.5 U of Ampli*Taq* DNA polymerase (Applied Biosystems). After a 1:20 dilution of preselective PCR products, selective PCR (program as in Gaudeul *et al*. 2000) was carried out in a 25 μL volume containing 2.5 mM $MgCl_2$, 200 μM of each dNTP, 1.25 μM of each primer, and 1 U of Ampli*Taq* Gold DNA polymerase (Applied Biosystems). PCR products were purified using spin-columns and diluted (1:10). One microlitre of the diluted products was mixed with 10 μL of HiDi formamide and 0.05 μL GENESCAN ROX 500 size standard (Applied Biosystems), and electrophoresed on an ABI PRISM 3100 Genetic Analyser capillary sequencer (Applied Biosystems). PCR products from each primer combination were run separately.

Raw data were collected and sized using the ABI PRISM GENESCAN 3.7 software (Applied Biosystems). The AFLP profiles were scored using the software GENOGRAPHER 1.6 (available at http://hordeum.oscs.montana.edu/genographer/) and coded as presence (1) or absence (0) of markers. Only variable markers in the size range 75–500 bp were scored (50–500 for the CE data set). Replicates were included in all data sets. Based on these controls, reproducibility was estimated as the average proportion of correctly replicated bands (Table 1; Bonin *et al*. 2004). Markers with particularly low reproducibility were excluded; markers with a proportion of presences or absences lower or similar to the error rate were checked carefully and only used if they were unambiguous.

In order to take into account all genetic variation present in the northern samples without excluding markers which may be too close to other markers or ambiguous in other regions, the AE data set was scored in two steps. First, all the northern individuals were scored together with a subset of the southern samples. Second, a subset of the northern individuals including all the identified different multilocus genotypes were scored together with all southern individuals, resulting in a data set with more markers (Table 1). In the data analysis, the second scoring was used except for the estimation of genetic diversity in the northern populations.

Data analysis

Within each of the three data sets, the genetic structure of populations was investigated using several clustering approaches detailed below. The distribution of genetic diversity was quantified by estimates of variation at different hierarchical levels: within localities, between localities and between regions. In order to determine the origin of the northern populations, the most similar individuals in other regions were identified from clustering results, the distribution of individual markers was examined, and an assignment test was performed.

The genetic relationship among individuals was represented graphically by principal coordinate analyses (PCO) and further investigated with neighbour-joining analyses. We used the software NTSYS (Rohlf 1990) to calculate matrices of pairwise DICE distances (equivalent to Nei & Li 1979; that only takes into account the sharing of present bands) and principal coordinates of all individuals along three axes. The software TREECON (Van de Peer & De Wachter 1994) was used to construct a neighbour-joining (NJ) tree from pairwise distances estimated after Nei & Li (1979). Support for resulting groups was calculated from 1000 bootstrap replicates.

As an alternative approach, we identified genetically homogeneous groups by genetic mixture analysis. For each data set, the appropriate number of groups (K) and the group each individual was most likely to belong to were estimated using both the software STRUCTURE 2.1 (Pritchard *et al*. 2000) and BAPS 3.2 (Corander *et al*. 2006), as recommended by Latch *et al*. (2006). STRUCTURE performs model-based clustering based on a Bayesian Markov chain Monte Carlo (MCMC) approach, and estimates the likelihood of the data for user-defined *K* values. We used a model with no admixture (as recommended for dominant data), and uncorrelated allele frequencies. For the AE and AFR data sets, we performed 10 runs for each *K* value ranging from 1 to 10 and 1 million MCMC replicates (200 000 additional replicates as burn-in). For the CE data set, we used K from 1 to 20 and increased the length of the MCMC chains to 1.5 million replicates and 2 million additional replicates as burn-in for K between seven and 14 in order to reach better convergence. All STRUCTURE runs were carried out at the Bioportal of the University of Oslo (www.bioportal.uio.no). Similarity among runs was calculated according to Rosenberg *et al*. (2002) using an R-script available from www.nhm.uio.no/ncb. This allowed assessing whether the clustering solutions obtained for each *K* value were consistent. In complex data sets, the genetic structure is often hierarchical, and several numbers of groups can thus be adequate to describe the data. Following recommendations from the STRUCTURE manual, we chose to build our interpretations on the *K* value with the highest likelihood, for which the 10 runs gave a consistent result and for which individuals were clearly assigned to nonempty groups. For the AE and AFR data sets, the groups determined by the first STRUCTURE analysis were subsequently analysed separately, in order to identify subgroups (see

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Rosenberg *et al*. 2002). BAPS 3.2 treats *K* as a variable to estimate and returns one optimal partitioning as well as the probability of the corresponding *K* value and other possible *K* values.

Genetic diversity within local samples and within regions was estimated as the average number of pairwise differences between individuals (Kosman 2003) using the R-script AFLPDAT (Ehrich 2006). The level of differentiation among local populations and among regions was estimated in an analysis of molecular variance (AMOVA) using the software ARLEQUIN 3.01 (Excoffier *et al*. 2005). The number of pairwise differences between individuals was used as genetic distance.

The most likely origin of the northern populations was identified by an assignment test performed with the software AFLPOP1.1 (Duchesne & Bernatchez 2002). All groups identified by the methods described above [e.g. PCO, NJ trees, STRUCTURE and BAPS], were considered as potential source populations. A fixed frequency of 0.01 was assumed for markers that were not observed in the source population. In addition, the distribution of individual AFLP markers was examined, in order to identify the populations with which the northern individuals shared most markers.

Results

Amphi-Atlantic and European data set

In Europe and the amphi-Atlantic region, genetic variability showed a strong geographical structuring. On the PCO plot, the individuals from most geographical regions formed distinct clusters (Fig. 2). The northern populations were clearly different from all the others and the populations from Germany formed three distinct groups. Individuals from the Alps and the northernmost sample from Germany were closest to the northern populations. The NJ-tree confirmed the strong regional structure in this data set, but whereas many local samples had high bootstrap support, there was little support for grouping of populations at a higher level (Fig. 3). The northern individuals clustered along with the population from northern Germany, but with low bootstrap support (57%).

The results of the genetic mixture analyses confirmed the clear regional structuring of the data. All STRUCTURE runs performed with $K = 3$ showed consistent results. The three groups were: the north, the east (Carpathians, Tatra and Crete), and the Alps, Pyrenees and Germany (Fig. 1). Higher values of *K* had higher likelihoods, but the clustering results differed among runs and were consequently not considered (Appendix III). Further analysis of the group comprising the Alps, Germany and the Pyrenees resulted in the distinction of seven groups, four in Germany, one in the Pyrenees and two in the Alps. Interestingly, the division in the Alps followed a latitudinal line. The eastern group was further subdivided into four groups corresponding to the four local samples. In total, STRUCTURE thus delineated 12 distinct groups (Fig. 1). BAPS identified 11 regional groups (probability = 1), which were in accordance with the result from STRUCTURE, except that the two subgroups in the Alps were considered one group. Both STRUCTURE and BAPS attributed all individuals sampled in one locality to the same cluster.

Genetic diversity was extremely low in the northern area, with a regional estimate of 0.0014 (232 individuals from 27 localities; Table 2). The estimated mean diversity was thus lower than the error rate. In total, 17 different genotypes were found and the most common genotype occurred in 197 individuals. The diversity was higher in all other regions, except in the small populations from Germany (Table 2). There was clear differentiation between the Alps and the Carpathians (including the Tatra; accounting for 30.6% of the total genetic variance) but even more genetic variation was found among local populations within regions (45.1% of the total genetic variance on average, Table 3). Local differentiation accounted for > 60% of the genetic variance within both regions (Table 3).

In an assignment test with 11 potential source populations corresponding to the groups identified by STRUCTURE, all northern individuals were clearly assigned to the group from the central Alps (green circles on Figs 1 and 2, log-likelihood difference > 7). Grouping the source populations into three larger regions, Germany, the Alps, and the Tatra and Carpathians, did not change this result: all northern individuals were clearly assigned to the Alps (log-likelihood difference > 4). The distribution of individual AFLP markers confirmed both the distinctness of the northern populations and their relatedness to the plants from the Alps and Germany. There were seven private markers in the north (of which two were fixed) and three fixed markers with only one or two occurrences in the rest of the data set. Seventy-nine of the 95 markers occurring in the northern populations were found in the Alps, 78 in Germany (all populations together) and fewer elsewhere. Among the German populations, the northern populations shared most markers with northern Germany (65 shared markers).

Central European data set

The geographical pattern of genetic variability was less clear for the CE data set than for the AE data set, but there was nevertheless a genetic structure. On the PCO plot, the first two axes mainly separated several large regional groups in the Alps (Fig. 2). The Tatra, the Carpathians and the north overlapped with different alpine groups. However, the third axis clearly separated the northern individuals from the rest (not shown). On the NJ-tree (Fig. 3), the three individuals sampled from one square usually formed a group with > 50% bootstrap support, but

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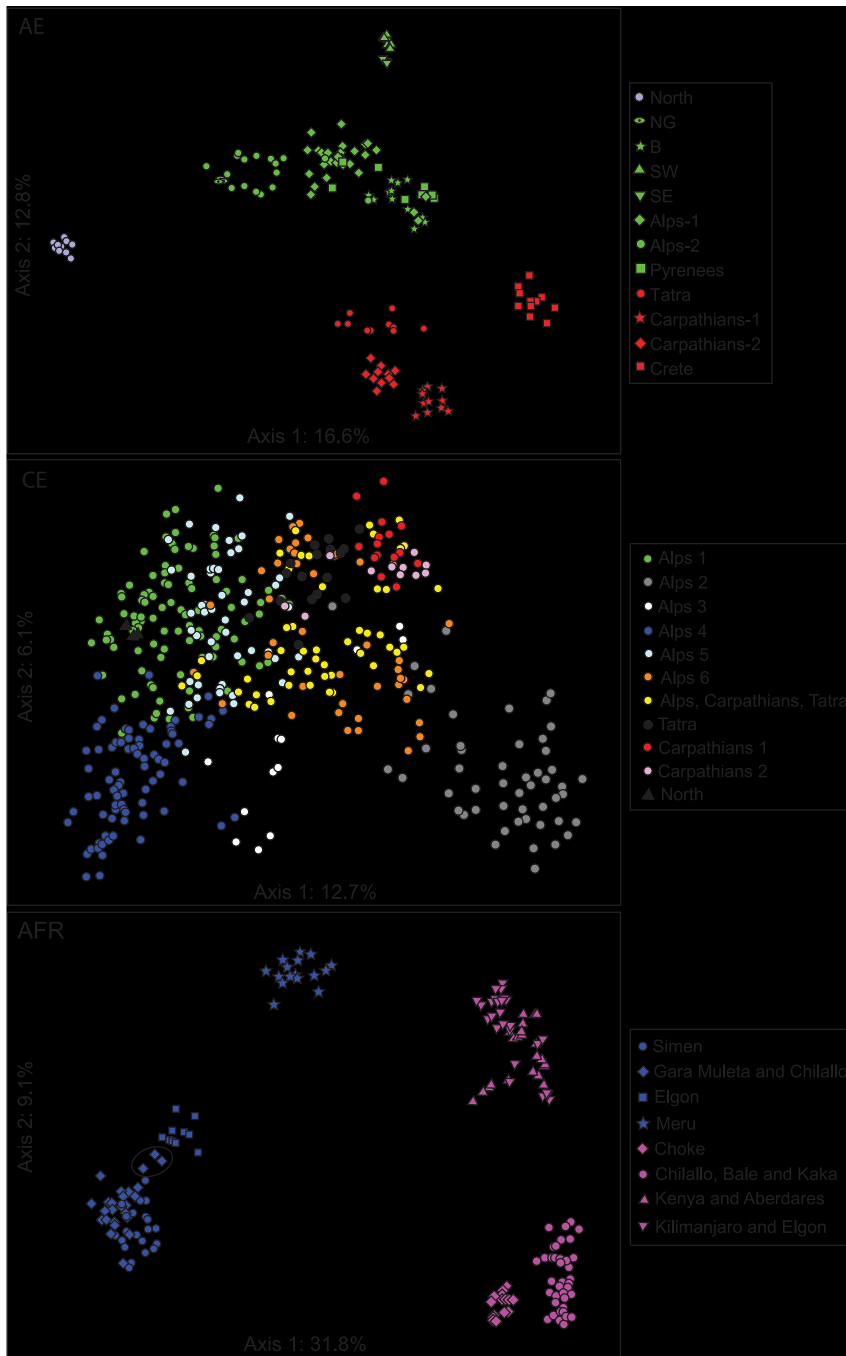

Fig. 2 Plots of the first two axes from principal coordinate analyses (PCO) of the three AFLP data sets obtained for *Arabis alpina*. The percentage of variation explained by each axis is indicated. Colours identify main genetic groups and shapes of symbols identify subgroups as determined by STRUCTURE, identical to those in Fig. 1. AE refers to the amphi-Atlantic and European data set, CE to the central European data set, and AFR to the African data set. For the AE data set, NG designates the northernmost population in Germany, B the population from Bavaria in southeastern Germany, and SW the western and SE the eastern of the two sampling sites in southern Germany (SG), respectively (see Fig. 1). For the AFR data set, the circle highlights the three individuals from Chilallo, which belong to the same genetic group as the individuals from Gara Muleta.

there was little support for larger groups (Fig. 3). The individuals from the north were mostly grouped with individuals from the northern Alps, however, without bootstrap support.

The genetic mixture analyses revealed a complex structure with several groups, some of which had disjunctive geographical distributions. With STRUCTURE, we obtained consistent results among runs for $K = 2$ and $K = 11$ (Fig. 1; Appendix III; runs with $K = 10$ included an empty group and were thus not considered). For $K = 2$, the southwestern Alps and the southern edge of the entire Alps formed a group together with the Tatra and Carpathians, whereas the northern populations grouped with the rest of the Alps. The northern individuals were assigned to a distinct cluster for $K \geq 6$. For lower values of K, they were assigned to a quite large cluster in the central Alps. BAPS suggested 12 genetic groups (probability = 1), which were in agreement with the 11 STRUCTURE groups except that the group from the southwestern Alps (grey in Fig. 1) was subdivided into two, and the individuals from the southeastern Carpathians

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Fig. 3 Neighbour-joining trees of individuals of *Arabis alpina* based on Nei & Li's (1979) distances between individual AFLP profiles. Trees were mid-point rooted. Bootstrap values above 50% (estimated from 1000 replicates) are indicated. To simplify presentation, bootstrap support for groups of two to three individuals were omitted. AE refers to the amphi-Atlantic and European data set, CE to the central European data set, and AFR to the African data set. For the AE data set, NG designates the northernmost population in Germany, B the population from Bavaria in southeastern Germany, and SW the western and SE the eastern of the two sampling sites in southern Germany (SG), respectively (see Fig. 1). For the CE data set, the star indicates a single individual from the Alps which clustered within a group from the Carpathians.

(yellow) belonged to the same group as the Tatra, instead of being attributed to a group occurring in the southeastern Alps. In most cases, all three individuals sampled in a square were assigned to the same cluster (in 125 of 151 squares for STRUCTURE and 133 of 151 for BAPS), indicating that despite a complex spatial distribution of genetic groups, there was little mixture at the local level.

Genetic diversity ranged from 0 to 0.150 within squares, and was on average 0.055 (SD = 0.033) in the Alps and 0.063 (SD = 0.030) in the Tatra and Carpathians (Table 2). Diversity was relatively low in the southern Carpathians and in some parts of the central Alps, but seemed otherwise randomly distributed. Among the 19 individuals from the north, the genetic diversity was only 0.006, confirming the low diversity observed in the AE data set. The AMOVA showed high levels of genetic differentiation among local samples within geographical regions: 66.5% of genetic variation was found between localities in the Alps and 66.7% in the Tatra and Carpathians (Table 3). There was also high differentiation among local samples within all groups identified by the genetic mixture analysis (ranging from 29.7% in the Tatra to 92% in a small disjunct group in the Alps — in white on Fig. 1; Table 3).

As for the AE data set, we carried out an assignment test to address the origin of the northern populations. Among 10 potential source regions (the data set was divided according to the results of STRUCTURE for $K = 11$), all northern individuals were clearly assigned to the northern Alps (green group in Figs 1 and 2, log-likelihood difference > 3). Most of the 47 markers found in the north were widespread in the rest of the data set. All of them were found in the Alps and all but one in the Tatra and Carpathians. The fact that the northern populations appeared less distinct than in the AE data set may be due to different scoring procedures:

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Region	Central European data set (CE)		Amphi-Atlantic and European data set (AE)	
	Within locality	Within region	Within locality	Within region
North		0.006	0.001 (0.002)	0.001
Alps	0.055 (0.033)	0.165	0.053 (0.037)	0.115
Germany			0.009 (0.014)	0.124
NG and SG			0.001 (0.004)	0.099
B			0.024 (0.017)	0.046
Carpathians	0.063 (0.030)	0.183	0.064 (0.022)	0.170
Pyrenees			0.039	0.057
Crete			0.067	0.103

Region	African data set (AFR)	
	Within locality	Within region
Simen	0.081 (0.026)	0.102
Choke	0.032	0.032
Gara Muleta	0.066 (0.012)	0.067
Southern Ethiopia*	0.035 (0.016)	0.081
Elgon	0.045 (0.017)	0.146
Kenya	0.030 (0.028)	0.088
Kilimanjaro	0.049	0.049
Meru	0.060	0.060

*The three divergent individuals from Chilallo were excluded. When including them, the average diversity was 0.051 (SD = 0.052) and the regional diversity 0.104 (populations from Chilallo, Bale and Kaka).

Table 2 Genetic diversity (average proportion of pairwise differences) in several regions of the distribution range of *Arabis alpina*. Averages (and standard deviations) were estimated within localities and within regions. NG designates the northernmost population in Germany, SG the two sampling sites in southern Germany, and B the population from Bavaria in south-eastern Germany. In the Pyrenees and Crete, only one population with more than two individuals was collected and a few additional single individuals were included for the regional estimate

the northern individuals were added to the CE data set after the main scoring was done and no new markers were added.

African data set

There was a clear genetic structure with several distinct genetic groups in the African *A. alpina*. On the PCO plot, the samples were divided into at least four clusters (Fig. 2). The first axis (31.8% of variation) separated Simen and Gara Muleta, in northern and eastern Ethiopia, from most other regions. The plants from Meru had an intermediate position. Interestingly, two populations from Elgon and three individuals from Chilallo were placed close to Simen and Gara Muleta on the PCO plot. The second axis (9% of variation) mostly separated the Ethiopian plants from those from the southern mountain massifs. The third axis (6.8% of variation, not shown) separated the plants from Choke from the rest. In the NJ analysis, mid-point rooting resulted in an unresolved division between Gara Muleta–Simen (and the related individuals from Elgon and Chilallo), Meru, and the other mountain regions (Fig. 3). All these three main groups had high bootstrap support. The strong genetic structure was also reflected in high bootstrap support for several regional groups, such as southern Ethiopia (Bale, Kaka and Chilallo), Choke, Kilimanjaro or the two distinct groups from Elgon.

The same clear pattern was identified by genetic mixture analyses. The results from STRUCTURE were consistent over 10 runs for $K = 2$ only (Appendix III), and suggested the division of the data set into the two main groups observed along the first axis of the PCO plot (with Meru in the same group as Gara Muleta and Simen). Separate analyses of these two main groups resulted in eight subgroups in total, four in Ethiopia and four in the southern mountain regions (Fig. 1). BAPS identified nine distinct clusters (probability = 1), identical to the STRUCTURE subgroups except that Kilimanjaro was separated from Elgon. Except for the three divergent individuals from Chilallo, which were assigned to the group from Gara Muleta, all individuals sampled in one locality were attributed to the same cluster.

The level of genetic diversity within the African populations ranged from 0.002 to 0.111 and was in general similar to that observed in central Europe (both AE and CE data sets; Table 3). Genetic diversity within local samples was highest in one population from Chilallo in eastern Ethiopia, where plants from the two main groups were observed. Genetic diversity was also very high in the two divergent mountain regions in Ethiopia, Simen and Gara Muleta and the lowest diversity estimates were observed on Mount

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Table 3 Results of AMOVAs performed on the three AFLP data sets for *Arabis alpina*. CE refers to the central European data set, AE to the amphi-Atlantic and European data set, and AFR to the African data set. Genetic differentiation was significant at all levels ($P < 0.001$). The African data were divided into two major groups as suggested by the STRUCTURE analysis: group 1 included Simen, Gara Muleta, Meru and part of the individuals from Elgon while group 2 included the remaining localities. The three divergent individuals from Chilallo were excluded from this analysis

	CE	AE
Between Alps and Tatra-Carpathians	13.3%	30.6%
Among localities within mountain regions	57.7%	45.1%
Within local samples	29.0%	24.3%
Among localities in the Alps	66.5%	60.3%
Within local samples	33.5%	39.7%
Among localities in the Tatra-Carpathians	66.7%	71.0%
Within local samples	33.3%	29%
Among 10 genetic regions*	29.0%	
Among squares within genetic regions	40.3%	
Within squares	30.7%	
	AFR	
Between two major groups	40.7%	
Among localities within groups	38.4%	
Within local samples	20.9%	
Among localities in group 1	68.9%	
Within local samples	31.1%	
Among localities in group 2	56.3%	
Within local samples	43.7%	

*Results from STRUCTURE 2.1 for $K = 11$, where the 11th group comprised the northern individuals. A corresponding AMOVA could not be calculated for the AE and the AFR data sets, because several STRUCTURE groups were composed of only one local population.

Kenya and in Choke (Table 2). The regional diversity was highest on Mount Elgon, where populations belonging to both main groups occurred. As in the two other data sets, genetic differentiation was very high among local samples. The AMOVA showed that 68.9% of genetic variation was found among local samples in one of the two major groups (Gara Muleta, Simen, Meru and Elgon; in blue on Fig. 1) and 56.3% in the other (Table 3).

Discussion

In accordance with our expectations, the genetic structure observed in *Arabis alpina* differed among the three geographical regions that we investigated. In the formerly glaciated areas of northern Europe, Greenland, and eastern North America, there was almost no genetic diversity and most sampled individuals were identical. In the Alps and the Carpathians, on the contrary, we observed a mosaic of differentiated patches and considerable overall genetic diversity, both within and between local populations and genetically defined regions. Genetic variation was also observed in the Pyrenees and Crete. This structure is consistent with recolonization of the European mountains from several refugia around and possibly in the Alps (Stehlik 2003; Schönswetter *et al*. 2005), the Carpathians and the Tatra. In the Pyrenees and the Mediterranean region, which were characterized by a milder climate, populations may have persisted *in situ*. In the African high mountains, the phylogeographical groups were highly differentiated and geographically localized. However, as in the mountains of central Europe, the genetic structure was not strictly consistent with geography, as two divergent groups were observed on Mount Elgon, and a few individuals from Chilallo belonged to a divergent group found in Gara Muleta. Despite their long-term isolation, all African populations harboured levels of genetic diversity that were similar to those in the populations of the European mountain massifs.

Lack of diversity in the north

Our results showed that the northern part of the distribution area has been colonized mainly by a single genotype, observed in 85% of the individuals sampled in this vast region. The other 16 genotypes observed differed from the main type for at most three markers, resulting in average gene diversity that was well below the error rate. This low variability observed in the formerly glaciated areas in the north was in striking contrast to the high diversity found in the Alps, which also were extensively glaciated. While recolonization from several refugia is likely for the Alps, our data clearly indicate that all northern *A. alpina* descend from a single refugial population.

The difference between the patterns that we observed in the North Atlantic region and the Alps is consistent with the predictions from Hewitt's (1996, 1999) model of postglacial recolonization, stating that latitudinal colonization over large areas leads to a greater loss of genetic diversity than altitudinal range shifts. Lower genetic diversity in northern populations after postglacial recolonization has been observed in many species (Hewitt 2004), but usually not in such an extreme form and over such a vast area as in *A. alpina*. A similar pattern has, however, been observed based on AFLP in another arctic-alpine species, *Ranunculus pygmaeus* (Schönswetter *et al*. 2006). This species has virtually no genetic diversity throughout Scandinavia and Greenland, which were recolonized after the last glaciation from refugia in the Ural Mountains. A pronounced, although not as extreme, reduction in genetic diversity in formerly glaciated areas of the North Atlantic has also been observed in *Ranunculus glacialis* (Schönswetter *et al*. 2003). Schönswetter *et al*. (2006) interpreted both patterns as resulting from strong bottlenecks during early phases of colonization, and related the extreme case of *R. pygmaeus* to its autogamy.

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Arabis alpina, *R. pygmaeus* and *R. glacialis* are pioneer species typically occurring in snowbeds and in glacier forelands. They need open habitats to establish, and succession studies in glacier forelands showed that *A. alpina* grows best in the first 10–20 years after deglaciation (Whittaker 1993). This requirement of open habitat for establishment, and the ability of these plants to grow in the early successional stages right after the retreat of the ice sheets may have resulted in rapid leading-edge colonization. In addition, because a closed vegetation cover appeared rather rapidly after the retreat of the glaciers, pioneer populations 'running' northwards were quickly isolated from their conspecifics growing further south. *A. alpina* recolonized the large areas opened after deglaciation rather rapidly, as a macrofossil of *A. alpina* dated to 10.4 thousand years ago has been found in eastern Greenland (Bennike *et al*. 1999).

However, although leading edge colonization may be a plausible explanation for the reduced genetic diversity observed in the North Atlantic area, it seems unlikely that such a large area was colonized nearly by a single genotype. In this case, all successful colonizers should indeed by chance have originated from the same local population. Our data from central Europe show that the populations in this region are genetically highly structured and variable, and it is likely that this was also the case during the ice ages. The genetic variability of today's populations in the Alps and the high differentiation among the small populations in Germany clearly suggest that both the ice age populations around the Alps and a hypothetical periglacial population were genetically diverse. As the edge of the ice sheets extended over long distances, it is difficult to imagine that *A. alpina* spread northwards to colonize its large distribution range from a single local population. We thus suggest an alternative hypothesis for the lack of genetic diversity in the north: strong selection for colonization ability. Strong selective pressure for a particular trait may cause a selective sweep, decreasing genetic diversity throughout the genome. In *A. alpina*, differentiation among local populations within regions was very high for all three data sets, indicating very little dispersal once populations have established. It seems thus possible that a genotype with better dispersal or colonization abilities would be advantageous in such a species, especially when large new areas opened up after deglaciation (Baker 1955; Dynesius & Jansson 2000).

Origin of the northern populations

Our analyses did not provide a unanimous answer to the question of the origin of the northern populations. The NJ tree showed that the northern plants shared most bands with individuals from northern Germany, whereas the PCO plot, based on the same genetic distance, indicated that some individuals from the eastern Alps belonging to the northern subgroup were about equally closely related to the northern ones as those from northern Germany (Figs 2 and 3). All the German plants, except the Bavarian ones, were rather closely related to those from the Alps on both trees. The assignment test, however, very clearly assigned all northern individuals to the Alps, showing that the marker frequencies in the north were most similar to those in the Alps. Last, the distribution of individual markers was equivocal; similar numbers of northern markers were found in Germany and the Alps.

Both the potential relict populations in Germany, some of which are very small today, and the northern populations experienced strong genetic drift during the Holocene leading to the loss of nearly all genetic diversity. In these populations, marker frequencies may thus have changed considerably during the Holocene, and several markers have probably been lost. This, together with the large difference in genetic diversity among regions, may explain the discrepancy between the different statistical analyses. Assignment tests were primarily developed to identify the present source populations for particular migrant individuals (e.g. stock mixture analysis, Paetkau *et al*. 1995) rather than to identify the historical source for colonization. They may thus not always be fully adequate to determine the historical origin of a population. The assignment tests also gave contradicting results concerning the part of the Alps, to which the northern individuals were assigned. While for the AE data set they were assigned to the central Alps, they were assigned to the northern Alps in the CE data set (the assignment was highly significant in both cases). The regional extent of the groups was, however, not the same in the two data sets.

Considering these results, the most plausible scenario seems to postulate a periglacial population, which extended from the northern edge of the Alps to the margin of the northern European glaciers (Fig. 1). It is likely that this population was widespread along the glacial margins, as *A. alpina* is a glacier foreland plant, but restricted to locally humid habitats in the otherwise dry tundra covering the area north of the Alps, such as along rivers. Considering the high local differentiation that we observed in the geographically continuous alpine population, it is also likely that this periglacial population was subdivided into differentiated local genetic groups with little dispersal among them. The ancestors of the northern *A. alpina* may have lived in the northern part of this hypothetical population, and the small populations from Germany may descend from other local groups growing close to their present-day locations. Plants living at the southern edge of this hypothetical population may have colonized the northeastern Alps without losing much genetic diversity.

The East African mountains

The phylogeographical structure of *A. alpina* in the African mountains was highly fragmented, but the local populations

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harboured much genetic diversity, in agreement with expectations for isolated refugial populations. The observed high levels of differentiation among local populations also within genetically defined regions were in agreement with the structure observed in the Alps and the Carpathians. Assuming a hypothetical future range expansion of these isolated populations to a more or less continuous distribution as observed at present in the Alps might well lead to a patchy genetic structure, possibly with some disjunctive genetic groups, as observed in the latter mountain regions.

The cpDNA data of Koch *et al*. (2006) and Assefa *et al*. (2007) showed two very divergent lineages in Africa, the Asian lineage in Simen and Gara Muleta and the African lineage in the remaining localities. The distinct AFLP genotypes that we observed in Simen and Gara Muleta are in accordance with the cpDNA pattern. The high AFLP diversity observed in Simen is consistent with the fact that this was the only mountain region, where cpDNA haplotypes from both lineages were recorded. Contrary to the cpDNA data, however, the AFLPs suggest that individuals of the Asian lineage dispersed southwards and reached Elgon. There, they occurred together with plants from the African lineage belonging to the same STRUCTURE group as the population from Kilimanjaro (Fig. 1). Meanwhile, all analysed individuals form Elgon had identical cpDNA haplotypes (Assefa *et al*. 2007), indicating cpDNA introgression. The AFLP data showed also that some individuals related to the populations from Gara Muleta spread to Chilallo. Dispersal of the small, light and narrowly winged seeds by strong winds seems a plausible mechanism for long-distance dispersal among mountain massifs in Africa.

Overall, the AFLP data revealed much more differentiation among mountain massifs than the cpDNA data, which distinguished only two main phylogeographical lineages, of which one was subdivided. This may be explained by the relatively low mutation rate, and thus low resolution of the analysed cpDNA marker. Thus, Chilallo, Bale and Kaka in Ethiopia, and Kenya and Aberdares in tropical east Africa belonged to the same phylogeographical subdivision for cpDNA, but there were clear differences between the two geographical regions for the AFLP profiles, possibly reflecting restricted dispersal over lowlands in recent times. Similarly, whereas Elgon, Kilimanjaro, Meru and Choke formed the other group within the African lineage for cpDNA data, the AFLP data placed Meru and Choke in clearly divergent positions, in accordance with their remote geographical locations within the group. However, contrary to the cpDNA data, the AFLP data placed Kenya and Aberdares rather close to Elgon and Kilimanjaro. This may indicate some gene flow across the Great Rift Valley, not reflected in the small sample sizes analysed for cpDNA.

In general, the genetic structure observed in Africa highlights a paradox present throughout this study of *A. alpina*. On the one hand, high differentiation among geographically close populations (e.g. between Meru and Kilimanjaro, between the two populations on Elgon, or between divergent lineages in the Alps) indicates that once established in a region, *A. alpina* disperses little. On the other hand, some genetically defined groups had disjunctive geographical distributions both in Africa and in the central European mountains, suggesting occasional events of long-distance dispersal. Furthermore, the rapid colonization of the North Atlantic region from one source population is difficult to imagine in a species with poor dispersal abilities. Altogether, these results might indicate genetic variation for traits relevant to dispersal and colonization ability in *A. alpina*, a hypothesis which should be tested further.

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Appendix I

IntraBioDiv Consortium

The IntraBiodiv Consortium is composed of members of the IntraBioDiv project, as well as additional scientists, botanical experts, and technical assistants who participated to this project in relation with the official contractors and subcontractors.
The project has been financially supported by the Commission of the European Union Sustainable Development, Global Change and Ecosystems Programme (FP6).
Acronym: IntraBioDiv
Title: Tracking surrogates for intraspecific biodiversity: towards efficient selection strategies for the conservation of natural genetic resources using comparative mapping and modelling approaches.
Reference: GOCE-CT-2003-505376
Duration: 1st January 2004–31st December 2006
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Appendix II

Table of populations of *Arabis alpina* included in the AFLP analysis (amphi-Atlantic–European and African data sets only; the Central European data set was not included because it was collected according to a grid design). For each population, the country, locality, collectors, altitude (Alt) in metres above sea level (m a.s.l.), geographical coordinates, number of individuals (*n*) and genetic diversity are given (D, estimated as average number of pairwise differences)

PopID	Country	Locality	Collector	Alt (m a.s.l.)	Coordinates (°)	*n*	D
African data set							
AFR 001–006	Ethiopia	Bale Mountains	AA, MKT	4380	6.8268N, 39.8190E	7	0.039
AFR 131	Ethiopia	Bale Mountains	AA, MKT	3610	7.0615N, 39.6528E	11	0.022
AFR 036–037	Ethiopia	Mount Kaka	AA, MKT	4180	7.3650N, 39.1576E	10	0.046
AFR 038	Ethiopia	Mount Kaka	AA, MKT	4010	7.3713N, 39.1709E	9	0.052
AFR 307	Ethiopia	Mount Chilallo	AA, MKT	3700	7.9283N, 39.2179E	5	0.017
AFR 300	Ethiopia	Mount Chilallo	AA, MKT	3600	7.8292N, 39.3572E	5	0.019
AFR 347	Ethiopia	Mount Chilallo	AA, MKT	3620	6.9395N, 39.2180E	11	0.165*
AFR 259-262-263	Ethiopia	Mount Choke	AA, MKT	4100	10.4268N, 37.5059E	21	0.032
AFR 060	Ethiopia	Simien Mountains	AA, MKT	3760	13.2619N, 38.2010E	5	0.065
AFR 062	Ethiopia	Simien Mountains	AA, MKT	3900	13.2857N, 38.1426E	5	0.067
AFR 071	Ethiopia	Simien Mountains	AA, MKT	3220	13.2330N, 38.0388E	11	0.111
AFR-106–115	Ethiopia	Gara Muleta	AA, MKT	2680	9.2080N, 41.7983E	16	0.058
AFR 109	Ethiopia	Gara Muleta	AA, MKT	3020	9.2282N, 41.7934E	5	0.075
AFR 164–165	Kenya	Mount Kenya	AA, MKT	3900	0.1679S, 37.2510E	10	0.019
AFR 180	Kenya	Mount Kenya	AA, MKT	3740	0.0840S, 37.2863E	11	0.011
AFR 144	Kenya	Aberdares NationalPark	AA, MKT	3670	0.3339S, 36.6513E	5	0.002
AFR 145	Kenya	Aberdares NationalPark	AA, MKT	3730	0.3430S, 36.6532E	5	0.064
AFR 151	Kenya	Aberdares NationalPark	AA, MKT	3100	0.4545S, 36.7140 E	11	0.056
AFR 194–195	Kenya	Mount Elgon	AA, MKT	4070	1.1212N, 34.5973E	10	0.057
AFR 196	Kenya	Mount Elgon	AA, MKT	4050	1.1209N, 34.5862E	11	0.033
AFR 084-085-086	Tanzania	Mount Kilimanjaro	AA, MKT	4150	3.1190S, 37.4303E	21	0.049
AFR 095-096-097	Tanzania	Mount Meru	AA, MKT	3670	3.2209S, 36.7688E	19	0.060
Amphi-Atlantic and European data set							
AK-3173	Canada	Newfoundland, Northern Pen, Pistolet Bay (N**)	IGA, AKB	20	49.2042N, 66.1804W	11	0.002
AK-3172	Canada	Québec, Gaspé Pen, Sainte-Anne-des-Monts (N)	IGA, AKB	20	49.7020N, 57.9430W	11	0
AK-3030	Greenland	Tunu Province, Blosseville Coast, Cape Dalton (N)	OG		69.4600N, 24.1000W	8	0
AK-1111	Greenland	Greenland, God Haven (N)	RN	30	69.2333N, 53.5500W	5	0
AK-365	Greenland	Jameson Land, Constable Point, Hare River (N)	IS, LL	10	70.7118N, 22.6546W	11	0.003
AK-349	Greenland	Jameson Land, Constable Point, Primula River (N)	IS, LL	60	70.7463N, 22.6835W	9	0
AK-220	Greenland	West Greenland, Nuuk, Malenebukten (N)	PBE	70	64.1623N, 51.6696W	8	0.001
AK-857	Iceland	Akureyri (N)	IS, SK, LL	800	65.8149N, 17.9929W	11	0.001
AK-812	Iceland	Nordwestfjorde, Holmavik (N)	IS, SK, LL	290	65.8045N, 22.2679W	9	0
AK-842	Iceland	Reykjavik, Akrafjell, SE of Akrafjelle (N)	IS, SK, LL	150	64.4400N, 21.9782W	9	0
AK-713	Svalbard	Bjørnøya, Ymerdalen (N)	RS		74.3600N, 19.0100E	10	0.002
AK-3239	Svalbard	Wedel Jarlsberg Land, Hornsund (N)	MHJ, IS	50	77.0143N, 15.5810E	10	0
AK-425	Norway	Buskerud, Hemsedal, Bjørnstedåni (N)	PBE	1050	60.8699N, 8.6709E	11	0
AK-508	Norway	Hordaland, Finse (N)	MHJ, IS, GHJ	1400	60.6067N, 7.5317E	10	0.002
AK-426	Norway	Hordaland, Odda, SW of Ulevåvatnet (N)	PBE	1070	59.8452N, 7.0593E	8	0.002
SUP02-115	Norway	Nordland, Andøya (N)	IGA		69.2376N, 15.9322E	5	0.001
AK-449	Norway	Oppland, Lom, SE slope of Vardhø (N)	PBE	1325	61.6709N, 8.0447E	7	0
CB01-02	Norway	Sør Trøndelag, Oppdal, Drivdalen (N)	CB	850	62.3345N, 9.6235E	2	0.009
AK-474	Norway	Sør-Trøndelag, Oppdal, Vinstradalen (N)	PBE	1400	62.4372N, 9.7305E	9	0.002
SUP02-109	Norway	Troms, Kåfjord, Kåfjordfjellet (N)	KW	800	69.3500N, 21.0575E	5	0.002
N224	Norway	Troms, Målselv (N)	PS, AT		68.7800N, 19.0500E	11	0.001
AK-3518	Norway	Troms, Storfjord, Mount Lávkaslubbu (N)	PS, AT	900	69.2333N, 20.4333E	10	0
AK-4439	Russia	Komi Rep, Pripolyarnyy Ural, Yugyd-Va NationalPark (N)	IGA, AT, DE	380	65.3410N, 60.7120E	10	0
AK-4467	Russia	Komi Rep, Pripolyarnyy Ural, 40 km SE Inta (N)	IGA, AT	600	65.6707N, 60.6265E	9	0.001
AK-3203	Russia	Nenetskiy AO, Malozemel'skaya tundra, Nenetskaya Gryada (N)	IGA, AT	10	68.3237N, 53.2911E	11	0
AK-4500	Russia	Yamalo-Nenetskiy AO, Polar Ural, Mount Slantzevaya (N)	IGA, MVK	300	66.9220N, 65.7960E	9	0.002
SUP04-008	UK	Scotland, Skye, Cuillins (N)	CWM		57.6400N, 6.2000W	1	—
SUP02-723	Austria	Salzburg, Hohe Tauern, Glockner-Gruppe	PS	2300	47.1747N, 12.8556E	5	0.068
SUP02-721	Austria	Steiermark, Nordöstliche Kalkalpen, Rax	AT	1800	47.6917N, 15.6917E	5	0.069
AK-1042	Austria	Tirol, Zillertaler Alpen, Grafmartalm	PS, AT	2500	47.1750N, 11.5458E	9	0.013
AK-4515	Germany	Baden-Wüerttemberg, Schwäbische Alb	MK	900	48.2000N, 9.0167E	11	0.001
AK-4512	Germany	Baden-Wüerttemberg, Schwäbische Alb (SE)	MK	910	48.2000N, 8.9833E	11	0.002
AK-4513	Germany	Baden-Würtenberg, Königsbronn, Herwartstein (SW)	MK	550	48.7333N, 10.1167E	11	0.001
AK-4516	Germany	Bavaria, Pottenstein, Teufelshöhle (B)	MK	395	49.4522N, 11.2500E	10	0.012
AK-4517	Germany	Bavaria, Pottenstein, Klumpertal (B)	MK	415	49.4433N, 11.2568E	8	0.036
AK-4514	Germany	NRW, Hochsauerland, Bruchhausen, near Brilon (NG)	KM	710	51.3330N, 8.5000E	11	0.001

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Appendix II *Continued*

PopID	Country	Locality	Collector	Alt (m a.s.l.)	Coordinates (°)	*n*	D
AK-4210	Poland	Tatra Mountains, Pysznianska Przelecz pass	PS, MR	1430	49.1964N, 19.8544E	10	0.090
AK-398	Romania	Fagaras Mountains, Balea Lake	MP	2220	45.6456N, 24.7103E	11	0.050
AK-3035	Romania	Southern Carpathians, Muntii Bucegi	PS, OP	2250	45.4150N, 25.4711E	11	0.053
AK-1012	Spain	Central Pyrenees, Macizo La Maladeta	GMS, PS	2800	42.5936N, 0.6567E	10	0.039
AK-3122	Greece	Kreta	ACS		35.2800N, 24.0100E	11	0.067
SUP04-002	Greece	Kreta, Mount Idi	HB	1300	35.3000N, 24.0200E	2	0.065
IB-E23	Austria	Alps	CT	1648	47.6432N, 11.6738E	3	0.060
IB-F18	Germany	Bavaria, N Allgäuer Alps	MS	1590	47.4276N, 10.3263E	3	0.073
IB-F24	Austria	Alps	CT	1681	47.5135N, 12.1756E	3	0.042
IB-G34	Austria	Alps, Steiermark, Hochlantsch	TE, MVL	1010	47.3448N, 15.4024E	3	0.108
IB-H12	Switzerland	Alps, Luzern, Malters	PD, PK, PM	980	47.0239N, 8.2270E	3	0.092
IB-O07	France	Rhône-Alpes, Beaufort	TD	2200	45.6765N, 6.6518E	3	0.003
IB-Q09	Italy	Piemonte, Alps	DM	2510	45.2940N, 7.1495E	3	0.005
Osna-1	Russia	Murmanskaya Obl, Kola Pen, Khibiny Mountains	HH	1800	67.6500N, 33.5333E	1	—
Osna-2		Murmanskaya Obl, Kola Pen, 150 km S of Murmansk	HH	1700	67.7333N, 33.4500E	1	—
Osna-3	Spain	Pyrenées, Salardu, Baguergue	UP		42.7000N, 0.9167E	1	—
Osna-4	Spain	Pyrenées, Val d'Aran	UP	2100	42.6167N, 0.9833E	1	—
Osna-5	Spain	Pyrenées, Benasque	UP	1900	42.6000N, 0.5333E	1	—
Osna-6	France	Alps, Mount Pelvoux	HH	2200	44.9833N, 6.3500E	1	—
Osna-8	Switzerland	Alps, Sattelspitzen	BG Fribourg	1700	46.7333N, 7.2167E	1	—
Osna-9	Switzerland	Alps, Wildergalm	BG Fribourg	1825	46.2500N, 8.0167E	1	—
Osna-10	Switzerland	Alps, Valais, Furka	BG Basel	2400	46.6000N, 8.4333E	1	—
Osna-11	Austria	Alps, Vorarlberg, Kleinwalsertal	BG Münster		47.3167N, 11.1667E	1	—
Osna-12	Austria	Eastern Forealps, Schneeberg, Kalte Kuchel	HH		47.7667N, 15.8667E	1	—
Osna-13	Austria	Alps, Tirol, Paznaun	BG Berlin	1600	47.0167N, 10.2667E	1	—
Osna-14	Austria	Alps, Kärntner Alpen, Gasnitzerklamm	BG Graz	2100	46.7000N, 13.3333E	1	—
Osna-15	Italy	Alps, Julische Alpen, Sella Neva	UR	1600	46.3833N, 13.4167E	1	—
Osna-16	France	Alps, Col du Galibier	HH	2640	45.0500N, 6.1500E	1	—

*In this population genetic diversity was particularly high because of three individuals which are possible immigrants from Gara Muleta.

**N indicates population of the northern part of the distribution area, NG designates the northernmost population in Germany, B the population from Bavaria in southeastern Germany, and SW the western and SE the eastern of the two sampling sites in southern Germany.

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Appendix III

Summary of the analyses of the three AFLP data sets for *Arabis alpina* with the program STRUCTURE 2.1. AE refers to the amphi-Atlantic and European data set, CE to the central European data set, and AFR to the African data set. On the left, the likelihood of each number of groups (K) for each of 10 runs is plotted against the *K* values. On the right, the average similarity between runs is shown for each *K* value. Circles represent the mean of all pairwise comparisons among the 10 runs, whereas triangles indicate an interval of ± the standard deviation. Similarity among runs was calculated according to Rosenberg *et al*. (2002) using an R-script available from www.nhm.uio.no/ncb. The original formula was slightly modified. Instead of evaluating the similarity coefficient over all possible permutations of the columns of one of the matrices (that makes the calculations very time-consuming), matrices were first aligned column by column and the similarity coefficient was then calculated only once. According to Rosenberg *et al*. (2002), a similarity value above 0.85 corresponds to a generally similar population structure.

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