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ORIGINAL ARTICLE

The wide but disjunct range of the European mountain plant *Androsace lactea* L. (Primulaceae) reflects Late Pleistocene range fragmentation and post-glacial distributional stasis

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ABSTRACT

Aim Our aim was to reconstruct the spatio-temporal genetic diversification of *Androsace lactea*, a widely but disjunctly distributed European mountain plant, to test the hypothesis that its distribution is the result of vicariance, in the late Tertiary or during the Pleistocene, or alternatively of long-distance dispersal. We also addressed the phylogeographic history of the Alps, emphasizing the role of Pleistocene refugia at their northern margin.

Location The central and southern European mountain ranges.

Methods We gathered amplified fragment length polymorphism (AFLP) data and plastid DNA sequences from one to four individuals of each of 26 populations spanning the entire distribution area. AFLP data were analysed with Bayesian clustering approaches, neighbour-joining analysis and NeighbourNet. Plastid sequences were used to depict relationships among haplotypes in a statistical parsimony network, to test for population expansions, and to obtain age estimates in a Bayesian framework.

Results The AFLP data suggested that many populations were genetically strongly differentiated. The internal structure, however, was weak, and only two major groups of populations, from the north-western Alps and adjacent regions and from the easternmost Alps, were supported in the neighbour-joining analysis. One of the Bayesian clustering approaches differentiated three groups of populations: Northern Alps, easternmost Alps and the remaining distribution area. Eleven closely related plastid haplotypes were found, separated by maximally four mutational steps, resulting in a star-like parsimony network. None of several estimators suggested statistically significant population expansions. The diversification age was inferred to be (mean/median) 0.135/0.08 Ma (95% highest posterior density interval 0.364–0.006 Ma).

Main conclusions We found no evidence that long-distance dispersal shaped the disjunct distribution range; our data rather favoured a vicariance scenario. However, in contrast to the hypothesis that wide but disjunct distributions are old, we conclude that range fragmentation probably happened in the Late Pleistocene, perhaps during the last glaciation. In the Alps, most populations are at least close to formerly unglaciated areas. Our data support distributional stasis and suggest that important refugia were situated at the north-eastern, but also at the northern and north-western edges of the Alps, thereby strengthening the evidence for glacial refugia in this strongly glaciated region.

Keywords

Alps, *Androsace lactea,* European mountain ranges, long-distance dispersal, molecular dating, phylogeography, refugia, vicariance.

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INTRODUCTION

Quaternary climatic changes have had a major impact on the geographical distribution of many plant and animal species by triggering range shifts, expansions, contractions and fragmentations as well as population extirpations (Comes & Kadereit, 1998; Hewitt, 2000; Petit *et al.*, 2003). Cold-adapted species were widely distributed during glacial periods, but were restricted to high latitudes and, at lower latitudes, to high-elevation refugia during warmer intervals, whereas the reverse is true for temperate species (Stewart *et al.*, 2010). For instance, during glacials the arctic–alpine dwarf willow *Salix herbacea* was widely and continuously distributed in lowland Central Europe, while it became restricted to arctic and temperate-alpine habitats in the warm post-glacial (Alsos *et al.*, 2009). Similarly, in extensively glaciated mountain ranges such as the European Alps, species were forced into peripheral and possibly lowland refugia during cold periods, from where they (re)colonized deglaciated areas during warmer periods such as the post-glacial (Schönswetter *et al.*, 2005).

Although the Quaternary climatic oscillations are expected to translate into cyclical contractions and expansions of species ranges, some species were not able to expand their ranges in times of suitable climatic conditions (Stewart *et al.*, 2010); the biological causes for this failure, however, remain unknown. Strongly limited range expansion is evident, for instance, from a number of local and regional endemics in the European Alps, whose distribution ranges largely overlap with presumed glacial refugia (Aeschimann *et al.*, 2004; Tribsch, 2004) and from the as-yet incomplete range-filling of some European temperate tree species (Svenning & Skov, 2007). Other species, whose distributions in the European Alps are largely congruent with the locations of Pleistocene refugia, nevertheless have wide albeit disjunct distributions across the European mountains, for example *Artemisia eriantha* (Asteraceae) and *Androsace villosa* (Primulaceae: see Meusel *et al.*, 1978; Meusel & Jäger, 1992). Under the assumption that the 'degree of disjunction' is proportional to time (Ozenda, 2009), such distributions have often been interpreted as the result of Pleistocene range fragmentation of a wider and more continuous distribution formed in the climatically more benign Tertiary (Engler, 1903; Brockmann-Jerosch, 1926; Lüdi, 1927). However, the occurrence of climatically suitable phases in the Pleistocene renders more recent range fragmentation a plausible alternative. In the absence of fossil evidence, molecular genetic data may allow testing of the two alternative scenarios. Specifically, coalescence times of gene lineages from geographically isolated distributional areas will provide maximum ages for the range fragmentation. As coalescence times can be substantially older than the times of population separation, for example owing to large population sizes, this test will be conservative with respect to rejecting an early range fragmentation. Genetic data can also test whether disjunct distribution ranges are the result of vicariance, as in the above hypothesis of range fragmentation irrespective of its temporal framework, or of relatively recent long-distance dispersal (e.g. Schönswetter *et al.*, 2002; Dixon *et al.*, 2009b).

Glacial refugia and their consequences for species distributions have been intensively studied in the European Alps. Early biogeographic studies, which were based exclusively on distributional data, identified refugia both at the margins and in the central parts (nunataks) of the Alps (Brockmann-Jerosch, 1926; Merxmüller, 1952, 1953, 1954). In recent years, an array of phylogeographical studies has revealed that glacial refugia for both calcicolous and silicicolous plants were situated mainly at the south-western and southern to north-eastern margins of the Alps, and that nunatak survival only rarely needs to be invoked as an explanation for current genetic patterns (Stehlik *et al.*, 2002b; Schönswetter *et al.*, 2005). Additional peripheral refugia might have been located in the Northern Alps (Merxmüller, 1952, 1953, 1954; Stehlik *et al.*, 2002a; Paun *et al.*, 2008; Winkler *et al.*, 2010). Such refugia are expected to be of particular importance for limestone-dwelling species, which usually show a disjunct distribution encompassing the limestone ranges of the Northern Alps and the southern Eastern Alps. Genetic exchange between populations in the northern and southern ranges has probably been limited because of the scarcity of suitable substrate in the intervening, mainly siliceous, Central Alps. In addition, populations in the Central Alps probably became extirpated during cold-period glacial advances. Consequently, repeated range contractions during glacial periods should lead to genetic differentiation between population groups at the northern and southern margins of the Alps, a hypothesis thus far not tested.

A good study species for investigating Pleistocene range dynamics on different geographic scales is *Androsace lactea* (Primulaceae). This species grows on non-mineral soils on limestone in rocky, shady habitats in the upper montane and subalpine (more rarely lower alpine) belt (Lüdi, 1927; Aeschimann *et al.*, 2004) and is widely but disjunctly distributed from the Cordillera Cantábrica and the eastern Pyrenees via the Alps and Carpathians to the Dinaric and the Balkan mountains (Fig. 1a). In the Alps, *A. lactea* occupies a highly fragmented distribution area in peripheral regions in or close to formerly unglaciated areas (Fig. 1a). Whereas populations in the Western and Southern Alps are often rare and scattered, *A. lactea* is fairly continuously distributed in a narrow belt along the northern margin of the Alpine arc (Fig. 1a). Using amplified fragment length polymorphism (AFLP) and chloroplast DNA sequence data we explored the mode and time of range formation in this species. Specifically, we tested the hypothesis that the disjunct distribution is the result of range fragmentation (vicariance) and that the resultant lineage differentiation dates back to the late Tertiary to early Pleistocene (Lüdi, 1927), with more long-distance dispersal and more recent range fragmentation being the alternatives. Furthermore, we addressed the existence of Pleistocene refugia at the northern margin of the Alps (Merxmüller, 1952, 1953, 1954), which are expected to result in strong genetic differentiation between population groups north and south of the Central Alps.

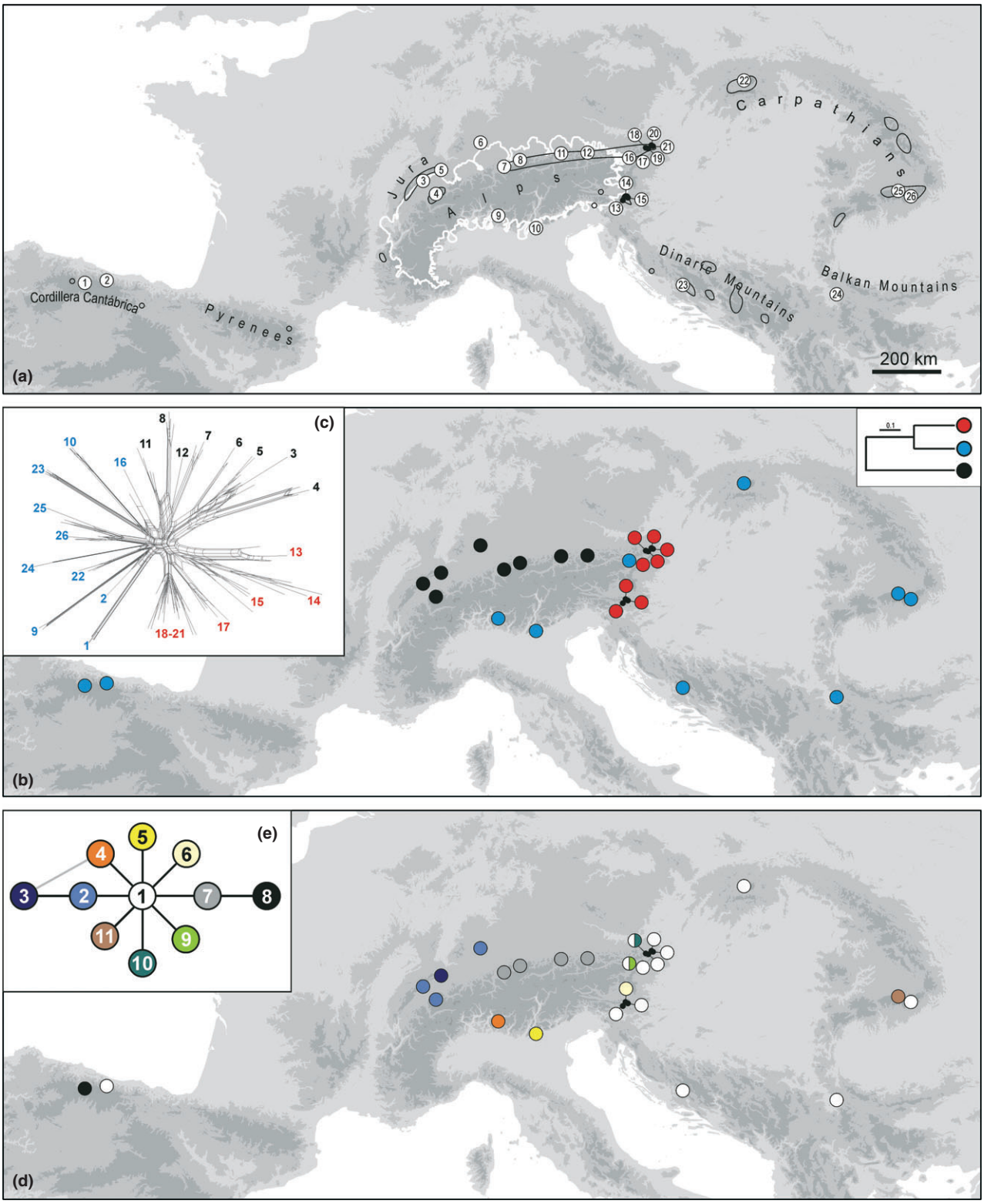

Figure 1 Distribution, sampled populations and genetic structure of *Androsace lactea*. (a) Geographic distribution (black line), sampled populations (see Appendix S1 for further information) and maximum extent of the Alpine ice sheet at the Last Glacial Maximum (white line; taken from van Husen, 1987, 1997 and Voges, 1995). (b) Genetic structure derived from Bayesian mixture analysis of AFLP markers. The top-right insert illustrates the relationships among the clusters (UPGMA-tree based on Kullback-Leibler distances). (c) NeighbourNet (simplified from Fig. 2; colour coding of population numbers as in b). (d) Geographic distribution of the 11 chloroplast DNA haplotypes. (e) Relationships among the chloroplast DNA haplotypes visualized as a parsimony network. Lines correspond to mutational steps. The loop involving haplotypes 1–4 is resolved based on geographic considerations (see text for details).

MATERIALS AND METHODS

Sampling and DNA extraction

Leaf material from one to four plants from 26 populations of *A. lactea* (Fig. 1a; and see Appendix S1 in the Supporting Information) was sampled and dried in silica gel. Voucher specimens are deposited at the Institute of Botany, University of Vienna, Austria (WU). Total genomic DNA was extracted from dried tissue (*c.* 10 mg) following a cetyl trimethyl ammonium bromide (CTAB) protocol (Doyle & Doyle, 1987) with a few modifications (Schönswetter *et al.*, 2002).

AFLPs

The AFLP procedure followed Schönswetter *et al.* (2009). Initially, selective primers were screened using 12 selective primer combinations. The five final primer combinations for the selective polymerase chain reaction (PCR) (fluorescent dye in brackets) were *Eco*RI (6-Fam)-ACA/*Mse*I-CAT, *Eco*RI (VIC)-AAG/*Mse*I-CTG, *Eco*RI (6-Fam)-ACA/*Mse*I-CAC, *Eco*RI (VIC)-ATG/*Mse*I-CTT and *Eco*RI (NED)-AAC/*Mse*I-CTT. 5 μL of each of the differently labelled selective PCR products was purified using Sephadex G-50 Fine (GE Healthcare Bio-Sciences, Uppsala, Sweden) applied to a Multi Screen-HV plate (Millipore, Molsheim, France). 1.2 μL of the elution product was mixed with 10 μL of formamide (Applied Biosystems, Foster City, CA, USA) and 0.1 μL of GeneScan 500 ROX (Applied Biosystems), the internal size standard, and run on an ABI 3130x automated capillary sequencer (Applied Biosystems). Nine individuals were replicated to calculate the error rate and to exclude non-reproducible fragments from the analyses.

AFLP analyses

Raw AFLP data were aligned with the internal size standard using ABI Prism GeneScan 3.7.1 (Applied Biosystems), and imported into Genographer 1.6.0 (available at http://hordeum.oscs.montana.edu/genographer) for scoring. The error rate (Bonin *et al.*, 2004) was calculated as the ratio of mismatches (scoring of 0 vs. 1) over matches (1 vs. 1) in AFLP profiles of replicated individuals. A neighbour-joining (NJ) analysis based on a matrix of Nei–Li distances (Nei & Li, 1979) including 2000 pseudo-replicates was conducted with TreeCon 1.3b (Van de Peer & De Wachter, 1997). Using the program SplitsTree 4.6 (Huson & Bryant, 2006), a NeighbourNet was constructed based on a matrix of *p*-distances [i.e. the distance is the proportion (*p*) of sites at which two samples being compared are different].

Population mixture analysis was conducted with baps 5.2 (Bayesian Analysis of Population Structure; Corander *et al.*, 2003, 2004). Because AFLPs are dominant markers, only AFLP phenotypes can be analysed, but this procedure does not violate the assumptions of baps (Corander *et al.*, 2004). We conducted a mixture analysis of individuals with the geographic origin of the samples used as an informative prior ('spatial clustering of individuals') or without this prior ('clustering of individuals'). baps was run with the maximal number of groups (*K*) set from 2 to 10. Each run was replicated 10 times, and the results were averaged according to the resultant likelihood scores. Results of the mixture analysis were used as input for population admixture analysis (Corander & Marttinen, 2006), with the default settings in order to detect admixture between clusters. A UPGMA (unweighted pair group method with arithmetic mean) tree was inferred based on Kullback–Leibler distances (Kullback & Leibler, 1951) among clusters as implemented in baps.

structure 2.2 with a Bayesian clustering approach developed for dominant markers (Pritchard *et al.*, 2000; Falush *et al.*, 2007) was used with an admixture model with uncorrelated allele frequencies and recessive alleles. Twenty replicate runs for each *K* (number of groups) ranging from 1 to 9 were carried out at the Bioportal of the University of Oslo (http://www.bioportal.uio.no/), using a burn-in of 10^5 iterations followed by 10^6 additional Markov chain Monte Carlo (MCMC) iterations.

Sequencing of plastid DNA

The three plastid regions, *ccmp*3f–*trn*R, *rpl*20–5′-*rps*12 and *trn*$S_{(UGA)}$–*trn*f$M_{(CAU)}$, successfully employed for intraspecific comparisons in other *Androsace* species (Dixon *et al.*, 2007, 2008, 2009a,b), were sequenced for three individuals per population (where available), usually including the individuals used for AFLPs (Appendix S1). In case of intrapopulational sequence variation (see Results), the respective region was sequenced for up to eight individuals. PCR was conducted as described in Dixon *et al.* (2009a). The PCR products were cleaned with Exonuclease I and Calf Intestine Alkaline Phosphatase (Fermentas, St. Leon-Rot, Germany) according to the manufacturer's instructions. BigDye Terminator chemistry (Applied Biosystems) was used according to the manufacturer's instructions for cycle sequencing following electrophoresis with an ABI 3130x capillary sequencer.

DNA analyses

Sequences were edited with SeqMan II 5.05 (DNAStar Inc., Madison, WI, USA) and aligned manually using BioEdit 7.0.4.1 (Hall, 1999). Prior to all analyses, an inversion in the *ccmp*3f–*trn*R region was manually reversed, as it would introduce substitutional mutations, which in fact are the result of a structural mutation (Löhne & Borsch, 2005). A haplotype network was constructed using statistical parsimony as implemented in tcs 1.21 (Clement *et al.*, 2000). For this analysis, all structural mutations (insertions/deletions of motifs of more than 1 bp and the inversion) were treated as single-step events.

Reconstruction of the demographic history and molecular dating were conducted using beast 1.4.8 (Drummond &

Rambaut, 2007) on the University of Vienna computer cluster (Schrödinger III, http://www.univie.ac.at/ZID/schroedinger/). As the plastid genome behaves as a single linkage group and our data consisted mostly of non-coding spacer and intron regions, we applied a single substitution model to all data. Substitution model uncertainty was substantial. The best model had an Akaike weight (determined with ModelTest 3.6; Posada & Crandall, 1998) of only 0.16, and 21 models (out of 56 tested) were included until the cumulative Akaike weight exceeded 0.95. The included models ranged from having only three (F81) to up to nine (GTR + Γ + I) free parameters, and for the final analysis we used HKY + Γ, which is a model of medium complexity with five free parameters, subsuming a proportion of invariable sites in the gamma-distribution modelling rate heterogeneity across sites, using six rate categories instead of the default four. For the substitution model parameters, we used Jeffrey's priors. Given the low level of sequence variation (see Results), we used a strict clock model with a prior on the substitution rate modelled as a normal distribution. Based on previously published substitution rates for plastid markers (Yamane *et al.*, 2003; Smith *et al.*, 2008), we used a mean of 4.0×10^{-3} substitutions site^{-1} Myr^{-1} and a deliberately wide standard deviation of 2.0×10^{-3} substitutions site^{-1} Myr^{-1}. After initial analyses, the root of the tree was constrained to be maximally 5 Myr old. As the Bayesian skyline plot (Drummond *et al.*, 2005) did not show any detectable sign of population-size changes (data not shown), we used a model of constant population size as our demographic model. The Markov chain was run twice for 3×10^7 generations each time, sampling every 1000th generation. After removing the first 10% of sample points as burn-in, parameter estimates and their 95% highest posterior density (HPD) intervals were obtained from 54,000 generations.

In order to test for population expansion we used neutrality tests (Tajima, 1996; Fu, 1997) and the R_2 statistic (Ramos-Onsins & Rozas, 2002) as implemented in DnaSP 5.10 (Rozas *et al.*, 2003). Specifically, significantly negative Tajima's D (Tajima, 1996) and Fu's F_S (Fu, 1997) and small values for the R_2 statistic (Ramos-Onsins & Rozas, 2002) indicate population expansion. Significances were assessed by 1000 samples simulated under a model of constant population size.

RESULTS

AFLPs

We scored 300 AFLP fragments ranging from 60 to 537 bp, of which 65 (21.7%) were monomorphic and were excluded from further analyses. The error rate was low (0.65%).

In the NJ analysis [not shown, but bootstrap (BS) values are plotted onto the NeighbourNet in Fig. 2] individuals from the investigated populations did not intermix, except for the geographically close populations 18–21. Many populations were strongly distinct and had long branches and maximal BS support. The backbone of the tree was unresolved, but two major groups of populations were recognized. The first comprised populations 3–6 from the north-western Alps, the Jura Mountains and the Danube valley (BS 78), and the second comprised populations 13–15 and 17–21 from the easternmost Alps (BS 75). In accordance with the NJ analysis, the NeighbourNet (Figs 1c & 2) illustrates the divergence of most populations and a weak internal structure.

Whereas structure did not return congruent clustering solutions for any value of K (Appendix S2), baps mixture analyses with or without spatially informative priors resulted in congruent assignment of the investigated individuals to three clusters (Fig. 1b). The best partitions received log marginal likelihoods of −5058 at $P = 0.99998$ (spatial clustering of individuals) and −4948 at $P = 1$ (without using geographic coordinates as informative priors). In the former case, population admixture analysis revealed that a single individual from population 16 was admixed among all three clusters. The cluster from the Northern and north-western Alps plus Jura and the Danube valley (populations 3–8, 11–12), which includes one of the well-supported groups from the NJ analysis (populations 3–6), was most distinct as judged from the reciprocal Kullback–Leibler divergences (Fig. 1b). The cluster in the easternmost Alps (populations 13–15, 17–21) is congruent with the second group supported by the NJ analysis (Fig. 2).

Plastid DNA sequences

Sequence variability was low: *ccmp*3f–*trn*R (484 bp) with one inversion; *rpl*20–5′-*rps*12 (761–762 bp) with two substitutions and one single base pair insertion/deletion in a multi-A motif; *trn*S$_{(UGA)}$–*trn*fM$_{(CAU)}$ (992–1014 bp) with three substitutions and two insertions (duplications of motifs 6 and 22 bp long, respectively). After concatenation and conversion of the inversion, the alignment of 2266 bp included (in parentheses the numbers including the insertions and inversions counted as single step changes) five (nine) variable characters, of which four (eight) were parsimony-informative.

The network analysis using statistical parsimony resulted in a single network connecting all 11 haplotypes (Fig. 1e). After resolving the single loop following considerations of geographic proximity, thus connecting haplotype 3 from the Swiss Jura with haplotype 2 from the same region rather than with haplotype 4 from the Southern Alps, the topology was star-like. All haplotypes were separated from the central haplotype 1 by single mutational steps with the exception of haplotypes 3 (Swiss Jura) and 8 (western Cordillera Cantábrica), which themselves were separated by another single mutational step from haplotypes 2 (Swiss Jura and adjacent areas) and 7 (northern Eastern Alps), respectively. Whereas the central haplotype 1 was widespread in the easternmost Alps, the Carpathians and the Dinaric mountain ranges as well as in the Picos de Europa of the Cordillera Cantábrica, the other haplotypes were usually restricted to single populations either exclusively so or more rarely co-occurring with haplotype 1 (Appendix S1), which then might be rare (in one out of eight individuals checked in population 16) or not (in three out of six individuals checked in population 18). The two other more

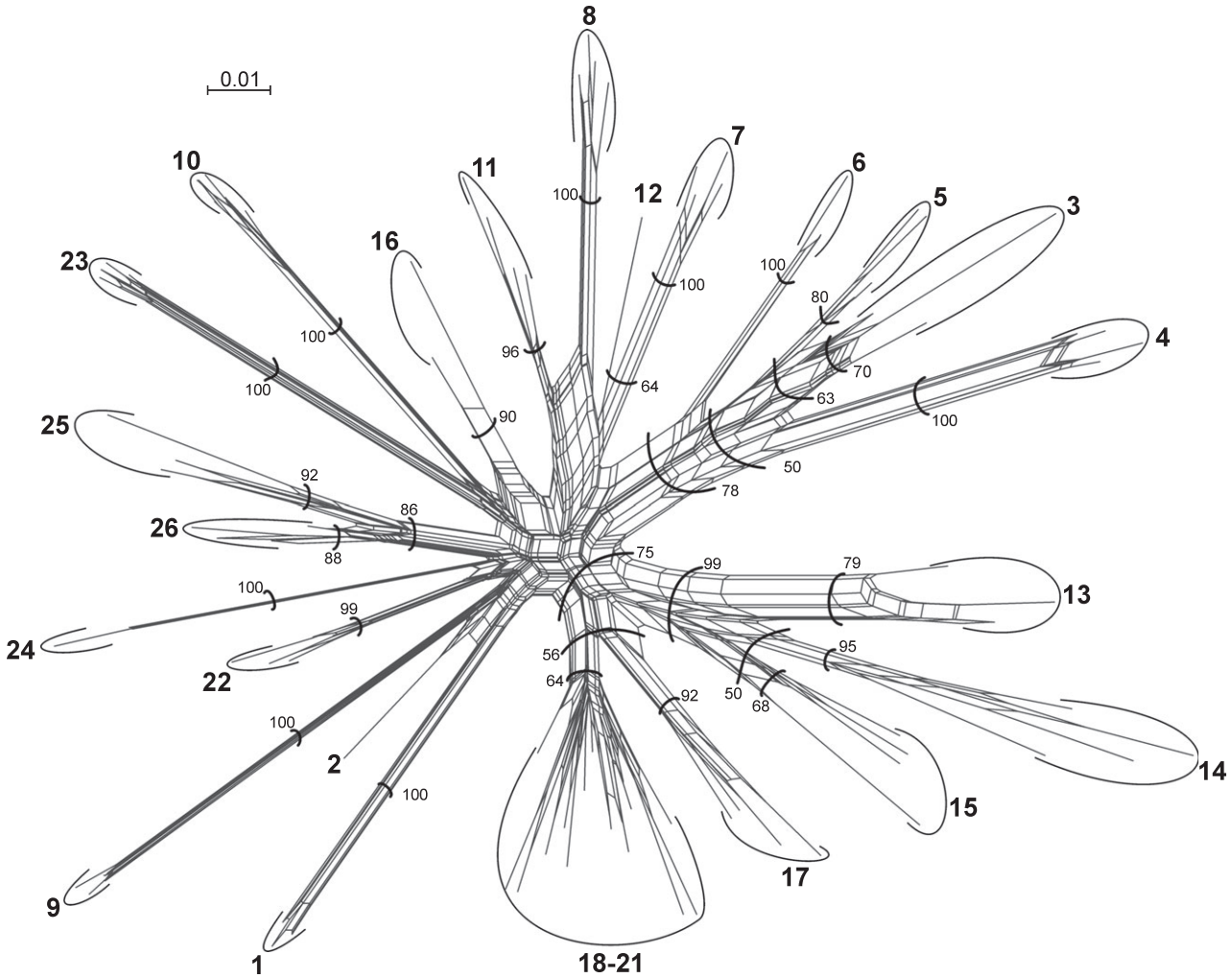

Figure 2 NeighborNet derived from AFLP data of *Androsace lactea*. Numbers along branches are bootstrap values from neighbour-joining analysis (2000 replicates). Populations are coded as in Fig. 1 and Appendix S1.

widespread haplotypes were haplotype 2 in the north-western Alps, Swiss Jura and Danube valley and haplotype 7 in the northern Eastern Alps (Fig. 1d).

Using a model of constant population size, the diversification age of *A. lactea* was inferred to be (given as mean/median) 0.135/0.08 Ma (95% highest posterior density interval 0.364–0.006 Ma), although, owing to the low level of sequence variation, these estimates should be viewed with appropriate caution. In agreement with a hypothesis of population expansion, both Tajima's D and Fu's F_S were negative (−1.143 and −2.841, respectively), but they were not significant ($P = 0.131$ and $P = 0.056$, respectively). Similarly, the R_2 statistic of 0.052 was not statistically significant ($P = 0.127$). Qualitatively identical results were obtained when structural mutations were coded as base substitutions (data not shown).

DISCUSSION

Androsace lactea is a widely but disjunctly distributed species of the southern European mountain system, and its distribution, especially in the European Alps, is tightly associated with putative glacial refugia (Fig. 1a), suggesting Quaternary climatic oscillations as an important force for range formation in this species. Specifically, it has been hypothesized that the current distribution is the result of the Pleistocene fragmentation of a once more continuous distribution range (Lüdi, 1927), and thus that it is strictly attributable to vicariance. On the other hand, *A. lactea* is absent from large areas with ample availability of suitable habitat and less severe glaciation during glacial periods, most notably major parts of the Pyrenees, suggesting that geographically highly isolated occurrences, such as those in the Cordillera Cantábrica, might be the result of long-distance dispersal, as suggested for other *Androsace* species (Dixon *et al.*, 2008, 2009b). A connection between the western Cordillera Cantábrica and the Northern Alps is indicated by the plastid sequence data, as the Cantabrian haplotype 8 appears to be derived from the Northern Alpine haplotype 7 (Fig. 1e). These two haplotypes are joined by an identical number of bases in a mononucleotide repeat region (10 instead of 11 A's). As mononucleotide repeats are

homoplasy-prone characters (Estoup *et al.*, 2002), the more likely alternative is that haplotypes 7 and 8 are derived independently from the central haplotype 1. Likewise, the AFLP data (Figs 1b,c & 2) suggest strong genetic divergence of all geographically isolated populations and do not yield any evidence for unintuitive geographical relationships. Hence, there is no unambiguous evidence for long-distance dispersal from the molecular data, supporting range fragmentation (vicariance) as the main cause for the currently highly disjunct distribution area, as seen also in many other mountain plant groups (Kropf *et al.*, 2002, 2006).

Wide but disjunct distributions are usually interpreted as old (Ozenda, 2009), and for *A. lactea* it has been suggested that a continuous range formed in the Tertiary and was fragmented in the Pleistocene (Lüdi, 1927). Pleistocene range fragmentation is supported by the inferred age of the haplotype differentiation, which is estimated to have occurred within the last 370,000 years. As age estimates for genetic differentiation provide maximum ages for the time of geographic separation, range fragmentation might actually date back only to the last glaciations (the median age of genetic differentiation is 80,000 years ago). These age estimates might be gross underestimates, if more divergent haplotypes remain unsampled, as is plausible given the comparatively low number of individuals investigated per population. However, the geographic isolation and small size of many populations (with the exception of the north-eastern Alps, where the species is fairly abundant; G.M. Schneeweiss, pers. obs.) render it unlikely that many haplotypes remained undetected. Low intrapopulational genetic diversity outside the easternmost Alps is also evidenced by the AFLP data (Fig. 2). A further complication may arise from the substitution rates used, as these might be inappropriate for the sequenced regions and/or for the investigated species. However, any bias is expected to be towards older ages owing to the time dependence of molecular rates (Ho *et al.*, 2005, 2007), even if this effect is of a smaller magnitude than initially anticipated (Debruyne & Poinar, 2009). Although we cannot exclude the possibility that *A. lactea* reached its wide distribution as early as the Tertiary, such a scenario requires that genetic differentiation, which is expected to have evolved during range fragmentations in the course of early Pleistocene glaciations, did not leave any traces in the current haplotype structure, which appears unlikely. Furthermore, because of its habitat requirements, *A. lactea* would, if present, have been restricted to higher mountain ranges in the late Tertiary, suggesting that a wider and more continuous distribution was established during colder periods. Therefore, we suggest that, in agreement with the level and structure of haplotype diversity (the most distinct haplotypes are only four mutational steps apart and none of the interior haplotypes in the star-like network remained unsampled: Fig. 1e), the wide distribution has a significantly younger origin, although the precise nature of range expansion in this species remains unknown. A similar pattern of strong AFLP-divergence among populations and young age as suggested by plastid markers was previously found in the disjunctly distributed southern European mountain plant *Papaver alpinum* (Schönswetter *et al.*, 2009).

Androsace lactea is widely distributed in limestone ranges of the Alps, but with the exception of the Northern Alps it is lacking from many regions with ample suitable habitat (Fig. 1a). Many populations are found in or close to formerly unglaciated areas (Fig. 1a), indicating that the currently scattered distribution is the result of failed post-glacial (re)colonization. Distributional stasis is also supported by the lack of a signal of range expansion in the plastid data and the restriction of derived haplotypes to single AFLP groups identified by the BAPS analysis (Fig. 1b,d), as well as by the strong geographic structure in the AFLP data on a regional scale (Fig. 2), indicating that genetic exchange has remained restricted to geographically close regions.

AFLP data (Figs 1b,c & 2) suggest the presence of two supported phylogeographic groups in the Northern Alps and northerly adjacent regions. The first includes populations 3–6 from the western Northern Alps plus those north of the Alps (Jura, Danube valley) and, according to the BAPS analysis, also populations 7, 8, 11 and 12 from the central Northern Alps (Fig. 1b), whereas the second is constituted by populations from the easternmost Alps (populations 13–15, 17–21; Fig. 1b). The AFLP pattern in the Northern Alps is congruent with the presence of distinct haplotype groups (haplotypes 2, 3 and 7 in the western and central Northern Alps; haplotypes 1, 9 and 10 in the easternmost Alps) in these regions (Fig. 1d,e). This is in agreement with the hypothesis of one or more Pleistocene refugia west of the early hypothesized north-eastern Alpine refugium at the northern margin of the Alps, which may have been situated in the deep incisions between glacier tongues (Fig. 1a; Merxmüller, 1952, 1953, 1954; Paun *et al.*, 2008; Winkler *et al.*, 2010). As is also the case for *Erinus alpinus* (Stehlik *et al.*, 2002a), the data available do not permit us to determine whether the populations in the Swiss Alps are the result of *in situ* persistence or of post-glacial immigration from an extra-Alpine refugium, such as the Jura Mountains, as suggested previously (Wirth, 1914), or even from one further north, such as the Danube valley (Fig. 2). Whereas the pattern of latitudinal genetic differentiation, as predicted for limestone-dwelling species, holds true for most of the Alpine arc, it does not do so in the easternmost Alps. There is instead a strong genetic tie between the north-eastern and south-eastern Alps (Figs 1b,c & 2), as has been suggested previously based on haplotypes of the low-copy nuclear RPA2 region in *Papaver alpinum* (Schönswetter *et al.*, 2009). As the eastern margin of the Alps was not glaciated during cold periods (van Husen, 1987, 1997), continuous gene flow across the central ranges of the Alps, probably via isolated limestone ranges in the Central Alps (population 17), may have been possible. This contrasts with the case for refugia further west, which were separated both latitudinally by the strongly glaciated Central Alps and longitudinally by valley glaciers reaching far into the foreland (Fig. 1a), the latter possibly also being responsible for the strong population differentiation between the Northern and the north-eastern Alps (Figs 1b & 2).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Appendix S1 Information on the sampled populations of *Androsace lactea*, numbers of analysed individuals, number of plastid haplotypes and GenBank accession numbers.

Appendix S2 Analysis of the AFLP data set of *Androsace lactea* with STRUCTURE 2.2.

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