LETTER

History or ecology? Substrate type as a major driver of spatial genetic structure in Alpine plants

Nadir Alvarez,[1][†] Conny Thiel-Egenter,[2][†] Andreas Tribsch,[3] Rolf Holderegger,[2] Stéphanie Manel,[4] Peter Schönswetter,[5] Pierre Taberlet,[4] Sabine Brodbeck,[2] Myriam Gaudeul,[6] Ludovic Gielly,[4] Philippe Küpfer,[1] Guilhem Mansion,[7] Riccardo Negrini,[8] Ovidiu Paun,[9] Marco Pellecchia,[8] Delphine Rioux,[4] Fanny Schüpfer,[1] Marcela Van Loo,[9] Manuela Winkler,[10] Felix Gugerli[2]* and IntraBioDiv Consortium[‡]

Abstract

Climatic history and ecology are considered the most important factors moulding the spatial pattern of genetic diversity. With the advent of molecular markers, species' historical fates have been widely explored. However, it has remained speculative what role ecological factors have played in shaping spatial genetic structures within species. With an unprecedented, dense large-scale sampling and genome-screening, we tested how ecological factors have influenced the spatial genetic structures in Alpine plants. Here, we show that species growing on similar substrate types, largely determined by the nature of bedrock, displayed highly congruent spatial genetic structures. As the heterogeneous and disjunctive distribution of bedrock types in the Alps, decisive for refugial survival during the ice ages, is temporally stable, concerted post-glacial migration routes emerged. Our multispecies study demonstrates the relevance of particular ecological factors in shaping genetic patterns, which should be considered when modelling species projective distributions under climate change scenarios.

Keywords

Alps, amplified fragment length polymorphisms, comparative phylogeography, ecological requirements, genetic structure, geology, glacial refugia, recolonization pathways, soil reaction.

Ecology Letters (2009) **12**: 632–640

INTRODUCTION

A fundamental question in biology is what factors determine the distribution of species (von Humboldt 1805; De Candolle 1820; Darwin 1859). After life-history traits, climatic history and ecology have been considered the most important factors influencing species' distribution and biogeographic patterns (Cox & Moore 2005). Past climatic fluctuations caused by orbital and tectonic forces (Williams *et al*. 1998) have continuously modified landscapes and biodiversity on the planet. For instance, glacial cycles have shaped biogeographic patterns through processes such as dispersal, vicariance and extinction (Bennett 1990; Hewitt 1996, 1999, 2000; Comes & Kadereit 1998; Taberlet & Cheddadi 2002). During the last two decades, phylogeographic studies at the intraspecific level have revealed that

[1]*Institute of Biology, University of Neuchâtel, 2000 Neuchâtel, Switzerland*
[2]*WSL Swiss Federal Research Institute, 8903 Birmensdorf, Switzerland*
[3]*Department of Organismic Biology, University of Salzburg, 5020 Salzburg, Austria*
[4]*Laboratoire d'Ecologie Alpine, UMR UJF-UdS-CNRS 5553, University Joseph Fourier, 38041 Grenoble, France*
[5]*Department of Biogeography and Botanical Garden, University of Vienna, 1030 Vienna, Austria*
[6]*Department of Systematics and Evolution, National Museum of Natural History, 75005 Paris, France*
[7]*Institute for Systematic Botany, University of Zürich, 8008 Zürich, Switzerland*
[8]*Institute of Zootechnics, Catholic University of Sacred Heart, 29100 Piacenza, Italy*
[9]*Jodrell Laboratory, Royal Botanic Gardens, TW9 3AB Kew, UK*
[10]*Department of Systematic and Evolutionary Botany, University of Vienna, 1030 Vienna, Austria*
[†]*These two authors have contributed equally to this work and are considered joint first authors.*
[‡]*http://www.intrabiodiv.eu/IMG/pdf/IntraBioDiv_Consortium.pdf*
*Correspondence: E-mail: felix.gugerli@wsl.ch

glacial refugia and post-glacial dispersal along colonization pathways strongly moulded spatial genetic patterns of species (Hewitt 1996, 1999; Taberlet *et al.* 1998; Petit *et al.* 2002; Avise 2009). Furthermore, it seems likely that the ecological requirements of species or intraspecific lineages have considerably influenced their glacial fate.

As early as in the beginning of the 20th century, it had been proposed that species are distributed deterministically with respect to each other and to ecological gradients (Clements 1916; Whittaker 1967; Diamond 1975; Gotelli & McCabe 2002). Populations of distinct species might therefore have survived in the same refugium and have consequently followed the same migratory routes during climatic changes (i.e. species assemblages moving as complete communities; McGill *et al.* 2005). A contrasting view is that species are distributed independently since their ecological requirements never overlap precisely (Gleason 1926; Whittaker 1967). Thus, post-glacial migration pathways and extant species distributions are the results of predominantly stochastic processes affected by, e.g. climatic clines (Graham *et al.* 1996; Wiens & Donoghue 2004) or topographical barriers (Hewitt 1996).

An approach to further understanding whether recolonization of deglaciated areas might result from factors other than climate and topography is to relate species' phylogeographies to their ecological requirements. To explore the possibility that ecological factors have affected species' large-scale phylogeographic patterns, we used high-mountain plant species of the European Alps as a study system. As the Alps were strongly and repeatedly glaciated during the Quaternary (Cox & Moore 2005), species faced major range shifts and, if at all, survived in refugia along the border of the Alps (Schönswetter *et al.* 2005) or rarely so in some ice-free nunatak areas within the intensively glaciated range (Stehlik *et al.* 2002; Bettin *et al.* 2007). Hence, we expect that species followed migration routes into and out of refugial areas directionally guided by their ecological requirements. This would have driven species that share the same ecological traits to exhibit similar spatial genetic patterns i.e. similar spatial distributions of major gene pools. In other words, if two species share key ecological requirements, their past migration, as inferred from respective refugial locations and recolonization pathways, should resemble each other.

Based on the above assumptions, we compared the phylogeographic patterns of 27 widely distributed Alpine plant species and related these to their respective ecological characteristics. To assess these patterns, we exhaustively sampled all species on a regular large-scale grid across the entire Alps and determined genetic variation through genetic fingerprinting. We found that among the ecological factors tested, only substrate type, which relates to calcareous or siliceous bedrock, revealed a significant effect on the genetic structure in these study species. Thus, we conclude that bedrock distribution and availability had a major effect on the migration pathways during post-glacial re-colonization of the Alps.

MATERIALS AND METHODS

Sampling design and genotyping

We investigated the phylogeography of 27 Alpine plant species sampled across the European Alps. These mostly widespread, abundant species represented a broad range of characteristics with regard to, e.g. life history, ecological niche, habitat type and taxonomy (Thiel-Egenter *et al.* 2009; Tribsch *et al.* 2009). Sampling was accomplished according to a grid with cell size of 20′ longitude by 12′ latitude (*c.* 20 km × 22.5 km; Gugerli *et al.* 2008). Plant material of three individuals was collected in one location of every second cell if the respective species occurred there, resulting in sample sizes ranging from 47 (*Cerastium uniflorum*) to 137 locations (*Carex sempervirens*; for location numbers of all species see Gugerli *et al.* 2008). The three samples per location represented spatial replicates within a grid cell. All samples were genotyped using amplified fragment length polymorphisms (AFLP) with three primer/enzyme combinations (for details see Gugerli *et al.* 2008). This genome-wide characterization of neutral genetic variation (Vos *et al.* 1995) allows an accurate assessment of the species' spatial genetic structures (Alsos *et al.* 2007; Skrede *et al.* 2009). Based on individual fingerprinting patterns, we generated matrices of presence/absence of markers independently for every species.

Identification of genetic structure

Within the software STRUCTURE (Pritchard *et al.* 2000), individuals were assigned to genetic clusters using model-based Bayesian clustering. With this population genetic approach, we aimed at finding genetically homogeneous clusters of sampling locations and identifying contact zones, irrespective of the species' phylogenetic relationships. STRUCTURE builds an *a priori* number of clusters, K, aiming at minimizing deviations from Hardy–Weinberg equilibrium and linkage disequilibrium within these clusters, and provides the probability of individuals to be assigned to either of the K clusters. A preliminary assignment was performed for a subset of species, using both the standard version of STRUCTURE 2.0 (Pritchard *et al.* 2000) and a modified version for dominant markers (STRUCTURE 2.2; Falush *et al.* 2007). Both methods yielded highly similar results when the uncorrelated allele frequencies and the no-admixture models were selected

($R^2 > 0.99$ when correlating assignment probabilities obtained with both methods). We therefore used the standard version of the software with uncorrelated allele frequencies and no-admixture parameters. After a burn-in period of 200 000 cycles, 1 000 000 Markov chain Monte Carlo simulations were performed for K ranging from 1 to 5. $K = 5$ was set as the maximum number of clusters for the following reasons. First, we wanted to compare large-scale genetic structures among species rather than to consider local gene pools, which typically arise at higher K-values. Second, the within-species spatial genetic structures of Alpine plants is generally assumed to be less than five on the scale of the Alpine range (Schönswetter *et al.* 2005). For each K, 20 independent runs were conducted and we selected the run with the highest likelihood for further analysis. We stopped using the resulting assignments at the K-value at which the following criteria were met: (i) The likelihood of $K = n + 1$ is lower than that of $K = n$ or (ii) the maximum value for individual assignment probabilities in at least one cluster within $K = n + 1$ is lower than 0.5 (i.e. according to the majority-rule concept). Therefore, between one and five genetic clusters were finally obtained for each of the 27 species (Table 1). Mean probability of assignment to each of the K clusters was recorded per sampling location, and the respective patterns were visualized for each species in ARCGIS 9.1 (ESRI, Redlands, CA, USA).

Interspecific comparisons of genetic structures

To compare the phylogeographic patterns among species, we used the STRUCTURE outputs for each K-value, namely the average assignment probabilities of a sample location to each cluster. We applied SIMIL to compute the similarity between genetic structures of species pairs (genetic-structure-similarity index, GSS; Alvarez *et al.* 2008), obtaining a GSS matrix for each of the four K-values. The SIMIL algorithm tests all cluster combinations per species pair and considers the combination that maximizes the final average value of GSS for each species pair. That is, in each cell we computed the between-species absolute differences of assignment probabilities and averaged over all cells. To down-weight cases in which the overlapping fraction of the sampling areas of each species pair was very low, we adjusted the corresponding similarity indices using the weighting procedure suggested by Alvarez *et al.* (2008; i.e. GSS mean). Subsequently, a matrix comprising the weighted similarity indices for all species pairs was constructed for each K-value. For each species pair, one (i.e. when only $K = 2$ yielded a suitable spatial structure) to four different patterns (i.e. when $K = 2$ to 5 allowed a comparison of the spatial structure) were compared.

Ecological indicators

Ecological requirements of a large number of Alpine plants have been documented by Landolt (1977) using, among others, the five indicators moisture, soil reaction (i.e. a proxy for soil pH), temperature, light and nutrient availability (i.e. nitrogen). These ecological indicators, which define a species' common habitat under competitive interactions, are based on a compilation of ecological data from the last century and are widely used in vegetation ecology (Diekmann 2003). Prior Mantel tests based on the trait similarity matrices showed a correlation of a further indicator, namely continentality, with the indicators moisture and light ($r = 0.20$ and 0.25, respectively). Thus, continentality was not considered in further analyses. As successional status (see below) and moisture were only weakly correlated ($r = 0.13$), we kept both of them in the analyses. For each of the above five indicators, Landolt attributed values (LV) ranging from 1 to 5, reflecting an increase in species' requirements (e.g. a soil reaction indicator value of 1 characterizes species adapted to very acidic substrate types whereas a value of 5 indicates species growing on very alkaline substrate types). To increase the power of our analysis and to obtain equal class sizes, indicator values were grouped so that classes comprised at least five observations, which resulted in a ternary classification for moisture and soil reaction, and a binary classification for nutrients, light and temperature (Table 1). We classified the indicators as follows: light: (i) moderately high (LV = 3–4) or (ii) very high (LV = 5); nutrients: (i) low (LV = 1–2) or (ii) moderately high (LV = 3–4); temperature: (i) very low (LV = 1) or (ii) low (LV = 2); moisture: (i) low (LV = 2), (ii) intermediate (LV = 3) or (iii) high (LV = 4–5). Soil reaction was classified according to the substrate type affinity as follows: (i) silicicolous species (i.e. mainly growing on substrates based on crystalline, mostly acidic bedrock; LV = 2); (ii) intermediate species (i.e. growing either on crystalline and limestone bedrocks or on bedrock of intermediate acidity; LV = 3) and (iii) calcicolous species (i.e. mainly growing on alkaline substrates on limestone bedrock; LV = 4–5). We used successional status as a further indicator, comprising the three classes early successional, intermediate successional and late successional. Early successional habitats are generally composed of a shallow A-horizon of soil, typically found in plant communities on scree and rocks, whereas late-successional habitats have developed soils as found in Alpine grassland communities. The classification of species was based on descriptions in Aeschimann *et al.* (2004) and on the authors' expert knowledge.

Based on Landolt's indicators and on successional status, similarity matrices were constructed for all species pairs, containing the values 1 for a shared indicator value and 0 for a different value. To investigate more thoroughly the effect

Table 1 Ecological requirements, expressed by modified Landolt's indicators and successional status, number of amplified fragment length polymorphisms and likelihoods of genetic clustering for each K = 1–5 phylogeographic clusters studied for 27 Alpine plant species

Species	Moisture	Soil reaction	Nutrients	Light	Temperature	Successional type	Marker number	1	2	3	4	5
Androsace obtusifolia All.	2	1	1	1	1	3	137	−4900.2	−4395.0	−4209.0	**−4044.0**	(−3932.9)‡
Arabis alpina L.	2	3	1	1	2	1	142	−16 250	14 334.7	−13 604.1	−12 874.7	**−12 506.7**
Campanula barbata L.	2	1	1	1	2	3	114	−10 422.8	−8937.8	−8408.1	−8124.1	**−7948.1**
Carex firma Mygind	1	3	2	2	1	2	58	−5886.4	**−5623.9**	(−5657.3)†	(−6018.4)†	(−5908)†
Carex sempervirens Vill.	1	2	1	1	1	3	122	−10 816.3	−9131.1	−8419.7	**−8170.7**	(−8113.2)‡
Cerastium uniflorum Clairv.	2	1	2	2	1	1	93	−3955.0	−3417.1	−3221.1	−3063.8	**−2956.5**
Cirsium spinosissimum (L.) Scop.	3	2	2	1	2	2	95	−8315.2	−7802.2	−7541.9	−7350.9	**−7239.5**
Dryas octopetala L.	1	3	1	2	1	1	99	−9740.1	−9239.5	−9012.0	**−8941.0**	(−8944.1)†
Gentiana nivalis L.	2	2	1	1	1	3	160	−11 057.9	−10 097.6	−9345.0	−8735.8	**−8437.3**
Geum montanum L.	2	1	1	1	2	3	88	−7174.9	−6412.9	−6172.3	−5963.8	**−5801.3**
Geum reptans L.	2	1	1	2	1	1	57	−2645.2	−2262.2	−2074.7	−1963.1	**−1949.8**
Gypsophila repens L.	2	3	1	2	2	1	94	−7511.3	−7366.3	−7322.4	**−7184.6**	(−7341.8)†
Hedysarum hedysaroides (L.) Schinz & Thell. s.l.	2	3	2	1	2	3	124	−12 104.4	−9970.6	−9650.6	−9529.7	**−9450.2**
Hornungia alpina (L.) Appel s.l.	3	3	2	2	1	1	225	−16 160.1	−14 862.9	−13 991.3	−13 599.1	**−13 217.2**
Hypochaeris uniflora Vill.	2	1	1	1	2	3	79	−3854.7	−3689.6	−3603.2	**−3536.0**	(−3514.7)‡
Juncus trifidus L.	1	1	2	2	1	2	87	−6919.5	−6110.8	−5761.0	−5554.5	**−5479.9**
Ligusticum mutellinoides (Cr.) Vill.	2	2	2	2	1	3	97	**−7185.8**	(−7215.7)†	(−7222.6)†	(−7246.7)†	(−7350.4)†
Loiseleuria procumbens (L.) Desv.	1	1	2	2	1	2	117	−13 050.6	−12 115.8	−11 858.7	−11 655.0	**−11 581.5**
Luzula alpinopilosa (Chaix) Breistr.	3	1	2	2	1	2	219	−11 263.2	−10 372.1	−10 024.5	−9804.2	**−9624.8**
Peucedanum ostruthium (L.) W.D. Koch	2	2	2	1	2	2	113	−16 102.3	−15 646.8	−15 331.2	−15 209.9	**−15 087.7**
Phyteuma betonicifolium Vill. s.l.	2	1	1	2	2	3	165	−15 330.9	−12 379.5	−11 471.0	−11 302.5	**−11 247.8**
Phyteuma hemisphaericum L.	1	1	1	1	1	2	234	−15 047.2	−12 900.1	−12 557.0	−12 255.2	**−12 069.3**
Ranunculus alpestris L. s.l.	3	3	1	1	1	1	429	−22 121.7	−20 163.3	−18 926.9	−18 527.6	**−18 118.7**
Rhododendron ferrugineum L.	2	1	1	2	2	3	119	−14 794.3	−13 503.2	−12 414.4	−12 236.8	**−11 973.6**
Saxifraga stellaris L.	3	2	1	2	2	1	196	−12 432.2	−11 562.5	−11 015.7	−10 634.7	**−10 321.5**
Sesleria caerulea (L.) Ard.	1	3	1	1	2	3	70	−12 298.3	**−11 796.0**	(−11 824.8)†	(−12 170.1)†	(−11 797.9)†
Trifolium alpinum L.	1	1	1	1	2	3	97	−7174.6	−6635.6	−6354.2	−6268.4	**−6177.1**

Ecological requirements were characterized based on five Landolt (1977) indicators collapsed to two or three categories per indicator (see text). Successional type was classified based on Aeschimann et al. (2004) and on the authors' expert knowledge. Phylogeographic clusters were computed with STRUCTURE (Pritchard et al. 2000).
†Results discarded because likelihood of $K = n + 1$ was lower than likelihood of $K = n$.
‡Results discarded because maximum probability of assignment to a genetic cluster for this value of K was lower than 0.5.

of substrate type affinity of species on the GSS pattern, we inferred a more detailed similarity matrix in which species pairs were split into four types, i.e. both species calcicolous, both species intermediate, both species silicicolous or both species with different substrate type affinities. Among the six indicators describing ecological requirements, the nutrients values did not substantially vary among species, because alpine plant species mostly grow on nutrient-poor soils. This indicator was therefore discarded from the analysis. All other indicators varied sufficiently and could be compared with the phylogeographic patterns.

Correlations between genetic structure and ecological indicators

For each K-value, we independently tested the influence of ecological requirements on genetic structures by performing Mantel tests using Congruence Among Distance Matrices (CADM) (Legendre & Lapointe 2004), and further determining Mantel Spearman's correlation coefficients r based upon ranks and one-tailed probabilities of the Mantel statistics after 100 000 permutations. We used the four matrices of GSS indices, each corresponding to a given value of K, and the five matrices of ecological trait similarities. Only those species were considered at each K for which a reliable phylogeographic signal was obtained in the STRUCTURE analysis, hence, the higher the K-value, the lower the number of species. Furthermore, we computed a global test of congruence among all distance matrices, considering only the species for which a satisfactory pattern was obtained with $K = 5$. Thus, the method applied in CADM for the global test of congruence can be seen as an extension of the Mantel test of matrix correspondence by correlating more than two similarity matrices. Finally, we compared GSS values at different K-values, among groups of species showing different substrate type affinities, by performing multivariate analyses of variance (MANOVA) in JMP 5.0.1 (2003, SAS Institute, Cary, NC, USA). Kolmogorov–Smirnov one-sample tests were significant for GSS values with $K = 2$, but not for GSS values with $K = 3$–5. We therefore assumed that the assumption for multivariate normality was not substantially violated.

RESULTS

Phylogeographic patterns

The AFLP genotyping resulted in 58–429 polymorphic markers per species. STRUCTURE analyses indicated distinct phylogeographic patterns in 26 of the 27 species (Table 1). Only *Ligusticum mutellinoides* showed the highest likelihood with one single cluster (henceforth the number of clusters will be defined as the K-value; Table 1). The genetic

structures obtained showed striking geographic consistency, i.e. the clusters within a species were spatially aggregated and admixture areas were often restricted to narrow zones (Fig. 1). *Carex firma* and *Sesleria caerulea* exhibited highest likelihoods with $K = 2$ and were therefore not considered for the analysis with higher K-values. Twenty-four species revealed clear phylogeographic patterns at $K = 3$ or 4. At $K = 5$, maximum probabilities of assignment to a cluster were lower than 0.5 in *Androsace obtusifolia*, *Carex sempervirens* and *Hypochaeris uniflora*, wherefore theses species were discarded for $K = 5$. *Dryas octopetala* and *Gypsophila repens* exhibited highest likelihoods at $K = 4$ and were likewise discarded for $K = 5$. Thus, 19 species revealed clear clustering results at $K = 5$ (Table 1).

Correlations between phylogeography and ecology

Computations of GSS indices were performed between pairs of species. Whereas pairwise similarity of the ecological indicators moisture, temperature, light and of successional type were not correlated with GSS, the effect of soil reaction was highly significant. This held true both when performing individual Mantel tests between the GSS matrices (i.e. different GSS matrices were designed according to the K number of clusters considered) and the ecological indicator matrices (Table 2), and when computing a global test of congruence among all distance matrices (Table 3; Legendre & Lapointe 2004). The pattern found in silicicolous species mainly showed an arrangement of phylogeographic clusters along the East–West axis, i.e. contact zones were perpendicular to the principal orientation of the Alps. In contrast, calcicolous species showed a predominant arrangement of clusters in the North or the South, i.e. along the Alpine arc (Fig. 1). Species with unspecific substrate type affinities revealed patterns with components of both types of cluster orientations.

When comparing GSS, we found a significant effect of soil reaction at all K-values tested (Table 2). Species pairs sharing substrate type affinities showed significantly higher GSS than species pairs growing on different substrate types (Fig. 2). Generally, the level of GSS increased with increasing K, except for calcicolous species which showed a lower similarity at $K = 5$ than at $K = 4$. Calcicolous species showed a trend to higher similarity than did silicicolous species at $K = 3$–4. Unspecific species again showed an intermediate position (Fig. 2).

DISCUSSION

In this study, we used a large-scale comparison of single-species phylogeographic patterns to identify the potential ecological parameters that correlate with the spatial genetic structure of populations in a heterogeneous environment.
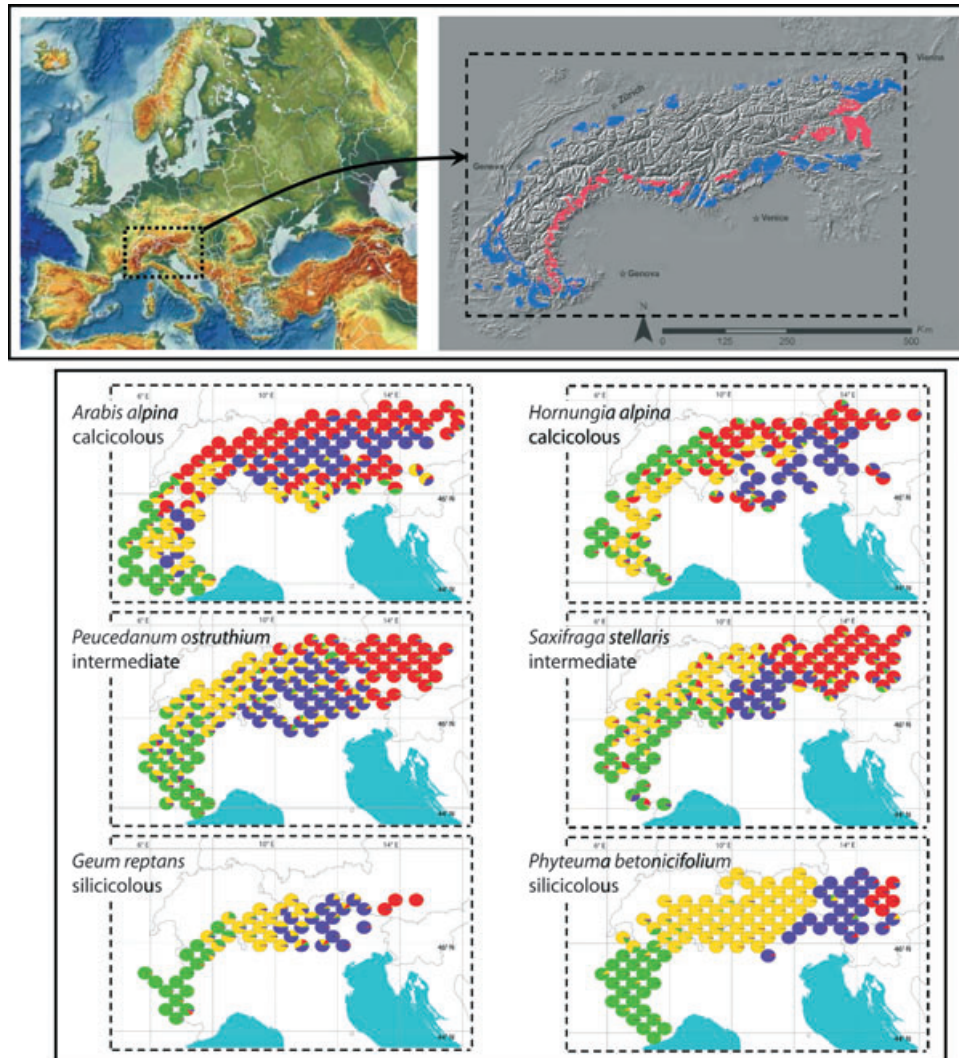
Figure 1 Sampling area, distribution of refugia and phylogeographic patterns of six representative Alpine species. The European Alps (above) with potential peripheral refugia of Alpine plants on siliceous (red) and calcareous (blue) bedrock during the last glacial maximum (map modified from Schönswetter *et al.* 2005). Phylogeographic structures (below) based on amplified fragment length polymorphisms (AFLPs) exemplified by pairs of Alpine plant species with the same substrate type affinities (calcicolous, intermediate, silicicolous species). In each species, pie charts correspond to the Bayesian assignment probabilities per sampling location (Pritchard *et al.* 2000) exemplified for each of $K = 4$ phylogeographic clusters.

Whereas previous studies were unable to address convincingly this long-debated issue, we found that one specific ecological factor, the substrate type affinity, i.e. soil reaction *sensu* Landolt (1977), may be considered a major driver of plant phylogeography. Hence, this study shows that the phylogeographic patterns found in widespread Alpine plants demonstrate a high level of congruence when considering species growing on the same substrate type. While the phylogeographic clusters of those species confined to acidic soils on siliceous bedrock were arranged in an East–West direction, species occurring on alkaline soils on calcareous bedrock exhibited a North–South alignment of phylogeo-

graphic clusters (Fig. 1). Moreover, soil reaction significantly explained differences in the similarity of spatial genetic structures among species pairs (Tables 2 and 3). Species pairs growing on the same substrate type showed phylogeographic patterns that were more similar than those of pairs growing on different substrates (Fig. 2).

Even though it has been argued that present-day climatic factors, e.g., summer drought, may be the cause of current phylogeographic patterns (Piñeiro *et al.* 2007), explanations for our results can be given in the light of the putative location of Quaternary refugia and re-colonization routes, which both were distributed differently according to

Table 2 Mantel Spearman's correlation coefficients *r* between ecological trait similarity and genetic-structure similarity (Alvarez *et al.* 2008)

Ecological indicator	$K = 2$ (26 species)	$K = 3$ (24 species)	$K = 4$ (24 species)	$K = 5$ (19 species)
Light	−0.0567	0.0412	−0.0185	0.0281
Moisture	−0.0688	−0.0481	−0.0602	0.0549
Soil reaction	0.2287*	0.2853*	0.2609*	0.3757*
Temperature	0.0687	0.0375	0.0233	0.0365
Successional type	−0.0302	0.0578	0.0331	0.0755

Correlation coefficients *r* are based upon ranks as calculated with CADM (Legendre & Lapointe 2004). One-tailed probabilities of the Mantel statistics after 100 000 permutations are computed separately for the different numbers of phylogeographic clusters, *K,* as obtained with STRUCTURE (Pritchard *et al.* 2000). *Significant test statistics at $P < 0.05$ after Bonferroni correction (20 tests; *P*-value cut-off = 0.0025).

Table 3 Results of the global Congruence Among Distance Matrices (Legendre & Lapointe 2004) test computed among distance matrices of genetic structure and ecological indicators

| Factor tested	*P*-value
Global test (H0: incongruence of all matrices)	0.00001*
Genetic structure similarity with $K = 2$	0.00001*
Genetic structure similarity with $K = 3$	0.00001*
Genetic structure similarity with $K = 4$	0.00001*
Genetic structure similarity with $K = 5$	0.00001*
Light	0.31469
Moisture	0.11147
Soil reaction	0.00494*
Successional type	0.02233
Temperature	0.12485

P-values represent probabilities of χ^2 after 10 000 permutations (Friedman's $\chi^2 = 465.99330$; Kendall's *W* with correction for tied ranks = 0.27411) and *a posteriori* tests of congruence were calculated for each matrix separately. *Significant test statistics at $P < 0.05$ after Bonferroni correction (9 tests; *P*-value cut-off = 0.0056).

bedrock type. Putative refugial areas of calcareous and siliceous bedrock show marked patchiness along the Alpine arc (Fig. 1), and species with different substrate type affinities have been hypothesized to have survived glacial periods in different refugia owing to the distinctive geological pattern of the Alps (Schönswetter *et al.* 2005). Putative refugia with predominantly siliceous bedrock were mainly present along the southern and eastern Alpine border, whereas those with calcareous bedrock were located at the northern and south-western border of the Alpine arc. There is a striking coincidence between the distribution of putative refugia and the phylogeographic patterns found here in silicicolous and calcicolous species, respectively (Fig. 1; Brockmann-Jerosch & Brockmann-Jerosch 1926; Stehlik 2000; Tribsch & Schönswetter 2003; Schönswetter *et al.* 2005). Our results suggest that with the beginning of climate warming after the Last Glacial Maximum *c.* 18,000 years BP, populations followed suitable habitat patches on either bedrock type when spreading from their refugia. Since alpine soils are typically shallow and continuously ablated due to erosion, geologic bedrock often directly influences alpine plant life and species occurrence (Ozenda 1988; Körner 2003). Thus, substrate type and its spatial availability have shaped the migration pathways of species, probably according to the concept of concerted migration routes (Sanmartin *et al.* 2007) and with regard to interspecific competition related to physiological optima (Körner 2003). However, stability in niche realization and competitive interactions cannot be presumed on the long term. Therefore, plant communities changed over time, and former vegetation assemblages may have no present-day analogues (MacDonald *et al.* 2008). Nevertheless, our data imply that species with similar substrate type affinities were channelled to similar migration routes on the large scale, though possibly at different times. Given the above interpretation, our results suggest that large areas where a particular substrate type dominated may have acted as avenues potentially facilitating rapid expansion of those species favouring the respective substrate type. On the other hand, such areas may have acted as barriers for the admixture of different refugial genetic lineages in species with an affinity to the alternative bedrock type. This fact is exemplified in calcicolous species. Here, the Central Alpine massifs, which are dominated by siliceous bedrock, have strongly impeded the intermixing of southern and northern refugial lineages, enhancing the level of congruence in migration pathways among calcicolous species. Accordingly, re-colonization routes followed a longitudinal direction from calcareous peripheral refugia, resulting in a respective North–South alignment of the phylogeographic clusters (Fig. 1). In silicicolous species, on the other hand, the distinctiveness of phylogeographic clusters was likely caused by the distribution of refugia with siliceous bedrock at the Eastern and Southern Alpine borders.

In contrast to substrate type, the other ecological factors studied here either do not vary at large spatial scales or they are not constant over time and, thus, do not leave a prominent imprint on species' migration routes and genetic structures. Nevertheless, we do not rule out that these or other factors, such as life-history traits, further impacted phylogeographic patterns and genetic diversity (Hamrick & Godt 1996; Nybom & Bartish 2000; Thiel-Egenter *et al.* 2009) in Alpine plants. An example might be successional type, for which we observed a trend to affect GSS (Table 3). However, species characteristics related to substrate, such as

Figure 2 Least-square means of genetic-structure-similarity (GSS) values (Alvarez *et al.* 2008) between pairs of species with the same or different substrate type affinities. Calculations were done in function of the kind of species pair considered for different *a priori* numbers of genetic clusters (*K* ranging from 2 to 5). *P*-values refer to the results from multivariate analysis of variation (MANOVA); letters 'a' and 'b' indicate significant differences between soil reaction types (at $P < 0.05$) within a single *K*-value.
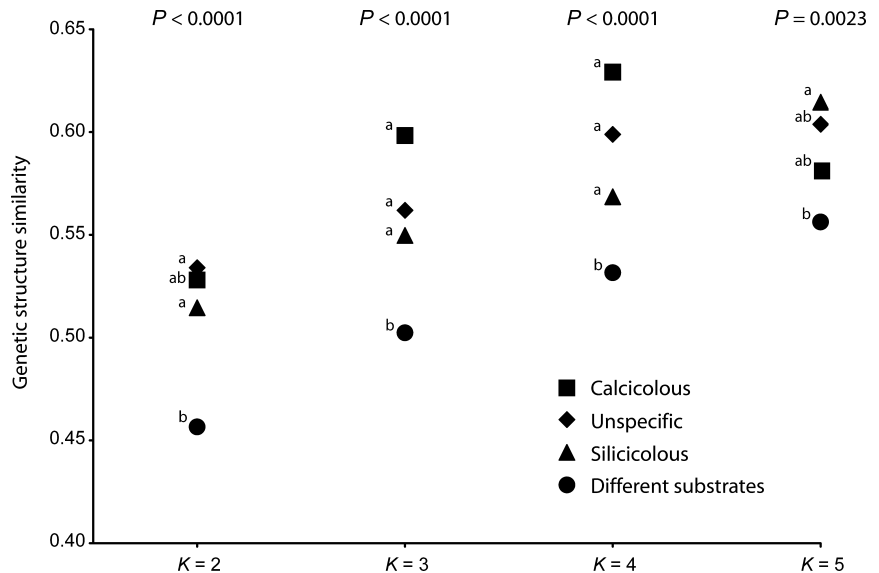
physiology and interspecific competition, seem sufficiently restrictive to have strongly driven plant migration along certain pathways following climatic changes.

CONCLUSIONS

Our similarity-based approach contributes to the understanding of the long-debated relative roles of stochastic processes and ecological determinants in shaping biogeographic patterns. The finding that some plant species may migrate in concert according to their substrate type affinity provides valuable information to understand the determinants of community structure and to predict responses to future climate change. The availability and spatial configuration of habitats for species with different ecological demands, as shown for substrate type in this study, to a large degree may determine the species' ability for future migrations. Therefore, it would seem valuable to conduct large-scale studies in a wide range of organism groups to identify the potential role of ecological variables for the geographic distribution of gene pools under climate change scenarios. This study further substantiates the relevance of (post-)glacial history for the formation, survival and current distribution of evolutionary lineages in Alpine species. The respective spatial patterns should be considered in future conservation efforts, which are usually restricted to entire species as the relevant taxonomic unit.

ACKNOWLEDGEMENTS

REFERENCES

Aeschimann, D., Lauber, K., Moser, D.M. & Theurillat, J.-P. (2004). *Flora Alpina*. Haupt, Bern.

Alsos, I.G., Eidesen, P.B., Ehrich, D., Skrede, I., Westergaard, K., Jacobsen, G.H. *et al.* (2007). Frequent long-distance plant colonization in the changing Arctic. *Science*, 316, 1606–1609.

Alvarez, N., Arrigo, N. & IntraBioDiv Consortium (2008). SIMIL: an R (CRAN) scripts collection for computing genetic structure similarities based on STRUCTURE 2 outputs. *Mol. Ecol. Res.*, 8, 757–762.

Avise, J.C. (2009). Phylogeography: retrospect and prospect. *J. Biogeogr.*, 36, 3–15.

Bennett, K.D. (1990). Milankovitch cycles and their effects on species in ecological and evolutionary time. *Paleobiology*, 16, 11–21.

Bettin, O., Cornejo, C., Edwards, P.J. & Holderegger, R. (2007). Phylogeography of the high alpine plant *Senecio halleri* (Asteraceae) in the European Alps: *in situ* glacial survival with postglacial stepwise dispersal into peripheral areas. *Mol. Ecol.*, 16, 2517–2524.

Brockmann-Jerosch, H. & Brockmann-Jerosch, M. (1926). Die Geschichte der Schweizerischen Alpenflora. In: *Das Pflanzenleben der Alpen* (ed. Schröter, C.). Raustein, Zürich, pp. 1110–1215.

Clements, F.E. (1916). *Plant Succession: An Analysis of the Development of Vegetation*. Carnegie Institution of Washington, Washington.

Comes, H.P. & Kadereit, J.W. (1998). The effect of quaternary climatic changes on plant distribution and evolution. *Trends Plant Sci.*, 3, 432–438.

Cox, C.B. & Moore, P.D. (2005). *Biogeography: An Ecological and Evolutionary Approach*, 7th edn. Blackwell, Malden.

Darwin, C. (1859). *On the Origin of Species by Natural Selection*. John Murray, London.

De Candolle, A. (1820). *Essai Elémentaire de Géographie Botanique*. Levrault, Strasbourg.

Diamond, J.M. (1975). Assembly of species communities. In: *Ecology and Evolution of Communities* (eds Cody, M.L. & Diamond, J.M.). Harvard University Press, Cambridge, pp. 342–344.

Diekmann, M. (2003). Species indicator values as an important tool in applied plant ecology – a review. *Basic Appl. Ecol.*, 4, 493–506.

Falush, D., Stephens, M. & Pritchard, J.K. (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Mol. Ecol. Notes*, 7, 574–578.

Gleason, H.A. (1926). The individualistic concept of the plant association. *Bull. Torr. Bot. Club*, 53, 7–26.

Gotelli, N.J. & McCabe, D.J. (2002). Species co-occurrence: a meta-analysis of J.M. Diamond's assembly rules model. *Ecology*, 83, 2091–2096.

Graham, R.W., Lundelius, E.L. Jr, Graham, M.A., Schroeder, E.K., Toomey, R.S. III, Anderson, E. *et al.* (1996). Spatial response of mammals to late quaternary environmental fluctuations. *Science*, 272, 1601–1606.

Gugerli, F., Englisch, T., Niklfeld, H., Tribsch, A., Mirek, Z., Ronikier, M. *et al.* (2008). Relationships among levels of biodiversity and the relevance of intraspecific diversity in conservation – a project synopsis. *Perspect. Plant Ecol. Evol. Syst.*, 110, 259–281.

Hamrick, J.L. & Godt, M.J.W. (1996). Effects of life history traits on genetic diversity in plant species. *Phil. Trans. R. Soc. Lond. B*, 351, 1291–1298.

Hewitt, G.M. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.*, 58, 247–276.

Hewitt, G.M. (1999). Post-glacial re-colonization of European biota. *Biol. J. Linn. Soc.*, 68, 87–112.

Hewitt, G.M. (2000). The genetic legacy of the Quaternary ice ages. *Science*, 405, 907–913.

von Humboldt, A. (1805). *Essai sur la Géographie des Plantes*. Levrault, Paris.

Körner, C. (2003). *Alpine Plant Life*, 2nd edn. Springer, Berlin.

Landolt, E. (1977). Oekologische Zeigerwerte zur Schweizer Flora. *Veröff. Geobot. Inst. ETH Zürich*, 64, 1–208.

Legendre, P. & Lapointe, F.-J. (2004). Assessing the congruence among distance matrices: single malt Scotch whiskies revisited. *Aust. N. Z. J. Stat.*, 46, 615–629.

MacDonald, G.M., Bennett, K.D., Jackson, S.T., Parducci, L., Smith, F.A., Smol, J.P. *et al.* (2008). Impacts of climate change on species, populations and communities: palaeobiogeographical insights and frontiers. *Prog. Phys. Geog.*, 32, 139–172.

McGill, B.J., Hadly, E.A. & Maurer, B.A. (2005). Community inertia of quaternary small mammal assemblages in North America. *Proc. Natl. Acad. Sci. U.S.A.*, 102, 16701–16706.

Nybom, H. & Bartish, I.V. (2000). Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspect. Plant Ecol. Evol. Syst.*, 3, 93–114.

Ozenda, P. (1988). *Die Vegetation der Alpen im Europäischen Gebirgsraum*. Fischer, Stuttgart.

Petit, R.J., Brewer, S., Bordács, S., Burg, K., Cheddadi, R., Coart, E. *et al.* (2002). Identification of refugia and post-glacial colonisation routes of European white oaks based on chloroplast DNA and fossil pollen evidence. *Forest Ecol. Manage.*, 6, 49–74.

Piñeiro, R., Aguilar, J..F., Munt, D.D. & Feliner, G.N. (2007). Ecology matters: Atlantic-Mediterranean disjunction in the sanddune shrub *Armeria pungens* (Plumbaginaceae). *Mol. Ecol.*, 16, 2155–2171.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.

Sanmartin, I., Wanntorp, L. & Winkworth, R.C. (2007). West Wind Drift revisited: testing for directional dispersal in the Southern Hemisphere using event-based tree fitting. *J. Biogeogr.*, 34, 398–416.

Schönswetter, P., Stehlik, I., Holderegger, R. & Tribsch, A. (2005). Molecular evidence for glacial refugia of mountain plants in the European Alps. *Mol. Ecol.*, 14, 3547–3555.

Skrede, I., Borgen, L. & Brochmann, C. (2009). Genetic structuring in three closely related circumpolar plant species: AFLP versus microsatellite markers and high-arctic versus arctic-alpine distributions. *Heredity*, 102, 293–302.

Stehlik, I. (2000). Nunataks and peripheral refugia for alpine plants during quaternary glaciation in the middle part of the Alps. *Bot. Helv.*, 110, 25–30.

Stehlik, I., Blattner, F.R., Holderegger, R. & Bachmann, K. (2002). Nunatak survival of the high Alpine plant *Eritrichium nanum* (L.) Gaudin in the central Alps during the ice ages. *Mol. Ecol.*, 11, 2027–2036.

Taberlet, P. & Cheddadi, R. (2002). Quaternary refugia and persistence of biodiversity. *Science*, 297, 2009–2010.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.-G. & Cosson, J.F. (1998). Comparative phylogeography and postglacial colonization routes in Europe. *Mol. Ecol.*, 7, 453–464.

Thiel-Egenter, C., Gugerli, F., Alvarez, N., Brodbeck, S., Cieślak, E., Colli, L. *et al.* (2009). Effects of species traits on the genetic diversity of high-mountain plants: a multi-species study across the Alps and the Carpathians. *Glob. Ecol. Biogeogr.*, 18, 78–87.

Tribsch, A. & Schönswetter, P. (2003). Patterns of endemism and comparative phylogeography confirm palaeo-environmental evidence for Pleistocene refugia in the Eastern Alps. *Taxon*, 52, 477–497.

Tribsch, A., Englisch, T., Gugerli, F., Holderegger, R., Niklfeld, H., Steinmann, K. *et al.* (2009). Integrating data across biodiversity levels; the project IntraBioDiv. In: *Data Mining for Global Trends in Mountain Biodiversity* (eds Spehn, E.M. & Körner, C.). CRC Press, Taylor & Francis, Boca Raton (in press).

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M. *et al.* (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.*, 23, 4407–4414.

Whittaker, R.H. (1967). Gradient analysis of vegetation. *Biol. Rev.*, 42, 207–264.

Wiens, J.J. & Donoghue, M.J. (2004). Historical biogeography, ecology and species richness. *Trends Ecol. Evol.*, 19, 639–644.

Williams, M., Dunkerley, D., De Deckker, P., Kershaw, P. & Chappell, J. (1998). *Quaternary Environments*. Edward Arnold, London.