Break zones in the distributions of alleles and species in alpine plants

Conny Thiel-Egenter1†§, Nadir Alvarez3††, Rolf Holderegger1, Andreas Tribsch1,4, Thorsten Englisch1, Thomas Wohlgemuth1, Licia Colli5, Myriam Gaudeul6,7, Ludovic Gielly6, Nejc Jogan8, Hans Peter Linder9, Riccardo Negrini6, Harald Niklfeld3, Marco Pellecchia5, Delphine Rious6, Peter Schönswetter3††, Pierre Taberlet6, Marcela van Loo3††, Manuela Winkler3,10, IntraBioDiv Consortium1 and Felix Gugerli3†*

ABSTRACT

Aim We test for the congruence between allele-based range boundaries (break zones) in silicicolous alpine plants and species-based break zones in the silicicolous flora of the European Alps. We also ask whether such break zones coincide with areas of large elevational variation.

Location The European Alps.

Methods On a regular grid laid across the entire Alps, we determined areas of allele- and species-based break zones using respective clustering algorithms, identifying discontinuities in cluster distributions (breaks), and quantifying integrated break densities (break zones). Discontinuities were identified based on the intra-specific genetic variation of 12 species and on the floristic distribution data from 239 species, respectively. Coincidence between the two types of break zones was tested using Spearman’s correlation. Break zone densities were also regressed on topographical complexity to test for the effect of elevational variation.

Results We found that two main break zones in the distribution of alleles and species were significantly correlated. Furthermore, we show that these break zones are in topographically complex regions, characterized by massive elevational ranges owing to high mountains and deep glacial valleys. We detected a third break zone in the distribution of species in the eastern Alps, which is not correlated with topographic complexity, and which is also not evident from allelic distribution patterns. Species with the potential for long-distance dispersal tended to show larger distribution ranges than short-distance dispersers.

Main conclusions We suggest that the history of Pleistocene glaciations is the main driver of the congruence between allele-based and species-based distribution patterns, because occurrences of both species and alleles were subject to the same processes (such as extinction, migration and drift) that shaped the distributions of species and genetic lineages. Large elevational ranges have had a profound effect as a dispersal barrier for alleles during post-glacial immigration. Because plant species, unlike alleles, cannot spread via pollen but only via seed, and thus disperse less effectively, we conclude that species break zones are maintained over longer time spans and reflect more ancient patterns than allele break zones.

Keywords AFLP, allele distribution patterns, alpine plants, elevational range, European Alps, floristic areas, genetic structure, glacial survival, species distribution patterns.
INTRODUCTION

The history of the Northern Hemisphere was greatly affected by Pleistocene climatic oscillations. Vast ice shields periodically covered major areas, causing large-scale displacements of suitable habitat for species (Hewitt, 2000). During cold periods, species were restricted to refugia, mostly outside the glacial area, that were ice- and snow-free during at least part of the year (Taberlet & Cheddadi, 2002). Processes within these refugia and during range expansion/retraction involved local extinction, migration, drift and adaptation (Hewitt, 2004) at the genetic and/or the species level. Therefore, different glacial refugia have probably harboured different genetic lineages of formerly widely distributed species, as well as different sets of species.

With the retreat of glaciers after the Last Glacial Maximum about 18,000 years ago, species started to re-expand their distributional ranges out of refugia. A variety of post-glacial expansion routes have been proposed for different organisms. In Europe, species were shown to mostly migrate northwards from refugia in southern peninsulas (the Balkans, Italy, Iberia; Taberlet et al., 1998; Hewitt, 1999), but there were also range expansions from previously overlooked refugia north of the Alps which also led southwards to reach the Alpine range (Bhagwat & Willis, 2008; Hofreiter & Stewart, 2009). Mountain ranges may be seen as melting pots where species and genetic lineages from different refugia met after recolonization, leading to high levels of diversity (Körner, 2003; Petit et al., 2003; Gugerli & Sperisen, 2010).

The comparison of genetic patterns across species has revealed conflicting results, showing both congruent (Solits et al., 1997; Moritz & Faith, 1998; Swenson & Howard, 2005) and non-congruent patterns among species (Taberlet et al., 1998; Solits et al., 2006). Congruence in the biogeographical break lines between floristic and zoogeographic regions was described by early biogeographers (de Candolle & Sprengel, 1821; Wallace, 1876) and has recently been reconsidered by Cox (2001). The comparison of geographical patterns of genetic variation among multiple, co-distributed taxa (Avise, 2000) has strong parallels with historical biogeography (Crisci, 2001). In both fields, the assemblage of either genetic lineages or biota is explained by shared history (Arbogast & Kenagy, 2001). Hence, if there were a common set of historical events acting in parallel on alleles within species and species within regional floras, the resulting distribution patterns should be similar. Despite numerous studies at either level, congruence in the distribution patterns and break lines of alleles and species has not yet been investigated.

Congruence among allele distribution patterns or between allele and species distribution patterns is more likely when topographic characteristics (i.e. traits related to the physical geography) of the studied area are distinctive (Kuo & Avise, 2005), when species show similar ecologies (Alvarez et al., 2009), and when the same analytical methods are applied. Therefore, we used the European Alps to test whether alpine plant species showed congruent break zones at both allele and species distribution levels. Due to the heterogeneous nature of alpine topography, one might assume many potential barriers for dispersal of alleles and species. In addition, glacial history played a prominent role in modifying distributions of alleles and species. During several periods of the Pleistocene, the climatic snowline mostly reached down to the large glaciers that covered the valleys in the Central Alps, potentially leaving only a few very restricted locations for plant survival (Tribsch, 2004). The location of presumed glacial refugia of alpine plants has been debated since the last century, and a synopsis map has been published recently, with a focus on plants that occur on markedly siliceous soils (Schönswetter et al., 2005).

In the present study, we chose silicicolous taxa, i.e. those that are restricted to siliceous bedrock in the alpine belt, because substrate is a key factor determining species distribution in the Alps (Wohlgemuth, 2002; Ewald, 2003; Alvarez et al., 2009). We further avoided drawbacks and biases often encountered in meta-analyses and reviews, e.g. spatially inconsistent data sets, by adhering to a regular sampling scheme on an identical grid, using the same genetic marker type and the same statistical methods in data analysis at both allele and species levels.

We hypothesized that alleles and species sharing a common history of glaciations underwent parallel processes within refugia, were equally restricted by substrate ecology, and experienced the same distinctive alpine topography during range retractions and expansions. Taking silicicolous plants of the European Alps as a study system, we tested for congruence between break zones based on allele and on species distributions. Therefore, our null hypothesis was that there is no such congruence in the two patterns. Additionally, we expected that these break zones would correlate with a key topographic parameter, the local elevational range, again attempting to reject the null hypothesis of no correlation between the two variables.

MATERIALS AND METHODS

Sampling scheme

We adopted the regular grid system widely used for floristic mapping in the European Alps, with a cell size of 12° latitude and 20’ longitude (c. 22 × 25 km, c. 570 km²; Gugerli et al., 2008), resulting in 353 cells reaching a threshold of 1000 m a.s.l. In one location in every second cell (in total 140 cells; see Appendix S2 and Fig. 1), we sampled for genetic analyses three individuals of each of twelve abundant, silicicolous alpine plant species, widely distributed over the entire Alpine Arc (Table 1). Vouchers for each species and sampling location were deposited at the herbaria of Neuchâtel (NEU), Vienna (WU) and Zürich (Z + ZT). Species may be considered as phylogenetically independent replicates, as no hybridization has been reported between pairs of study species, except for the two Geum congeners. Only three of the twelve study species are known to hybridize with other taxa within the Alps (Hess et al., 1976–1980). Species chosen comprise a broad range of
taxa and life-history traits (Thiel-Egenter et al., 2009a) that make them a representative sample of widespread, common plants of alpine habitats.

**Genetic data collection**

DNA was extracted from 10 mg of dried plant material. DNA extractions and amplified fragment length polymorphism (AFLP) fingerprinting were conducted in five laboratories, each working with several full species data sets (Gugerli et al., 2008). All laboratories used replicates to conduct internal quality checks, following Bonin et al. (2004). DNA extractions were carried out using a cetyl trimethyl ammonium bromide (CTAB) protocol for *Trifolium alpinum* or the Qiagen DNeasy 96 Plant Kit (Qiagen, Hilden, Germany) for all other species (Gugerli et al., 2008). Each partner used lab-specific protocols and methods for AFLPs, but all following the principal protocol of Vos et al. (1995). The restriction fragments were separated by electrophoresis on 8% polyacrylamide gels (*Trifolium alpinum*) or on automated sequencers, together with internal size standards (all other species; Gugerli et al., 2008; Thiel-Egenter et al., 2009b). For further analysis, binary matrices of presence/absence of AFLP fragments were used for all 12 species.

**Floristic data collection**

Data on the distribution of alpine plant species from the European Alps were compiled for all grid cells based on: (1)
published and unpublished floristic data, (2) analyses of herbarium material, and (3) field surveys (Gugerli et al., 2008). For the analysis, we included 239 silicicolous species, described in Flora alpina (Aeschimann et al., 2004) as being common on siliceous substrates but not common on calcareous substrates. As we used silicicolous species only, we only included those 172 cells of the regular grid where siliceous bedrock is frequent (>10% of area above 1000 m a.s.l.; Fig. 1). For further analysis, we used a presence/absence matrix with 239 species (see Appendix S2).

Calculation of break zones in allele distribution patterns

We conducted separate break analyses for each of the 12 silicicolous species (Thiel-Egenter et al., 2009b). Briefly, we applied model-based Bayesian population clustering, designed to cluster population genetic data (structure 2.1; Pritchard et al., 2000) for K = 2–6 clusters, with 20 replicates at each level of K, and parameters set as described in Thiel-Egenter et al. (2009b). We did not consider K-values >6 because we wanted to identify multi-species large-scale genetic clusters rather than local gene pools, which typically arise at higher K values. Resulting cluster membership probabilities were used to calculate location pairwise Euclidean distances for all K-values and replicates. By integrating across the five K-values, we deliberately considered the largely hierarchical structure of clustering with increasing levels of K. Subsequently, we used Delaunay triangulation to identify geographically neighbouring locations and searched for the largest distances between them with Monmonier’s maximum difference algorithm (Monmonier, 1973) in barrier 2.2 (Manni et al., 2004). The number of barriers was set to five in concordance with the structure analysis (Kmax−1). Using all matrices obtained (i.e. different K-values and replicates), barrier calculates how many times a break line segment evolves and accordingly assigns a robustness value to each segment.

To search for zones of highest break densities among the 12 species, we imported the break lines of each species into ArcGIS 9.1 (ESRI, Redlands, CA, USA) and used the line density tool with a search radius of 40 km (comprising about nine grid cells) and an output cell size of 10 × 10 km (about half a grid cell). The length of the portion of each break line that falls within this circle was multiplied by the break line’s weight (robustness of break line segments). These values were then summed and the total was divided by the circle’s area. The resulting unit-free break densities were visualized by colour density.

Calculation of break zones in species distribution patterns

To conduct a similar cluster analysis for the species distribution data as for the allele data set, we applied fuzzy clustering (FANNY; Kaufman & Rousseeuw, 1990) for K = 2–6 clusters using the package cluster v.1.11.4 in R (Martin Maechler, ETH Zürich, Switzerland). The fuzzy clustering algorithm attempts to minimize a total dispersion function based on dissimilarities. The algorithm stops when the function converges, i.e. when two subsequent iterations show the same result. Fuzzy clustering allows a grid cell to be assigned to various clusters, thus showing a realistic pattern when cluster transitions are not discrete but admixed. As recommended for the analysis of biotic elements (Hausdorf & Hennig, 2003; Hennig & Hausdorf, 2004) in which it is important to adjust for differences in the number of species (Hennig & Hausdorf, 2006), we calculated Kulczynski distances between pairs of grid cells, based on presence/absence matrices. Using this distance measure, subsequent clustering will group areas with few species into the same biotic element as areas with many species if the former are a subset of them (Hennig & Hausdorf, 2006). We used cluster memberships of fuzzy clustering to calculate Euclidean distances for all pairs of clusters at all levels of K to integrate across K-values. As described for genetic break analysis, barrier 2.2 was used to detect breaks in the species data set, and zones of highest densities of species break lines were calculated in ArcGIS 9.1 as described above.

Since break density in species distributions did not meet assumptions of normality according to Kolmogorov–Smirnov at P < 0.05, we used Spearman’s ρ correlation coefficient to test for a relationship between mean break densities per grid cell in allele-based versus species-based distribution patterns, calculated in spss 11.0 (SPSS, Chicago, IL, USA).

Topography

A 200-m digital elevation model for the Alps (MONA-DEM, Geosys Data Inc., Balma, France) was used to infer elevational range per grid cell as a representative of alpine topography. Elevational range was calculated as the difference between the highest and lowest elevation per grid cell (see Appendix S3). A linear regression model was performed in spss with both mean allele-based and species-based break densities per grid cell as dependent variables and elevational range per grid cell as an independent variable. Residuals of the regression analysis of both break densities were normally distributed according to probability plots.

Dispersal capacity

To test whether dispersal mode may explain why some species are more widespread than others, the species of our floristic list were categorized into long- and short-distance dispersers (LDD and SDD, respectively) based on Landolt et al. (2010). Information for two species was inadequate, leaving 237 data entries. The study range was separated into four areas according to the three species-based break zones found in the previous analyses. Cells with break densities ≥0.06 were considered as belonging to either of the three break zones, and the remaining cells assigned to one of the four areas. For each species we recorded the number of areas in which it occurred.
We compared the proportion of LDD and SDD with the number of inhabited areas to see whether the former occurred across a higher number of species-based break zones than the latter. In parallel, independence of rows and columns in a $2 \times 4$ contingency table (with dispersal type – SDD or LDD – in rows and the exact number of covered areas – from one to four – in columns) was tested by means of a generalized Fisher’s exact test (Fisher, 1935) using the struc program (Raymond & Rousset, 1995) with $10^7$ iterations.

**RESULTS**

**Allele distribution patterns**

Analyses of individual allele distributions yielded distinct breaks in all of the 12 species studied (Fig. 2). Robustness of the break segments ranged from 1 to 100% per species. At the allele level, 1200 matrices (20 runs for each of the break segments ranged from 1 to 100% per species) were used to calculate break zones. When overlaying single species break lines, break densities ranged from 0 to 18.5. We found two main break zones in allele distribution patterns (Fig. 3a). One zone was in the western Alps, in the area of the two inner alpine valleys Aosta and Valais (referred to as the Aosta zone hereafter) and the other was on the transition between the western and eastern part of the Alps between Lake Garda and Innsbruck (Brenner zone).

**Species distribution patterns**

Break analysis of the 239 silicicolous species of the Alps resulted in distinct breaks mainly perpendicular to the main orientation of the Alpine Arc (Fig. 4). Robustness of single break segments ranged from 20 to 80%. When compared with the allele-based analysis, densities of species distribution breaks were lower, ranging from 0 to 0.10. However, relative to the number of matrices used, both levels showed a similar range of density, because only five matrices ($K = 2–6$) were incorporated at the species level. We found three break zones based on species distributions (Fig. 3b): one in the western Alps, corresponding to the Aosta zone, the second between the western and eastern part of the Alps (Brenner zone) and the third in the eastern Alps (referred to as the Tauern zone).

**Correlation of allele- and species-based break zones**

Allele-based and species-based break zones overlapped in two regions: the zone in the western Alps and the zone between a western and an eastern part of the Alps (Fig. 3). Hence, we found a significant correlation between the break zones of the allele and species data sets (Spearman’s $\rho = 0.28$, d.f. = 171, $P < 0.001$).

**Topography**

We tested whether the location of break zones was influenced by topography, as measured by the elevational range per grid cell. Across the 172 cells considered, we found a strong linear relationship between mean break density per grid cell based on allele distributions and elevational range per cell ($r = 0.712$, $P < 0.001$). In contrast, mean species-based break density per grid cell was not significantly correlated with elevational range ($r = 0.121$, $P = 0.114$).

**Dispersal capacity**

We found 150 LDD and 87 SDD in our list of high-mountain plant taxa. The frequencies of both categories logically decreased with increasing number of areas occupied, but more strongly so in SDD than in LDD (Table 2). Thus, among the silicicolous species of the Alps studied here, LDD tended to occur across a larger range than SDD. In the $2 \times 4$ contingency table, each class of dispersal mode was translated into the following vectors (composed of the taxon numbers covering exactly one, two, three or four areas): LDD = (35, 21, 27, 67) and SDD = (30, 19, 19, 19). As shown by the generalized Fisher’s exact test, the frequency of LDD significantly increased with the number of areas occupied (unbiased estimate of $P = 0.0041$).

**DISCUSSION**

Migration of species as a consequence of Pleistocene glaciation had a strong effect on both the phylogeographical and biogeographical patterns in the Northern Hemisphere, represented by distributions of alleles and species, respectively. Consequently, we expected and found clear signals that the patterns of allele and species distributions in silicicolous alpine plant species are largely congruent. This finding allowed us to reject our null hypothesis that patterns of alleles and species distributions show no correlation. Both allele and species distributions are interpreted to be the consequence of similar forces driving retraction to and re-colonization from glacial refugia. Knowledge about the nature and location of such refugia, which have been dealt with elsewhere (Tribsch & Schönswetter, 2003; Stewart et al., 2010) and probably differ among the species investigated here, is not required to test the hypotheses in this study. However, the European Alps represent a suitable system for studying the effects of plant colonization into previously uninhabited areas on patterns of allele and species distributions because of the wealth of detailed floristic and geological data available. Break densities as identified in our data sets may result from strong barriers in few species or from weak signals in most species. Indeed, we found individualistic patterns of genetic breaks in the 12 study species (Fig. 2), which amalgamated into zones of high-density breaks (Fig. 3). Identifying such a general trend based on a number of singular, possibly even contradictory results, is similar to performing a meta-analysis, in which an overall effect is quantified given the variation of outcomes in single experiments (Arnvist & Wooster, 1995). However, we argue that the spatial coincidence of break zones with phylogeographical and biogeographical breaks described previously (e.g.
Figure 2 Allele-based breaks in each of the 12 silicicolous alpine plant species analysed across the European Alps. The thickness of the break line refers to the robustness of breaks (see Materials and Methods).
Merxmüller, 1952; Ozenda, 1988) provides a strong argument for the reliability of our findings. Further support is given by the positive relationship between the occurrence of break zones and large elevational variation, indicating that topographical barriers to gene flow had a distinct effect on the patterns observed. As we outline below, historical processes such as range shifts, contractions and expansions complement ecological restrictions (Alvarez et al., 2009) to shape current allele and species ranges. We expect that the same will hold true for future range changes under global warming.

Figure 3 Break zones identified in the European Alps. (a) Density of allele-based break zones integrated across 12 silicicolous alpine plant species. (b) Density of species-based break zones in the distribution of 239 silicicolous alpine plant species.

Figure 4 Species-based breaks in the distributions of 239 silicicolous alpine plant species across the European Alps. The thickness of the break line refers to the robustness of breaks (see Materials and Methods).
et al. and Lake Como is based mainly on the boundary between the eastern Alps along a line between Lake Constance (Pampanini, 1903) or vegetation ecology (Ozenda, 1988). This latter division is often referred to in the context of biogeography as the Penninic–Savoyic break (Merxmüller, 1952) or the Brenner line in their study of phylogeographical structures in alpine plants. However, these authors only included regionally occurring species, whereas we sampled widespread species. As outlined below with respect to differences between patterns in species and allele distributions, the discrepancy in allele-based break zone occurrences observed between the two studies may originate from the differences in the ranges of the species studied.

We account for the difference between break occurrences in allele and species distribution patterns as the result of the different dispersal abilities of species and alleles. While species disperse via seeds only, alleles can additionally spread via pollen vectors. Given the historical records of pollen dispersal, we presume that the different dispersal abilities of species and alleles account for differences in the observed break zone patterns. 

Species-based analysis revealed a third break zone in the eastern Alps along a line between Salzburg and Trieste (Tauern zone), which was only weakly retrieved in the allele-based break analysis (Fig. 3). This break was also described by Vierhapper (cited in Merxmüller, 1952) and recently by Tribsch (2004) based on East Alpine endemism. Also, Schönswetter et al. (2005) identified an eastern Alpine break line in their study of phylogeographical structures in alpine plants. However, these authors only included regionally occurring species, whereas we sampled widespread species. As outlined below with respect to differences between patterns in species and allele distributions, the discrepancy in allele-based break zone occurrences observed between the two studies may originate from the differences in the ranges of the species studied.

We account for the difference between break occurrences in allele and species distribution patterns as the result of the different dispersal abilities of species and alleles. While species disperse via seeds only, alleles can additionally spread via pollen through insects and wind (ten and two species, respectively, in our genetic data set). Both pollen vectors have been shown to be efficient for gene flow (Webb & Bawa, 1983; Ellstrand, 1992; Nason et al., 1998; White et al., 2002; Lowe et al., 2005) and can blur phylogeographical patterns (Liepelt et al., 2002) in allele distributions. By contrast, species distribution patterns are generated only by seed dispersal, and are consequently changed more slowly by dispersal than are allelic patterns. Furthermore, our species selection for the allele-based break analysis was biased towards widely distributed taxa because we searched for allele distribution break zones across the entire range of the European Alps. The fact that these species are currently distributed over the entire Alps

Table 2 Proportions of long-distance dispersers (LDD) and short-distance dispersers (SDD) in 237 silicicolous alpine plant species occurring in a given number of areas as delimited by the species-based break zones of the European Alps.

<table>
<thead>
<tr>
<th>Number of areas</th>
<th>LDD</th>
<th>SDD</th>
</tr>
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<tbody>
<tr>
<td>More than one area</td>
<td>0.767</td>
<td>0.655</td>
</tr>
<tr>
<td>More than two areas</td>
<td>0.627</td>
<td>0.437</td>
</tr>
<tr>
<td>More than three areas</td>
<td>0.447</td>
<td>0.218</td>
</tr>
</tbody>
</table>

Two concordant break zones in the distribution of alleles and species

We found two significantly correlated break zones in the distribution of alleles and species for silicicolous plant species. The western break zone was centred on the Aosta Valley (Aosta zone; Fig. 3). In a recent phylogeographical review (Schönswetter et al., 2005), a break was shown in this area for several silicicolous alpine plant taxa, and at the species level it was highlighted by earlier authors as the most important floristic break in the Alps (Merxmüller, 1952; Ozenda, 1988) and called the Penninic–Savoyic break (Merxmüller, 1952). The eastern break zone was located in the area west of the Dolomites along a line from Innsbruck to Lake Garda (Brenner zone), separating a western and an eastern part of the Alps. This break zone was situated along the north–south oriented valley of the River Adige, which leads to Brenner Pass, forming the lowest transition through the central part of the Alps. Again, Schönswetter et al. (2005) described a phylogeographical break line in the same region. In the 19th century, a Brenner line was described as an important border between westerly and easterly distributed plant species (Kerner, 1870). In the 1920s, Vierhapper (cited in Merxmüller, 1952) highlighted a middle floristic area in the Alpine Arc, delimited by exactly the two break zones found here, and Pawłowski (1970) separated centres of plant endemism in the Alps by a boundary between Innsbruck and Lake Garda. Neither of the two break zones coincides with the traditional division between the western and eastern Alps along a line between Lake Constance and Lake Como, drawn by geographers (Krebs, 1928). This latter division is often referred to in the context of biogeography (Pampanini, 1903) or vegetation ecology (Ozenda, 1988). Besides orographic features, the line between Lake Constance and Lake Como is based mainly on the boundary between the Penninic and the Austroalpine tectonic systems (Schmid et al., 2004) and a gap in Central Alpine continentality (Ozenda, 1988). As we did not find any species-based breaks in this area (Fig. 4), the latter two factors do not seem relevant to the distribution of silicicolous alpine plant taxa in the European Alps.

Shared history

The history of Pleistocene glaciations may explain the congruence between alleles and species break zones. The three areas separated by the Aosta and Brenner zones are each characterized by a relatively uniform allelic and species composition in the silicicolous flora, which may be a result of having harboured refugia during glaciations. Our findings fit well with the theory of long-term survival in glacial refugia for silicicolous alpine plant species. Presumed refugia were interpreted based on the maximum extent of the glacial ice sheet, the glacial snowline and palaeoenvironmental data (Steinhil, 2000; Tribsch & Schönswetter, 2003; Schönswetter et al., 2005): one presumed refugium at the eastern border of the Alps (Lower Austria), where large siliceous mountain areas remained unglaciated; one at the south-western border of the Alps below the glacial snowline (between Nice and Aosta); smaller presumed refugia at the southern border (Aosta–Lake Como; Lake Como–Dolomites); and potentially nunatak areas within the glacial snowline in the middle Alps (Merxmüller, 1952; Ozenda, 1988; Steinhil, 2000; Tribsch, 2004). These refugial areas may thus be seen as having harboured source populations for the recolonization of the Alps, leading to the respective break zones in areas where advancing lineages came into contact.

Different dispersal abilities of alleles and species

Species-based analysis revealed a third break zone in the eastern Alps along a line between Salzburg and Trieste (Tauern zone), which was only weakly retrieved in the allele-based break analysis (Fig. 3). This break was also described by Vierhapper (cited in Merxmüller, 1952) and recently by Tribsch (2004) based on East Alpine endemism. Also, Schönswetter et al. (2005) identified an eastern Alpine break line in their study of phylogeographical structures in alpine plants. However, these authors only included regionally occurring species, whereas we sampled widespread species. As outlined below with respect to differences between patterns in species and allele distributions, the discrepancy in allele-based break zone occurrences observed between the two studies may originate from the differences in the ranges of the species studied.

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implies good dispersal abilities. Consequently, their alleles have the potential to spread rapidly, possibly even among refugia, and break zones in allele distributions within these species may disappear more quickly than in a random sample of species. In contrast, the broader species level included heterogeneous seed dispersers, with dispersal abilities ranging from limited to wide. In agreement with expectations, we found that species with high dispersal capacity tended to occur across a wider range, i.e. they had been able to cross more species-based break zones, than did short-distance dispersers (Table 2). These two dissimilarities between allele and species distributions, i.e. that (1) alleles spread by both pollen and seed versus species spread only by seed, and (2) only a subset of widely distributed species was studied at the allele level versus an exhaustive sampling at the species level, can explain the different number of break zones found for alleles and species.

Alpine topography

An evident rationale for the location of break zones may be found in the heterogeneous alpine topography. Indeed, break density in allele distributions was strongly and positively correlated with large elevational ranges. Here, distinct genetic lineages obviously faced dispersal barriers during post-glacial recolonization. The western allele-based Aosta zone lies in an area of large elevational ranges, reaching 4230 m between the lowest and the highest elevation in cells containing the highest Alpine elevations [Mont Blanc (4810 m a.s.l.), Dom (4545 m a.s.l.)], and extending towards the broad, deeply incised, low-elevation Aosta Valley. The Brenner zone in the eastern Alps contains elevational ranges up to 3150 m in the area of the lowest valley in the Alps (see above) with high adjacent mountain chains. Thus, both low and open valleys and high elevations above the permanent snowline in two of the three high-density break zones can be considered as barriers because these areas lack or only marginally harbour suitable habitats for alpine plants. Further, steep gradients in climatic conditions could hinder highly ecologically specialized genetic lineages from crossing a certain area. This may be the case in the Aosta zone, which marks the bend of the Alpine Arc and shows a distinct change in climate since the low summer precipitation of the Mediterranean extends to this area (Ozenda, 1988).

However, topography in terms of elevational range did not explain break density in overall species distributions. A likely explanation is the fact that the third, eastern-most Tauern zone, which is clearly present only in the analysis of species distributions, is not paralleled by large elevational variation. However, we consider that the species-based Aosta and Brenner zones are also affected by the respective large elevational range within grid cells in these areas because the two break zones correlate with those based on allele distributions. In the particular case of the Tauern zone, the species-based break zone could well be a consequence of the Carpathian species pools reaching into the eastern Alps.

CONCLUSIONS

The shared history of Pleistocene climatic fluctuations induced largely congruent break zones in the distribution of alleles and of species across the European Alps. Both alleles and species were probably subject to the same processes during the ice ages, which shaped distinct genetic lineages and floristic units. Such processes involve local extinction, drift and migration and have recently been shown to act in parallel on genes and species (Vellend, 2005; Vellend & Geber, 2005). However, the lower rate of dispersal of species (limited to seed dispersal) than of alleles (pollen and seed dispersal) may explain the additional species-based break zone in the eastern Alps, which was not present at the allele level. Large elevational differences probably acted as dispersal barriers for alleles, forming break zones during immigration. Thus, high concordance in allele distribution patterns and respective break zones was detected among the species investigated, as has been postulated by Kuo & Avise (2005) and shown by Alvarez et al. (2009) for alpine plant species. However, the additional species-based break was not related to topography. Thus, we conclude that species (i.e. biogeography) maintain break zones over longer time spans and reflect more ancient patterns than do alleles (i.e. phylogeography).

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REFERENCES


**SUPPORTING INFORMATION**

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

**Appendix S1** Members of the IntraBioDiv consortium.

**Appendix S2** List of alpine plant species included in the floristic data.

**Appendix S3** Geographical information on sampling cells.

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**BIOSKETCHES**

Conny Thiel-Egenter and Nadir Alvarez have studied the effects of historical and ecological processes on patterns in allele and species distributions, but also on genetic diversity and differentiation, in alpine plant species. This work was part of the EC-supported research project IntraBioDiv, which investigated large-scale and multi-species relationships among levels of plant biodiversity in mountain ecosystems.


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