RESEARCH PAPER

Effects of species traits on the genetic diversity of high-mountain plants: a multi-species study across the Alps and the Carpathians

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ABSTRACT

Aim To test the influence of various species traits, elevation and phylogeographical history on the genetic diversity of high-mountain plants in the Alps and Carpathians.

Location The regular sampling grid comprised the whole range of the European Alps and the Carpathians.

Methods Twenty-two high-mountain plant species were exhaustively sampled and their genetic diversity was assessed with amplified fragment length polymorphisms (AFLPs). ANOVAs were used to check for relationships between species traits and species genetic diversity, and to test whether genetic diversity was influenced by altitude and phylogeographical history (i.e. Alps versus Carpathians).

Results In both mountain systems, species dispersed and pollinated by wind showed higher genetic diversity than species with self or insect pollination, and with animal- or gravity-dispersed seeds. Only in the Alps did altitudinal range size affect species genetic diversity significantly: species with narrow altitudinal ranges in the highest vegetation belts had significantly higher genetic diversity than those expanding over wide altitudinal ranges. Genetic diversity was species specific and significantly higher in the Alps than in the Carpathians, but it was not influenced by elevation.

Main conclusions Wind pollination and wind dispersal seem to foster high genetic diversity. However, species traits are often associated and their effects on genetic diversity cannot be clearly disentangled. As genetic diversity is species specific, comparisons across species need to be interpreted with care. Genetic diversity was generally lower in the Carpathians than in the Alps, due to higher topographical isolation of alpine habitats in the Carpathians and this mountain massif's divergent phylogeographical history. Elevation did not influence genetic diversity, challenging the long-held view of decreasing genetic diversity with increasing elevation in mountain plants.

Keywords

AFLPs, alpine plants, Alps, Carpathians, elevation, genetic diversity, Nei's gene diversity, phylogeographical history, Quaternary glaciations, range-wide sampling grid.

INTRODUCTION

Among the factors influencing levels of genetic diversity, species traits and population history have been regarded as major determinants (Ellstrand & Elam, 1993; Hamrick & Godt, 1996). As a result of specific environmental conditions acting as selective forces, similar functional traits or life-history strategies such as life-form, mode of reproduction or type of seed dispersal are

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found in plants. These traits also influence genetic diversity (Hamrick & Godt, 1989; Hamrick *et al.*, 1991; Hamrick & Godt, 1996; Nybom & Bartish, 2000; Nybom, 2004; Aguinagalde *et al.*, 2005). Reviews by Hamrick & Godt (1989, 1996) demonstrated significant effects of life-form, breeding system, seed dispersal mode, successional status and geographical range on allozyme diversity. Outcrossed, animal-dispersed, long-lived and late successional species with large distribution ranges show higher genetic diversities than selfed, gravity-dispersed, short-lived and early successional species with small distribution ranges. Reviews based on random amplified polymorphic DNA (RAPD) markers confirmed that genetic diversity was higher in outcrossed and late successional than in selfed and early successional plant taxa (Nybom & Bartish, 2000; Nybom, 2004).

However, there are other factors potentially influencing the genetic diversity of plants. First, the elevation of a species' habitat has long been hypothesized to influence its genetic diversity. High elevations restrict plant life due to low temperatures and short growing seasons (Ellenberg, 1988; Körner, 1999). Likewise, a lower number and activity of pollinators at high versus low altitudes (Arroyo *et al.*, 1982) supposedly promote inbreeding and asexual reproduction with increasing altitude (Mosquin, 1966; Richards, 1997). Since the latter factors are well known to reduce genetic diversity (Ellstrand & Elam, 1993; Frankham & Ralls, 1998), one could deduce that plant species experience a decrease in genetic diversity along altitudinal gradients, with populations at high elevations being genetically less variable than their low-elevation counterparts. In contrast to these expectations, Bingham & Orthner (1998) demonstrated that more efficient pollination and longer stigmatic receptivity can compensate for lower pollinator visitation at high altitudes. Indeed, several studies recently showed that the genetic diversity of insect-pollinated plants was rather similar at low and high altitudes (Gugerli, 1998; Gugerli *et al.*, 1999; Bingham & Ranker, 2000; Till-Bottraud & Gaudeul, 2002). Second, the phylogeographical history also influences the genetic diversity of populations (Hewitt, 2000; Petit *et al.*, 2003). As landscapes differ in terms of topography and permeability, re-colonization after Quaternary glaciations as well as post-glacial gene exchange among populations can vary greatly in different landscapes (Hewitt, 2000; Lindenmayer & Fischer, 2006). Both of these processes affect genetic diversity (Frankham *et al.*, 2002; Leimu *et al.*, 2006) and one can assume that landscapes with different glacial histories will also exhibit different levels of genetic diversity. Colonization models, for example, suppose high genetic diversity in Pleistocene glacial refugia and a gradual loss of diversity along post-glacial colonization out of these source populations (Hewitt, 1996, 2000). On the other hand, some studies found the highest genetic diversity in contact zones where refugial gene pools intermixed (Petit *et al.*, 2003; Walter & Epperson, 2005).

One major shortcoming of generalizations about different factors influencing genetic diversity is that different studies applied different sampling strategies and laboratory techniques. Even though it has been recognized that such discrepancies may largely bias results (Hamrick & Godt, 1996; Aguinagalde *et al.*, 2005), this drawback has not been thoroughly accounted for in any empirical comparative study. Hence, we sampled 22 abundant high-mountain plant species on a regular grid over the entire European Alps and Carpathians and assessed their genetic diversity with amplified fragment length polymorphisms (AFLPs). We thus had a consistent sampling design and laboratory techniques. We were interested in how the species altitudinal range size, dispersal mode, distribution type, dominance, life-form and pollination mode influence genetic diversity. As the Alps and Carpathians differ in their phylogeographical history (Schönswetter *et al.*, 2005; Ronikier *et al.*, 2008), we were also able to evaluate the latter's influence on genetic diversity. Furthermore, we took into account the elevation of the sampling locations of populations.

Due to the patchy occurrence of species-specific habitats in alpine landscapes (Ellenberg, 1988), we expected that species traits related to dispersal and gene flow are most important in shaping the distribution of genetic diversity. More specifically, we hypothesized that species pollinated and dispersed by wind harbour high genetic diversity. Furthermore, we suspected that genetic diversity was not influenced by elevation and that species had different levels of genetic diversity in the Alps and Carpathians due to their different Quaternary histories.

MATERIALS AND METHODS

Sampling

For species sampling, we adopted the regular grid system used for the mapping of the European alpine flora, with cell sizes of 12′ latitude and 20′ longitude (*c.* 22.3×25.3 km or 563 km^2; Fig. 1). We only considered cells comprising land above 1500 m. We sampled 22 abundant, widespread high-mountain plant species occurring in both the Alps and the Carpathians during summer 2004 (Table 1). By choosing abundant and widespread species we obtained a large consistent sampling range that was as similar as possible for all species. Species were sampled exhaustively in both mountain systems (average sampling success = 80%; Table 1). As far as is known, all species have a consistent ploidy level across the study range. To minimize potential effects of phylogenetic relationships, we selected species from 21 different genera and 15 different families. In the Alps, sampling was conducted in every second cell of the grid (totalling 149 cells). In the Carpathians, all cells with land above 1500 m were sampled (totalling 32 cells) owing to the island character of alpine areas. At one location per grid cell per species, three individuals were collected and the exact GPS coordinates and elevation (m a.s.l.) were recorded. A detailed description of the sampling scheme is provided by Gugerli *et al.* (in press).

DNA extraction and AFLP analysis

DNA extractions and DNA fingerprinting with selectively neutral AFLP markers were conducted in six laboratories, each working with several full species samples (Gugerli *et al.*, in press). DNA extractions from 10 mg of dried plant material were carried out using either a cetyl-trimethylammonium bromide (CTAB)

Table 1 Plant species and family, number of polymorphic AFLP fragments, sampling success (percentage of expected species records based on the IntraBioDiv database and effectively sampled species in a cell; Gugerli *et al*., in press), classification of species traits (for explanations see Materials and Methods), lab that analysed a given species, number of sample cells and mean genetic diversity in the Alps and Carpathians, respectively.

				Species traits							Alps		Carpathians	
Species	Family	No. of fragments	Percentage sampling success	Altitudinal range size	Dispersal mode	Distribution type	Dominance	Life-form	Pollination mode	Lab	No. of cells	Mean genetic diversity	No. of cells	Mean genetic diversity
Arabis alpina L.	Brassicaceae	150	88.1	2	2	2	2	2	2	A	129	0.05	19	0.06
Campanula alpina Jacq.	Campanulaceae	155	82.6	1	2	1	2	1	2	F	13	0.09	19	0.09
Carex firma Mygind	Cyperaceae	58	80.6	2	1	1	1	1	1	E	76	0.24	3	0.24
Carex sempervirens Vill.	Cyperaceae	121	100	2	2	1	1	1	1	C	133	0.09	22	0.06
Dryas octopetala L.	Rosaceae	101	89.2	2	1	2	1	2	2	A	124	0.12	15	0.07
Gentiana nivalis L.	Gentianaceae	154	63.9	1	2	2	2	1	2	D	73	0.08	6	0.03
Geum montanum L.	Rosaceae	93	86	2	1	1	2	1	2	C	122	0.08	19	0.06
Geum reptans L.	Rosaceae	61	65.6	1	1	1	2	1	2	C	51	0.1	8	0.04
Hedysarum hedysaroides (L.) Schinz & Thell.s.l.	Fabaceae	123	71.4	2	1	1	2	1	2	E	76	0.15	11	0.12
Hornungia alpina (L.) Appel s.l.	Brassicaceae	225	76.5	2	2	1	2	1	2	B	97	0.08	3	0.03
Hypochaeris uniflora Vill.	Asteraceae	94	68.2	2	1	1	2	1	2	A	59	0.11	27	0.17
Juncus trifidus L.	Juncaceae	88	88.8	2	1	2	1	1	1	C	91	0.12	23	0.10
Ligusticum mutellinoides (Cr.) Vill.	Apiaceae	97	69	1	1	1	2	1	2	E	56	0.22	4	0.16
Loiseleuria procumbens (L.) Desv.	Ericaceae	121	78.9	1	2	2	2	2	2	A	90	0.18	13	0.14
Luzula alpinopilosa (Chaix) Breistr.	Juncaceae	218	83.5	2	2	1	2	1	1	D	82	0.08	19	0.07
Phyteuma confusum A. Kern.	Campanulaceae	152	100	1	2	1	2	1	2	B	2	0.19	7	0.14
Primula minima L.	Primulaceae	169	87.3	1	2	1	2	1	2	F	28	0.16	18	0.10
Ranunculus alpestris L. s.l.	Ranunculaceae	434	77.1	2	1	1	2	1	2	B	79	0.07	7	0.04
Saxifraga stellaris L.	Saxifragaceae	190	83.2	2	2	2	2	2	2	B	100	0.07	12	0.06
Sempervivum montanum L. s.l.	Crassulaceae	107	57.9	2	2	1	2	2	2	F	9	0.13	10	0.08
Sesleria caerulea (L.) Ard.	Poaceae	70	96.1	2	1	1	1	1	1	E	137	0.24	7	0.24
Soldanella pusilla Baumg.	Primulaceae	90	66.7	1	2	1	2	1	2	F	13	0.19	8	0.08

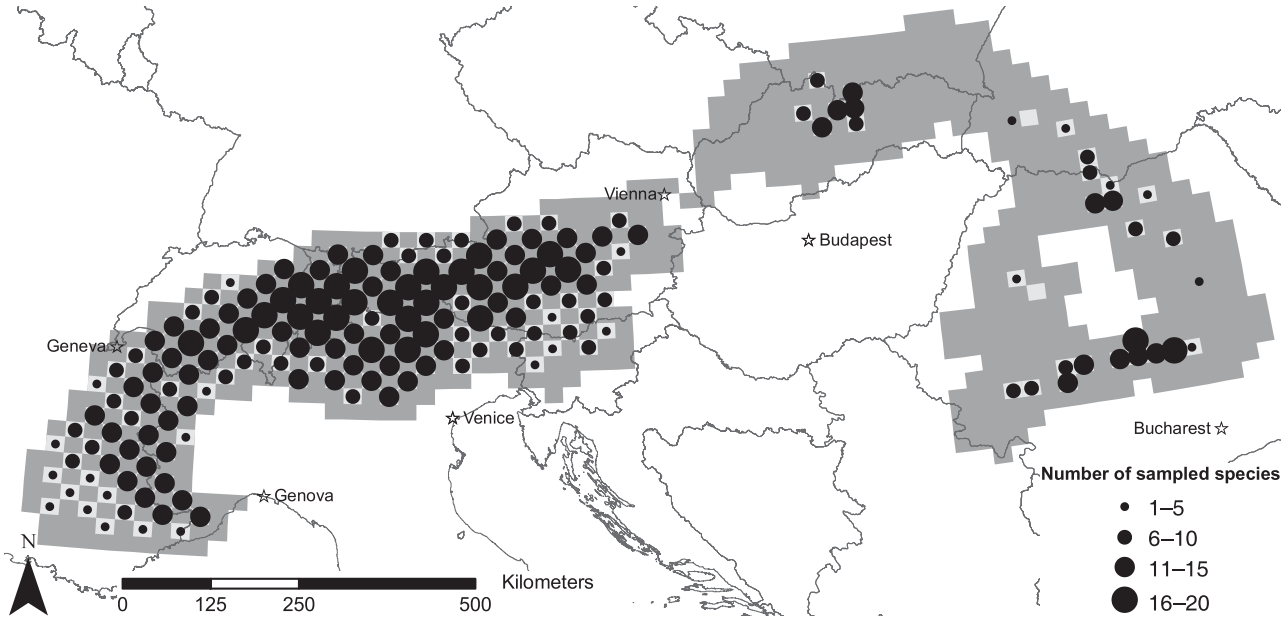

Figure 1 Study area showing the range of the Alps and Carpathians in grey shading. Rectangles in light grey represent grid cells of the sampling scheme. The size of the black dots indicates the number of species sampled per grid cell.

protocol (lab E; Table 1) or the DNeasy 96 Plant Kit (Qiagen; all other labs). A common AFLP protocol was established, largely following Vos *et al.* (1995) with lab-specific modifications (Gugerli *et al.*, in press). Three selective primer combinations were used per species. In lab E, AFLP fragments were separated by electrophoresis on 8% polyacrylamide gels, and the presence or absence of fragments was scored manually. In all other labs, fragment separation was conducted on automated capillary sequencers and the presence or absence of fragments was scored with GENEMAPPER 3.7 (Applied Biosystems) or GENOGRAPHER 1.6.0 (http://hordeum.msu.montana.edu/genographer/; Gugerli *et al.*, in press). All species were scored separately; species-specific matrices of fragment presence/absence only contained polymorphic AFLP fragments (140 per species on average). Fragments were considered monomorphic when present in all individuals or when present/absent in all but one individual. All labs applied a quality control following the recommendations of Bonin *et al.* (2004) using replicates from DNA extraction to selective polymerase chain reaction (PCR). When unstable fragments were detected among replicates, the respective fragments were excluded from analysis. Additionally, about 10% of all samples were duplicated as blind controls from extraction to PCR, resulting in a minimum reproducibility of 95% within the species analysed (Gugerli *et al.*, in press).

Calculation of genetic diversity

Genetic diversity was calculated as Nei's gene diversity, which is the mean number of pairwise differences between the three individuals sampled per species per grid cell (Nei, 1973; Kosman, 2003), using an R-script available from http://www.intrabiodiv.eu/. Nei's gene diversity is the most appropriate diversity measure for AFLP data (Bonin *et al.*, 2007). To obtain species genetic diversities, we used the mean over all locations of a species in the Alps and in the Carpathians. As we mainly analysed species means of genetic diversity, either for the Alps or the Carpathians separately, estimating gene diversity from only three individuals per cell was counter-balanced by using a large number of genetic markers (Nei, 1987) and a large number of locations per species.

Overall correlations with elevation and phylogeographical history

We tested whether genetic diversity: (1) differed among species, (2) was related to the elevation of the sampling location, and (3) was different in the Alps and the Carpathians. Since the AFLP data sets were generated in six different laboratories, we also tested for a possible lab effect on detected genetic diversity. We applied a mixed nested analysis of variance (ANOVA) in SPSS 12.0.2 (SPSS Inc.) with genetic diversity per location as the dependent variable. We considered elevation as a covariate and lab (six classes) and phylogeographical history (Alps versus Carpathians) as fixed factors. Species were nested within lab as a random factor. We only used interactions in the ANOVA model that were significant, i.e. the interaction between phylogeographical history and species. In particular, the interaction elevation × species, potentially pointing to nonlinear reactions to elevation varying among plant species, was non-significant. Residuals were normally distributed, and variances met criteria of homogeneity.

Correlations with species traits

We used six species traits that had previously been shown to be related to genetic diversity (Hamrick & Godt, 1989, 1996;

Table 2 Mixed nested analysis of variance (ANOVA) testing the influence of elevation (covariate), lab (fixed effect), phylogeographical history (Alps–Carpathians; fixed effect) and species nested within lab (random effect) on genetic diversity.

Source	Type III Sums of Squares	d.f.	Mean Square	*F*	*P*
Elevation	0.002	1	0.002	1.261	0.262
Lab	0.945	5	0.189	4.006	0.015
Phylogeographical history	0.123	1	0.123	10.631	0.004
Species [lab]	0.755	16	0.047	32.140	≤ 0.001
Alps-Carpathians × species [lab]	0.242	21	0.012	7.858	≤ 0.001
Error	2.751	1875	0.001		

Nybom & Bartish, 2000), each classified into two categories. For altitudinal range size and distribution type we referred to the *Flora alpina* (Aeschimann *et al.*, 2004). We defined altitudinal range size as the number of vegetation belts in which a species occurs (i.e. colline, montane, subalpine, alpine or nival belt). Vegetation belts where a species occurs frequently were counted as one, and vegetation belts where a species occurs less abundantly were counted as half. Altitudinal range size (= sum per species) were then classified as: (1) narrow (1–2 vegetation belts), or (2) large (2.5–4 belts). Altitudinal range size per species thus reflects a species' potential to grow in a restricted or broad ecological amplitude associated with elevation. As a measurement of distribution type, we used the global distribution range of a species, assuming that species with a larger global distribution would show higher genetic diversity than more regionally distributed species. We classified the distribution types of species as either: (1) a European distribution, or (2) an Arctic–Alpine/European–west Asiatic distribution. Species were also classified into two dominance categories according to their dominance within their main distribution range (A. Tribsch, M. Ronikier & T. Englisch, unpublished data), i.e. (1) dominant or (2) non-dominant. For all other traits, we used the classification in S. Ertl and T. Englisch (unpublished data; for references see Appendix S1 in Supporting Information). Seed dispersal mode was classified into: (1) anemochorous dispersal (seeds or fruits showing morphological adaptations to wind dispersal such as hairs, wings, balloons), or (2) zoochorous (dispersal via attachment to animals or via digestion) and boleochorous (gravity dispersal) dispersal. Life-form was divided into: (1) therophytes and hemicryptophytes (e.g. forbs, grasses), or (2) chamaephytes and nanophanerophytes (e.g. cushion plants, dwarf shrubs) (Ellenberg, 1988). Pollination mode was grouped into: (1) wind-pollinated species, and (2) self- or insect-pollinated species.

We tested for associations among the six traits by using Fisher's exact tests in 2 × 2 tables in SPSS 12.0.2 for each trait combination. Fisher's exact test probabilities are two-tailed tests of the null hypothesis of no association. This test is used for nominal data when cells have expected frequencies of less than 5 (Sokal & Rohlf, 1995). To check for associations of species traits with laboratory, we used a chi-square likelihood ratio in 2 × 6 tables in SPSS 12.0.2. These tests are used in tables with any number of rows and columns when cells have expected frequencies of less than 5 (Sokal & Rohlf, 1995). In both these statistical tests, we accounted for multiple testing by applying sequential Bonferroni corrections (Holm, 1979).

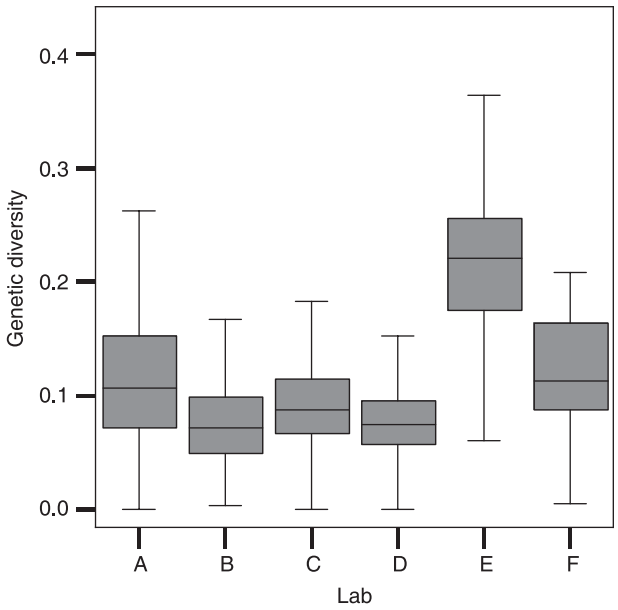

Figure 2 Box-plots indicating medians and quartiles of genetic diversity over the species analysed in six different labs.

Lab E used electrophoresis on polyacrylamide gels for fragment separation and manually scored marker presence or absence, whereas all other labs used capillary electrophoresis on automated sequencers and common software for marker scoring. The factor lab indeed significantly influenced genetic diversity (Table 2) with lab E showing genetic diversities that were two-fold higher than those found in the other labs (Fig. 2). As the reproducibility of markers was generally high, erroneous marker scoring is unlikely to have contributed to the higher diversity in lab E. An alternative explanation lies in the species allocated to this lab. All species investigated in this lab were wind-dispersed, and thus expected to harbour high genetic diversity (Hamrick *et al.*, 1991; Hamrick & Godt, 1996). In accordance, we found a significant association between lab and dispersal mechanism (Table 3). However, we cannot finally disentangle the factors lab, species and dispersal mechanism and are therefore not able to determine whether laboratory differences or species dispersal mechanism caused the observed differences in genetic diversity.

For further analysis, we only used those traits that did not show significant associations with each other. For both the Alps and

Table 3 Significance values of pairwise tests of association among six species traits (Fisher's exact test) and between species traits and lab in which a given species was analysed (likelihood ratio chi-square test).

	Altitudinal range size	Dispersal mode	Distribution type	Dominance	Lab	Life-form
Dispersal mode	0.204					
Distribution type	1.000	0.646				
Dominance	0.115	0.135	0.585			
Lab	0.603	0.008*	0.090	0.157		
Life-form	0.613	0.323	0.009*	1.000	0.073	
Pollination mode	0.115	0.624	1.000	0.003*	0.084	0.290

*Significant associations after sequential Bonferroni correction (at $\alpha = 5\%$).

Table 4 Analysis of variance (ANOVA) testing the influence of four unassociated species traits (fixed factors) on mean genetic diversity of plant species in the Alps.

Source	Type III Sums of Squares	d.f.	*F*	*P*
Altitudinal range size	0.021	1	8.535	0.010
Dispersal mode	0.012	1	4.906	0.041
Life form	0.003	1	1.171	0.294
Pollination mode	0.012	1	4.948	0.040
Error	0.041	17		

Table 5 Analysis of variance (ANOVA) testing the influence of four unassociated species traits (fixed factors) on mean genetic diversity of plant species in the Carpathians.

Source	Type III Sums of Squares	d.f.	*F*	*P*
Altitudinal range size	0.007	1	2.200	0.156
Dispersal mode	0.013	1	4.384	0.052
Life form	0.002	1	0.670	0.424
Pollination mode	0.015	1	4.920	0.040
Error	0.051	17		

the Carpathians we applied an ANOVA to analyse the influence of traits on mean genetic diversity per species. We considered all traits as fixed factors. Distributions of residuals showed normality, and variances met criteria of homogeneity.

RESULTS

Overall correlations with elevation and phylogeographical history

There was no significant overall effect of elevation on genetic diversity (Table 2). Since there was no significant interaction of species × elevation (see Materials and Methods), we can exclude a species-specific but counterbalancing effect of elevation on genetic diversity. A visual examination of the relationship of elevation and genetic diversity supported this finding (examples given in Fig. 3 and Appendix S2). Genetic diversity was species specific and was significantly different in the Alps and the Carpathians (Table 2). Species showed significantly higher genetic diversity in the Alps than in the Carpathians (interaction Alps–Carpathians × species in Table 2; Fig. 4). The latter fact justified separating the data sets of the Alps and the Carpathians for further analyses.

Correlations with species traits

After sequential Bonferroni correction, Fisher's exact tests showed significant associations between the explanatory variables distribution type and life-form and between dominance status and pollination mode (Table 3). Likelihood ratio chi-square tests showed a significant interaction of lab and dispersal mode. Therefore, we only kept the four unassociated traits altitudinal range size, dispersal mode, life-form and pollination mode for further analyses.

The ANOVA with the four uncorrelated species traits showed that mean genetic diversity per species in the Alps was significantly influenced by altitudinal range size, dispersal mode and pollination mode (Table 4). Mean genetic diversities were higher for species with narrow altitudinal ranges (0.152 ± 0.019 SE, $n = 8$) than for species with large altitudinal ranges occurring over several vegetation belts (0.117 ± 0.015, $n = 14$). Genetic diversity was also higher in the wind-pollinated (0.153 ± 0.035, $n = 5$) and in the wind-dispersed species (0.147 ± 0.020, $n = 10$) than in the insect/self-pollinated (0.123 ± 0.012, $n = 17$) and in the animal- or gravity-dispersed species (0.116 ± 0.015, $n = 12$). Life-form had no significant effect on mean genetic diversity in the Alps (Table 4).

Species mean genetic diversity was significantly influenced by pollination mode in the Carpathians (Table 5), with higher genetic diversity in wind-pollinated (0.143 ± 0.041, $n = 5$) than in insect- or self-pollinated species (0.087 ± 0.011, $n = 17$). There was a marginally significant effect of dispersal mode on genetic diversity ($P = 0.052$; Table 5), with higher diversities in wind-dispersed (0.125 ± 0.024, $n = 10$) than in animal- or gravity-dispersed species (0.079 ± 0.010, $n = 17$). Neither altitudinal range size nor life-form influenced genetic diversity in those species sampled in the Carpathians (Table 5).

DISCUSSION

Influence of species traits on species mean genetic diversity

Dispersal and pollination mode influenced species mean genetic diversity both in the Alps and the Carpathians. It has been shown before that outcrossed and animal- or wind-dispersed plant species have a higher genetic diversity than do selfed and gravity-dispersed species (Hamrick & Godt, 1996; Nybom & Bartish, 2000; Nybom, 2004). We found that wind-pollinated and wind-dispersed species show higher genetic diversity than species with self or insect pollination as well as species dispersed by animals or gravity. Since nuclear markers such as AFLPs are dispersed by both pollen and seed, it is reasonable to find a strong influence on genetic diversity of both gene flow-related traits. Furthermore, pollination mode and dominance were associated, since all wind-pollinated species considered are widely distributed graminoids, and all but one of the graminoids are dominant within their habitat.

The altitudinal range size of species was not correlated with any other species trait, but it significantly influenced species mean genetic diversity in the Alps. Here, species with a narrow altitudinal range size, i.e. occurring across only one or two vegetation belts, surprisingly revealed higher diversities than did species with wider altitudinal range size occurring in up to four altitudinal vegetation belts. These studied species with a narrow altitudinal range size grow at high elevations, i.e. in the alpine or nival belt, and exhibit large differences in genetic diversity between the Alps and the Carpathians (Fig. 4). In the Carpathians, where the altitudinal range of species is restricted owing to the generally lower elevations in this mountain system, altitudinal range size had no effect on species genetic diversity.

There is no straightforward explanation for why species restricted to the highest vegetation belts should show higher genetic diversities in the Alps. In contrast, and at a latitudinal gradient, species with larger distribution ranges show higher genetic diversity than do more narrowly distributed plant species (Hamrick & Godt, 1989). A potential explanation for this counterintuitive result is that high-mountain species survived Quaternary glaciations within the Alpine ice shield on high mountain tops or southerly exposed slopes, i.e. on nunataks (Stehlik *et al.*, 2002). Therefore, they might have conserved substantial intraspecific genetic diversity during the ice ages in different nunatak areas of the Alps. Post-glacially, these nunatak populations intermixed with populations immigrating from peripheral refugia and distributed their high genetic diversity by gene flow.

In most previous studies on the influence of species traits on genetic diversity, traits were tested separately (Hamrick & Godt, 1989; Nybom & Bartish, 2000; Nybom, 2004; but see Hamrick & Godt, 1996). However, species traits are often related to each other, which could confound their relationship with genetic diversity. Our results showed that pairs of species traits, namely life-form and distribution type as well as pollination mode and dominance, were associated (Table 3). This demonstrates the importance of considering the relationships among the factors studied before analysis. A further problem in testing effects

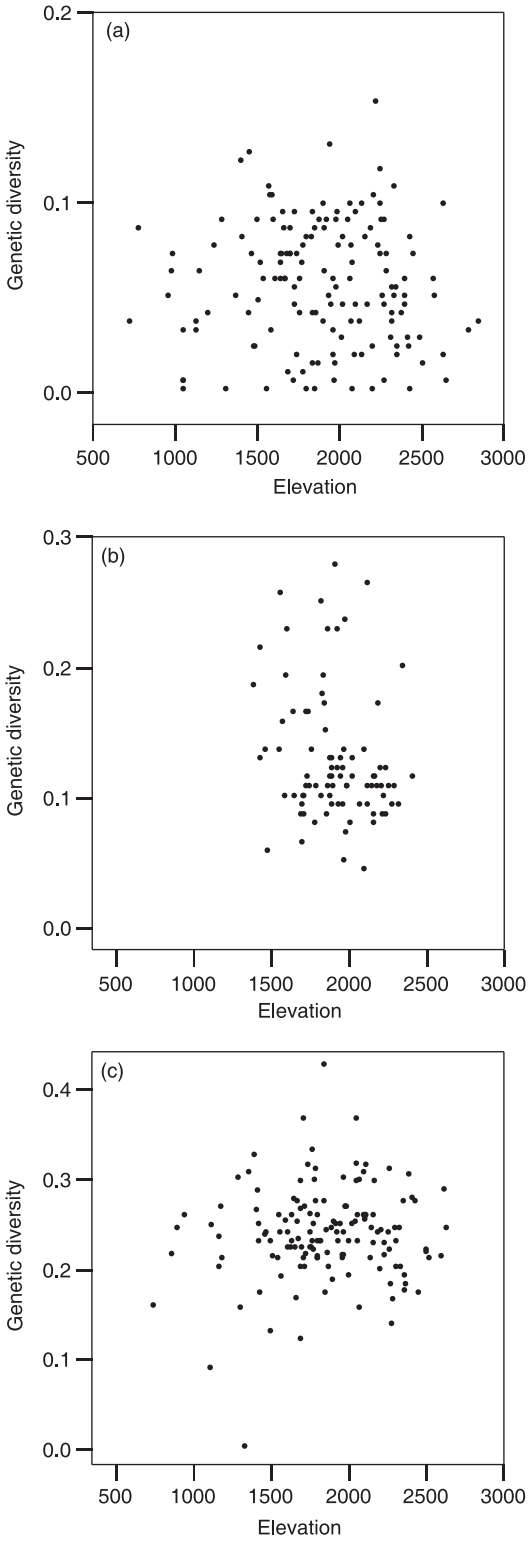

Figure 3 No effect of elevation (m a.s.l.) on genetic diversity as exemplified by *Arabis alpina* (a), *Hypochaeris uniflora* (b) and *Sesleria caerulea* (c).

of species traits on genetic diversity separately is the multiplicity of pairwise testing, which results in an increased type I error rate (i.e. observing a significant difference when there is none). Even though this problem can be solved by adjusting significance

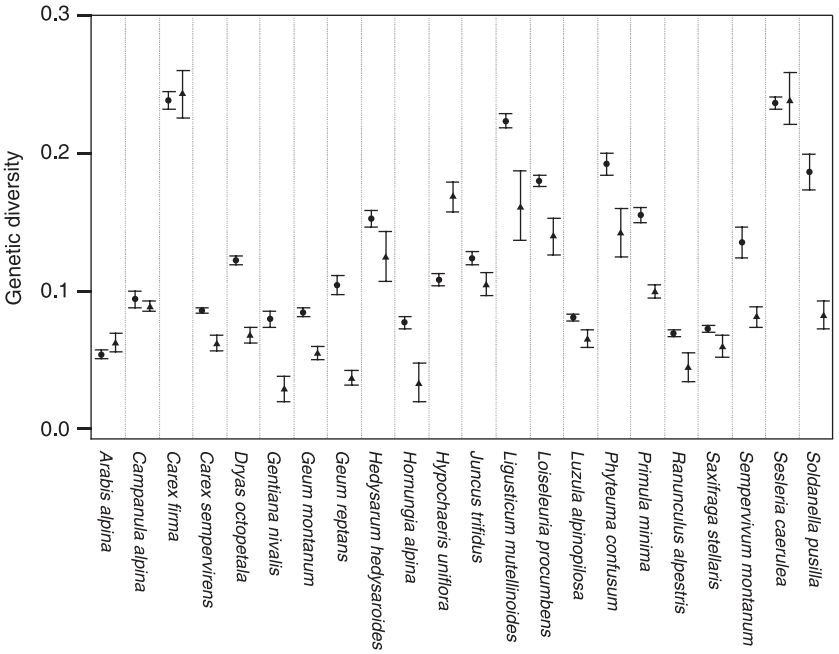

Figure 4 Mean genetic diversity (± SE) of species in the Alps (circles) and Carpathians (triangles).

levels, most published studies ignored this fact. To overcome this latter drawback, we applied multi-way ANOVA testing unassociated species traits synchronously.

No influence of elevation on genetic diversity

There was no effect of elevation (i.e. altitude at which a population was sampled) on the genetic diversity of high-mountain plant species in the Alps and the Carpathians, even though populations were sampled over a wide range of elevations (e.g. a range of 2300 m in *Arabis alpina* and *Gypsophila repens*). Even at the highest elevations, genetic diversity was not reduced compared with locations at the lower end of a species' altitudinal sampling range. Our findings are consistent with the results of several other genetic studies on insect-pollinated species (Gugerli *et al.*, 1999; Bingham & Ranker, 2000; Plüss & Stöcklin, 2004) and wind-pollinated species (Zhao *et al.*, 2006). However, a recent review of studies on mainly tree species showed that genetic diversity was often highest at intermediate elevations, decreasing towards high and low elevations (Ohsawa & Ide, 2008). This finding, explained by higher genetic drift (due to limited gene flow, smaller population sizes and founder effects) in peripheral as compared with core populations, could not be corroborated in our study. We found no correlation of genetic diversity and elevation (Table 2). Further, the interaction of elevation and species, which would point to a species-specific reaction to elevation, was not significant either (see Materials and Methods). Insect-pollinated plants have mechanisms for compensating lower pollinator abundance at high altitudes (Bingham & Orthner, 1998), and selfing rate and clonal growth do not seem to be generally increased at high elevations (Gugerli, 1998; Plüss & Stöcklin, 2004). One could therefore conclude that in alpine plants neither gene flow nor sexual reproduction seems to be restricted in high-elevation as compared to low-elevation habitats.

Genetic diversity in the Alps and the Carpathians

Our results showed that the level of genetic diversity was species specific and differed significantly between the Alps and the Carpathians. The fact that genetic diversity was generally higher in the Alps than in the Carpathians (in some species more than two-fold) can be attributed to the different phylogeographical history and physiography of the two mountain ranges. There are generally fewer populations of high-mountain plants in the Carpathians than in the Alps (Gugerli *et al.*, in press). In addition, the suitable habitat area for high-mountain plant species is smaller in the Carpathians than in the Alps owing to the former's generally lower altitude and the discontinuous, island-like conformation of high-mountain massifs (Pawłowski, 1970). Hence, species habitats were more isolated during the Quaternary glaciations as they are today in the Carpathians and less so in the Alps, which increases the influence of genetic drift on populations, especially during the ice ages and during post-glacial recolonization. Furthermore, founder effects during post-glacial re-colonization (e.g. from the Alps or from the east), as shown for *Dryas octopetala* (Skrede *et al.*, 2006), and a lack of post-glacial lineage mixing due to the island character of Carpathian high-mountain habitats (Ronikier *et al.*, 2008) have potentially contributed to the lower genetic diversity of high-mountain plants in the Carpathians as compared with those in the Alps.

Our findings are confined to a particular group of organisms, namely high-mountain vascular plants. It is not clear to date whether other taxonomic groups, such as alpine lichens, insects or mammals, would confirm our findings of different levels

of genetic diversity in mountain areas with divergent phylogeographical histories. Our sampling design and analytical methods may thus serve as a reference for the conduction of further studies of the influence of phylogeographical history on the genetic diversity of organisms.

CONCLUSIONS

As hypothesized, genetic diversity was influenced by pollination and seed dispersal modes in high-mountain plants of the Alps and Carpathians. In the Alps, species with narrow altitudinal range sizes, mostly growing at the highest elevations, showed the highest genetic diversity. These species are adapted to adverse climatic conditions, and hence, are likely candidates for *in situ* glacial survival during the Quaternary glaciations. This result is in marked contrast to long-held views on the genetic diversity of high-mountain plants and clearly deserves further investigations. In fact, genetic diversity was not reduced at high compared with low elevations, suggesting that high-mountain populations possess mechanisms to ensure genetic diversity over the long term. Our hypothesis of a generally lower genetic diversity in the Carpathians than in the Alps was confirmed. This result was probably caused by a higher degree of topographical isolation of high-mountain plant populations in the Carpathians, but also due to the divergent phylogeographical history of the two mountain systems. The standardized sampling across the whole range of two different mountain systems and the application of the same molecular marker type strengthen our findings that genetic diversity is species specific. This fact should be considered in any application comparing levels of genetic diversity, such as in conservation management.

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SUPPORTING INFORMATION

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

Appendix S1 References for the three species traits that were classified according to S. Ertl and T. Englisch (unpublished).

Appendix S2 Relationship of genetic diversity and elevation of all 22 species.

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AUTHOR CONTRIBUTIONS

C.T.E., F.G., P.T., R.H., A.T. and I.B.D. designed the study; C.T.E., F.G., N.A., S.B., E.C., L.C., T.E., M.G., L.G., G.K., R.N., O.P., M.P., D.R., M.R., P.S., F.S., P.T., A.T., M.vL., M.W., R.H. and I.B.D. sampled and gathered the data and/or conducted AFLP analysis; C.T.E., F.G. and R.H. analysed the data; C.T.E., F.G., N.A., T.E., P.S., P.T., A.T., R.H., M.R., O.P., M.vL., L.C. and I.B.D. discussed the results and drafts of the manuscript; C.T.E., F.G. and R.H. wrote and revised the paper.

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