BIODIVERSITY RESEARCH

No positive correlation between species and genetic diversity in European alpine grasslands dominated by *Carex curvula*

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ABSTRACT

The distributional range of alpine plants experienced dramatic changes during the Quaternary ice ages. These changes offer many opportunities for studying the impact of habitat contraction and fragmentation on both species and genetic diversity. Here, we examined the amplified fragment length polymorphism-based genetic diversity in the sedge *Carex curvula* All. in relation to the species diversity of siliceous European alpine grasslands in which the sedge is dominant. We found no relationship or even a negative relationship between genetic and species diversity indices. Local species richness was associated with the regional pool size of siliceous alpine species, which was itself dependent on the extant area of suitable habitats for these species. Genetic diversity of *C. curvula* was primarily shaped by the presumed location of glacial refugia and the routes of post-glacial colonization. We conclude that the two levels of diversity are not positively correlated because genotypes and species do not respond similarly to the spatial dynamics of suitable habitats induced by Quaternary temperature changes.

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INTRODUCTION

The relationship between two fundamental levels of diversity – genetic diversity and species diversity – has recently gained renewed interest (Antonovics, 2003). This topic originated in long-standing efforts to relate ecological and evolutionary drivers of diversity patterns in nature. It has been hypothesized that species diversity and genetic diversity might be positively correlated, either because both levels of diversity respond similarly to the same driving factors or because of direct causal relationships between the two at a local scale (Vellend & Geber, 2005). This view tends to be supported by both modelling approaches (Vellend, 2006) and a few empirical studies (reviewed in Vellend & Geber, 2005). However, the results show that the patterns of covariation are much stronger for island systems with patchy distribution of habitats (Vellend, 2003) than for mainland systems (Odat, Jetschke *et al.*, 2004). Overall, it has been noted that the available data are still too sparse to confirm or deny the underpinning theoretical arguments for such a positive relationship (Vellend & Geber, 2005).

On an ecological time scale, drift and migration are two processes proposed as the dominant drivers of diversity patterns (Kimura, 1983; Hubbell, 2001). It has been recently hypothesized that these neutral processes more readily lead to correlated patterns of diversity at different levels than non-neutral processes (Whitham, Young *et al.*, 2003; Vellend & Geber, 2005). Noteworthy,

the relative importance of drift and migration is affected by landscape-scale distribution of suitable habitats, as emphasized in metapopulation models (Hastings & Harrison, 1994). The balance between gene flow and drift at the genetic level, or the balance between immigration and extinction at the community level, is finely tuned by the spatial arrangement of suitable patches. The process of habitat contraction and habitat fragmentation is generally considered to be a cause of extinction of genes, populations, or species (Fahrig, 2003). Patch size reduction triggers a reduction in population size, thus increasing the risk of local extinction (MacArthur & Wilson, 1967; Tilman *et al.*, 1994). The breaking apart of favourable habitat reduces the relative importance of migration and may favour species endemism or high genetic drift. Rare and long-distance founding events have important consequences on population genetic divergence and on differences among species assemblages in a highly fragmented landscape (Avise, 1998; Comes & Kadereit, 1998). Spatial covariation between the two levels of biodiversity may thus result from these parallel influences of the landscape dynamic on genes and species (Vellend & Geber, 2005). Species assemblages for which the distributional range experienced periods of contraction and expansion should provide a relevant biological model to test this hypothesis. We know of only one case study addressing this question in a context of land use induced habitat fragmentation (Odat, Jetschke *et al.*, 2004).

The evolutionary consequences of long-term climatic oscillations have been highlighted (Dynesius & Jansson, 2000). Because temperature is the key driver of species distribution in mountains, Quaternary climatic variations have often triggered dramatic changes in the ranges of alpine species (Theurillat & Guisan, 2001). The sites where alpine plant communities currently occur were covered with ice during glacial periods. The impact of these repeated events on species diversity remains largely unknown. Biogeographers have postulated that cold periods might have favoured endemism as a consequence of habitat fragmentation or alternatively increased exchanges between the regional pool of different species (Ozenda, 1985). Population geneticists have examined the consequences of these cold periods for lowland plants, especially trees (Petit, Brewer *et al.*, 2002; Coart, Van Glabeke *et al.*, 2005; Magri, Vendramin *et al.*, 2006) and more occasionally for alpine species (Holderegger, Stehlik *et al.*, 2002; Tribsch, Schönswetter *et al.*, 2002; Schönswetter & Tribsch, 2005). The locations of refugia and post-glacial migration routes have been shown to strongly shape the contemporary population genetic structure of these species. Attempts have been made to compare the post-glacial history of alpine species with overlapping niches using genetic markers (Tribsch & Schönswetter, 2003). To our knowledge, no study has undertaken a comparative approach of both species and genetic diversity for a well-defined floristic assemblage.

Here, we consider the siliceous alpine grasslands dominated by the sedge *Carex curvula*. These grasslands have long been recognized as the dominant vegetation of the alpine belt on acidic bedrocks in the European Alpine System – a biogeographical entity comprising the main temperate mountain ranges in Europe (Ozenda, 1985). The floristic assemblage of *C. curvula* grasslands exhibits striking similarities throughout these mountain ranges (Theurillat, 1996). Most of the above- and below-ground phytomass of these grasslands consists of *C. curvula* (Klug-Pümpel, 1982; Grabherr, 1989). As a dominant species, *C. curvula* has major implications on the structure and functioning of the ecosystem (Grime, 1998; Whitham, Young *et al.*, 2003). In this study, we present a combined analysis of two data sets: (1) a floristic survey of species richness in *C. curvula*-dominated siliceous grasslands, and (2) a phylogeographical study of *C. curvula* based on amplified fragment length polymorphism (AFLP) genetic markers. Our primary aim was to test whether local species diversity and genetic diversity of the dominant species were positively correlated or not. Second, we attempted to identify the main historical drivers of the observed diversity patterns by focusing on the spatial dynamics of potential areas for siliceous alpine species driven by Quaternary temperature changes.

METHODS

Floristic surveys

The siliceous alpine grasslands dominated by *C. curvula* All. ssp. *curvula* are found predominantly in the Alps, Pyrenees, South-eastern Carpathians, and the Balkans, mainly between *c.* 2200 and 2700 m a.s.l. In this study, we focused on 29 sites distributed throughout the European distributional range of these alpine grasslands (Fig. 1). The Alps are subdivided into three different regions, i.e. South-Western, Central, and Eastern Alps on the basis of vegetation and floristic differences (Ozenda, 1985). In each of these 29 sites, a floristic survey was conducted by visually estimating the identity and relative abundance of
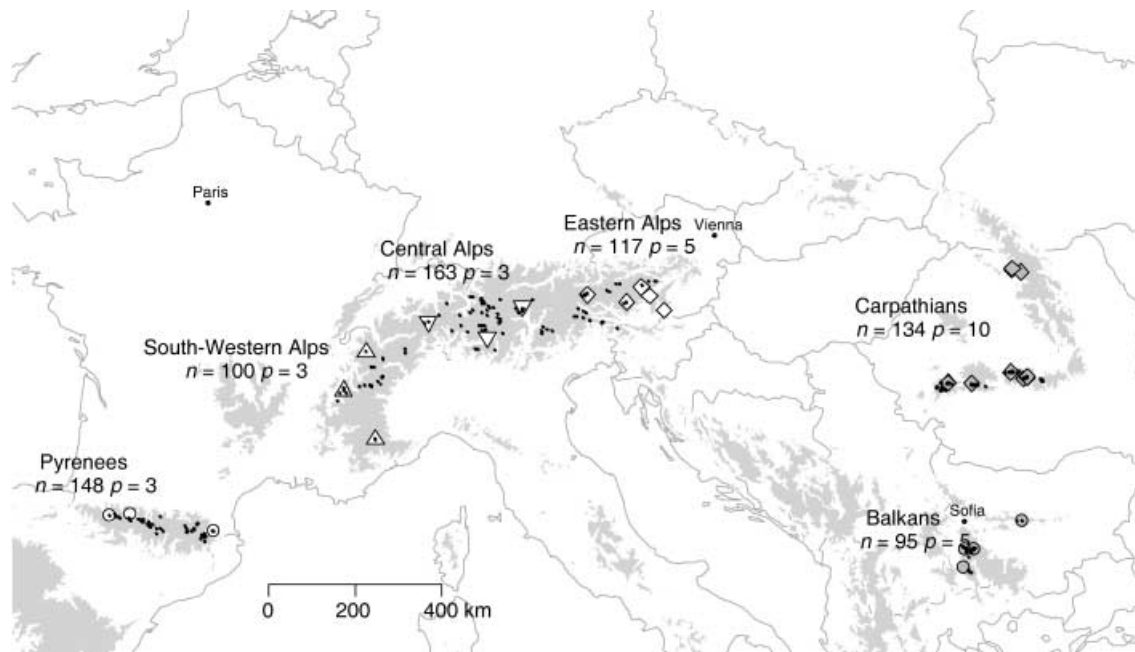

Figure 1 Map of the sampling sites in the six investigated European mountain regions. Within each region, large symbols correspond to the *p* sites where species and genetic analyses have been analysed, whereas small black dots represent the *n–p* additional plots where floristic surveys have been conducted in *C. curvula* alpine grasslands. The relief above 800 m is shown in grey.

Table 1 Summary of the hierarchical sampling design and the diversity indices computed at the plot scale and at the regional scale.

Sptial scale	Plot (25–50 m²)	Region (100–1000 km²)
Genetic data	*A population sample of 5 genotyped Carex curvula individuals*	*p Carex curvula populations*
AFLP band richness	Total number of AFLP bands	Asymptote of the AFLP band accumulation curve and estimated regional AFLP band pool
AFLP band diversity	Between and among individuals partitioning of the AFLP band diversity	Initial slope of the AFLP band accumulation curve
Floristic data	*A species list with abundance class*	*n species lists with abundance class*
Species richness	Total number of species present	Asymptote of the species accumulation curve and estimated regional species pool
Species diversity	Evenness index ($E_{1/D}$)	Initial slope of the species accumulation curve

vascular plant species within a contiguous, homogeneous area ranging from 25 to 50 m². Species cover was classified according to the following six-level scale: 1: <5%; 2: 5–10%; 3: 10–25%; 4: 25–50%; 5: 50–75%; and 6: >75%.

Genetic analyses

We analysed genetic variations in *C. curvula* populations using AFLP genetic markers. AFLP markers are randomly distributed over the entire genome. The AFLP technique has been widely used to assess genetic diversity and genetic relationships between populations, as it usually exhibits sufficient nucleotide variability at this taxonomic level (Despres, Gielly *et al.*, 2003). During the summers of 2001–04, populations of *C. curvula* were sampled at the same 29 sites as described above (Fig. 1). Young, green leaves of five randomly chosen plants, separated by at least 10 m, were collected in each population. Vegetative tissues were stored in tubes with silica gel until DNA was extracted. Total DNA was extracted from roughly 10 mg of dried leaf tissue using the DNeasy 96 Plant Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Six random individuals from the total sample set were extracted twice as blind samples (Bonin *et al.*, 2004). Double digestion of genomic DNA was performed for 2 h at 37 °C in a 20 μL mix using 2 U of MseI and 5 U of EcoRI (New England Biolabs, Ipswich, UK). Following this, adapters were ligated to DNA in a 40 μL volume for 2 h at 37 °C using 1 U of T4 DNA Ligase (New England Biolabs). Diluted 10 times, digested, and ligated DNA was subjected to a preselective amplification using EcoRI primer E.A (5′-GACTGCGTACCAATTCA-3′) and MseI primer M.C (5′-GATGAGTCCTGAGTAAC-3′) in a 25-μL volume containing 1.5 mм MgCl₂, 200 μм of each dNTP, 1.25 μм of each primer, and 0.5 U of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA, USA). Diluted 20 times preselective products underwent selective polymerase chain reaction (PCR) with the following primer combinations: E.ATC-M.CAC, E.ATC-M.CAT, and E.ATC-M.CTG. Selective amplifications were run in a 25-μL volume containing 2.5 mм MgCl₂, 200 μм of each dNTP, 1.25 μм of each primer, and 1 U of AmpliTaq Gold® DNA polymerase (Applied Biosystems). Following this step, excess labelled primers were removed by spin-column purification. Finally, 1.5 μL of diluted (10×)

purification was added to a 10-μL mixture (10 : 0.1) of HiDi formamide and GeneScan-500 ROX (Applied Biosystems). AFLP reactions were electrophoresed for 41 min on an ABI PRISM®3100 Genetic Analyser (Applied Biosystems) using 36 cm capillaries and POP-4™ polymer. The raw data were size-called using GeneScan Analysis software, version 3.7 (Applied Biosystems). The size-calibrated GeneScan files were imported into genographer (version 1.6.0, James J. Benham, Montana State University, 2001; http://hordeum.oscs.montana.edu/genographer) for allele scoring. The thumbnail option was used to evaluate a certain marker for all individuals at the same time. AFLP bands within a 50–500 bp range were scored, resulting in a presence/absence matrix with 145 individuals, i.e. five individuals from 29 populations, and 115 polymorphic bands.

Diversity measurements at the plot scale

Diversity measurements at the plot scale are summarized in Table 1. Species richness is defined as the number of species observed in a plot. In order to calculate an evenness index at the plot scale, we first transformed plant cover classes to the mean percentage cover (class means), hereafter x_i. Let S be the number of species in the plot and $p_i = x_i / \Sigma_{i=1,s} x_i$, the relative abundance of the *i*th species. We calculated evenness as $E = D^{-1}/S$, where D is the Simpson's index of species 'dominance' ($D = \Sigma\ p_i^2$). This index fulfils several important requirements for evenness calculations, specifically: (1) it is unaffected by an increase in species richness; (2) it decreases with the addition of a low-abundant species; and (3) it decreases with changes in the abundance of minor species (Smith & Wilson, 1996).

Because AFLPs are dominant markers, no attempt was made to derive allelic frequencies from the data. We first calculated the total number of AFLP bands present in a population, hereafter referred to as AFLP band richness or D_γ. For each population, we then partitioned the AFLP band richness into a within- and a between-individual component using an additive model (Lande, 1996; Crist & Veech, 2006). Within-individual AFLP band diversity (D_α) was calculated as the mean number of AFLP bands per individual. Between-individual AFLP band diversity (D_β) was calculated as the mean number of AFLP bands that were absent in an individual AFLP phenotype.

We estimated the line that best describes the bivariate scatter between species and genetic diversity indices using a model II regression, since our purpose was not to predict one variable from the other (Warton, Wright *et al.*, 2006). The standardized major axis (SMA) technique was chosen to determine this best fit line. Tests were conducted with or without the Pyrenean populations, due to the particular characteristics of this mountain range (see below). The analyses were performed using the 'smatr' R package (Warton & Ormerod, 2007).

Species and AFLP band regional pools

In an attempt to explain the floristic diversity patterns observed at the local scale, we estimated the species pool for each of the six regions investigated. The siliceous alpine grasslands dominated by *C. curvula* All. ssp. *curvula* were among the first plant assemblages described by European phytogeographers (the so-called *Caricetum curvulae* in the phytosociological studies (Braun-Blanquet & Jenny, 1926). An extensive grey literature is available on the floristic composition of these grasslands (Theurillat, 1996). Here, we used 60 different regional studies to generate a floristic data set covering the entire distributional range of the species (see Appendix S1 in Supplementary Material for a full list of the original sources used). The number of plots in each investigated region is given in Fig. 1. Importantly, there were at least three different sources of data for each sampling region to limit possible investigator biases. The main aim of each of these monographs was generally to describe all the types of alpine plant communities in a given area. Alpine grasslands dominated by *C. curvula* are easily recognizable and are systematically included in these works when locally present. We selected all the plots for which the species abundance of *C. curvula* was above 25%. The final matrix comprised 757 plots and 203 species, with about 100 plots at least for each region. Ten percent of the floristic surveys were conducted by the authors.

Because each region is represented by a different number of vegetation releves, we used species accumulation curves for standardization on the basis of the sampling effort. Sample-based rarefaction's curves – also known as smoothed species accumulation curves – can be viewed as the probability of adding a previously unsampled species when comparing two subsamples of size *n* and *n* + 1 (Olszewski, 2004). Means of accumulated species numbers were obtained from repeated resampling of all the pooled samples of a given region (Gotelli & Colwell, 2001). The asymptote of the accumulation curve was extrapolated using a bootstrapping algorithm implemented in the 'vegan' R package (Oksanen, 2007). It is an estimate of the regional species pool, i.e. the regional set of species that are able to coexist in *C. curvula*-dominated plant communities. The slope of the steepest part of the accumulation curve – the initial slope – is a measure of the between-plot diversity in a given region (Olszewski, 2004).

To estimate the regional pool of AFLP bands, we also used accumulation curves with individual genotypes in place of vegetation releves. In this case, we did not rely on supplementary data, and the accumulation curves were obtained from the AFLP genotypes of the 29 studied sites.

Spatial analysis of potential habitat

We combined the topographical data derived from the European digital elevation model at a 90-m scale (Jarvis, Reuter *et al.*, 2006), and the European Soil Data base (ESDB) (European Commission, 2004) at a 1-km scale to map the potential habitat of *C. curvula* in the six investigated regions. We based our modelling approach on the simple assumption that the occurrence of *C. curvula* is almost certain if the conditions of the alpine belt on siliceous bedrocks are met. Therefore, the spatial overlay between the digital elevation model and the distribution map of siliceous bedrocks was taken as a proxy for potential habitat of the species.

We simulated the spatial distribution of potential habitats using a moving elevation 'window' that was 800 m wide, which corresponded to the elevational amplitude of the alpine belt. This elevation window was centred at different mean elevations in order to mimic the upshift and/or downshift of the alpine belt in relation to cooling and warming periods (see Appendix S2 in Supplementary Material for examples of simulated ranges for the Carpathians). Eight different simulations were performed by increasing the mean elevation of the alpine belt by steps of 200 m, from 1400 m to 2800 m, corresponding to an alpine belt range of 1000–1800 m and 2400–3200 m, respectively. For each simulation, we analysed the spatial configuration of patches of suitable habitats in each region. Several regional-scale indices were calculated to compare the habitat loss/gain and the habitat fragmentation among the different scenarios. Among them, three were retained as they were presumably the most important for their impact on diversity indices: (1) the total surface of suitable patches; (2) the number of patches; and (3) the percentage of the total surface covered by the top 5% of largest patches, which is a measure of the inequality of the patch size distribution in a given region.

All spatial analyses were made using ArcView 3.2 software (ESRI, Redlands, CA, USA). The other calculations and all graphs were generated in the open-source R-CRAN software (R Development Core Team, 2006).

RESULTS

Local species richness ranged from 6 to 31 species (Fig. 2a). Species richness in Eastern European mountains was significantly lower compared to the Western European mountains (Fig. 3a). Conversely, species evenness, which ranged from 0.07 to 0.43, did not exhibit any regional differences (Figs 2 and 3b).

The AFLP band richness was between 55 and 75, except for the Pyrenean populations where it was much lower (Figs 2a and 3c). Overall, within individual AFLP band diversity accounted for nearly two-thirds of the observed AFLP band richness. This proportion was significantly higher for the Carpathian Mountains (Figs 2c and 3d).
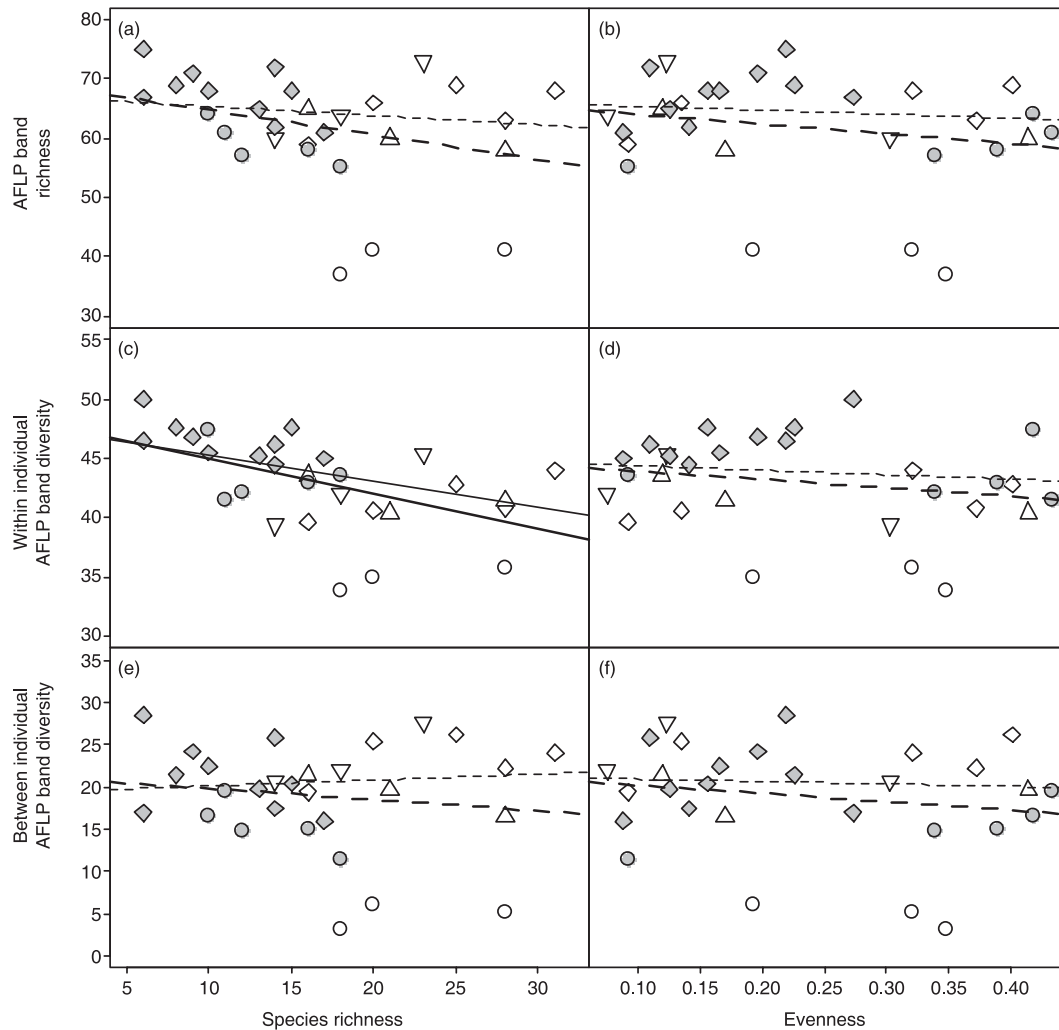
Figure 2 Bivariate plots between species diversity and genetic diversity. Species diversity indices include species richness (a,c,e) and species evenness (b,d,f), whereas the total AFLP band richness at the population level (a,b) is partitioned additively using separate richness indices calculated within (c,d) and between-individuals (e,f). Linear fits are shown for data sets including (grey lines) or excluding (black lines) the Pyrenees. Solid lines indicate a significant Pearson's product moment correlation at $P < 0.05$, and the symbol for each region is as shown in Fig. 1.

We did not find any evidence that species and genetic diversity indices were positively correlated (Fig. 2). The only significant correlation found was between the species richness and the within-individual AFLP band diversity (Fig. 2d). However, the correlation estimate was negative ($r = -0.52$, $P = 0.004$). Similar results were obtained after exclusion of the data from Pyrenees (Fig. 2).

AFLP band accumulation curves confirmed the particular situation of the Pyrenees. The initial slope of the curve indicated a low divergence between the sampled individuals (Fig. 4a). Marked differences in AFLP band richness were observed between regions, with Carpathian Mountains exhibiting the highest accumulated number of AFLP bands. On the other hand, there were no differences in the initial slope of the curves except for the Pyrenees. The species accumulation curves provided a totally different picture. Eastern European mountains were characterized by a lower floristic dissimilarity between plots compared with Western mountains (Fig. 4b). Moreover, the

estimated species pool in the Eastern European mountains was nearly two times lower than in the Alps (Fig. 4b). The floristic richness of the Pyrenees was intermediate between the Alps and the Eastern European mountains. The estimated regional species pools were in good agreement with the calculated area of potential habitat (Fig. 4c). The range dynamics of the siliceous alpine belt for each region is depicted in Fig. 5. For the Carpathians and the Balkan mountains, a tremendous increase in the total surface occupied by suitable patches was observed when the mean elevation of the alpine belt shifted from 2400 m to 1400 m. Based on these simulations, cold periods characterized by a downshift of alpine elevation belt should result in a much larger expanse of suitable areas for *C. curvula* in Eastern European ranges (Fig. 5a). In the Alps, a similar trend, although of lower magnitude, was only noticeable for the Eastern region. This variation in the total surface of suitable habitats was accompanied by changes in the spatial arrangement of patches (Fig. 5b). In the Eastern regions, a few large patches accounted for 80% of the total surface during
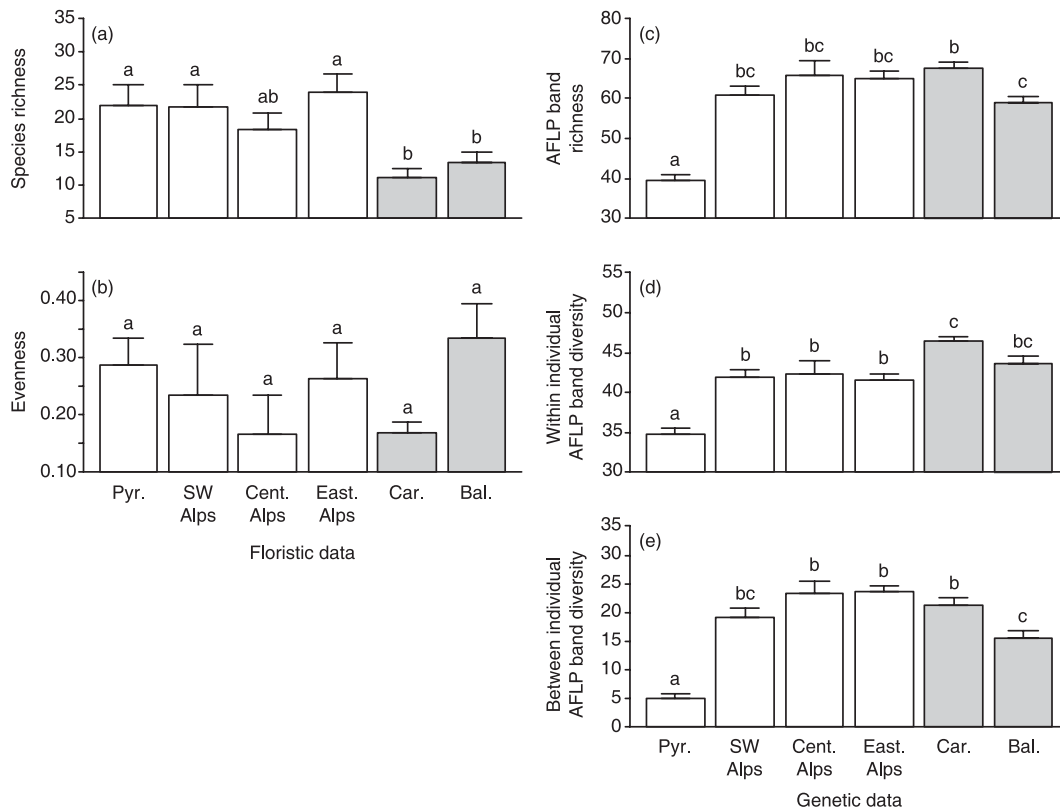
Figure 3 Regional means (+SE) of species (a,b) and genetic (c,d,e) diversity indices used in Fig. 2. Different lower case letters above bars indicate significant differences at the 5% level between regions after Tukey's *post hoc* tests.
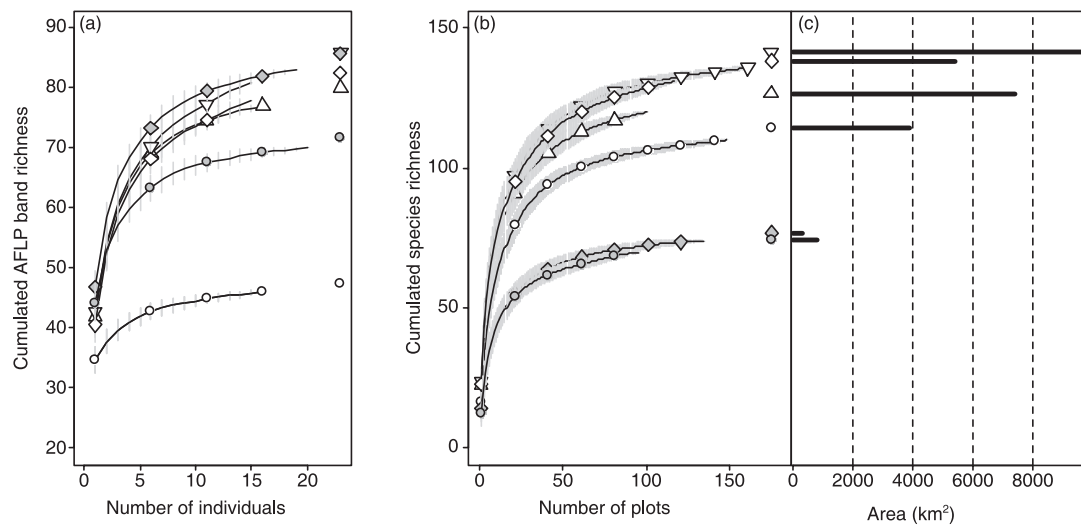

Figure 4 (a) AFLP band and (b) species accumulation curves for each region. Curves were obtained using 200 resamplings of AFLP individual profiles and species lists at the plot scale. Mean and standard deviation are shown for each region. (c) Estimate of the total surface covered by the suitable habitat for alpine siliceous grasslands in the different regions as resulting from a spatial modelling analysis. The symbol for each region is as in Fig. 1.

the cold periods, whereas this surface sharply declined in the warmer periods (Fig. 5b). Interestingly, a reverse trend was obtained for the South-Western Alps, in which an over-dominance of large patches was observed during warmer periods (Fig. 5b).

DISCUSSION

Our results provided evidence against a positive correlation between species and genetic diversity in the European alpine grasslands dominated by *C. curvula*. The highest genetic diversity
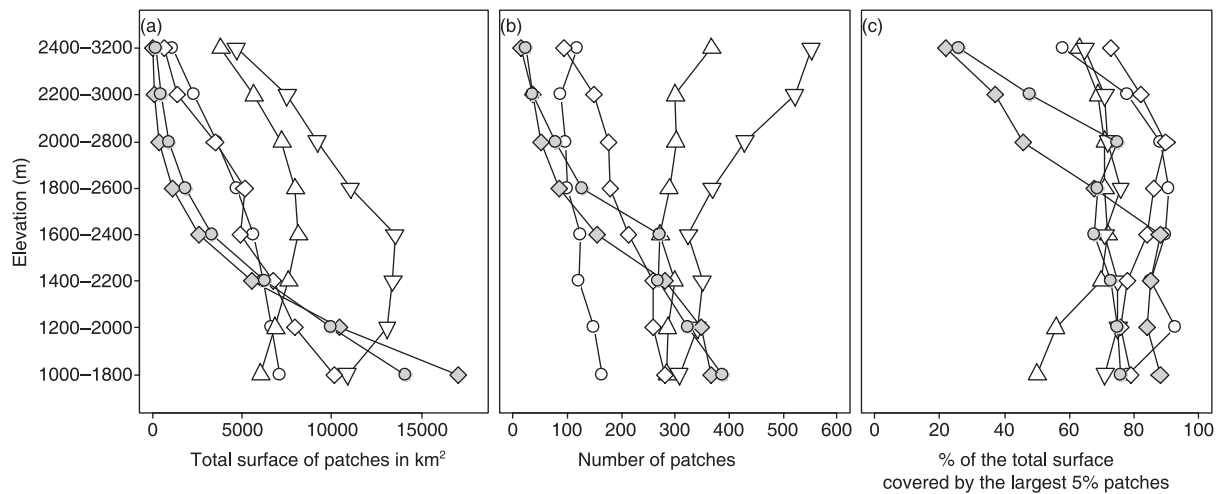
Figure 5 Regional-scale metrics of the suitable habitats for alpine siliceous grasslands. Indices were calculated for eight 800-meters altitudinal windows corresponding to eight different mean altitudes of the alpine belt. (a) The total surface covered by the suitable patches (b) the number of patches, and (c) the percentage of the total surface covered by the largest 5% patches are given for each region. The symbol for each region is as shown in Fig. 1.

was found in the species poor Carpathian grasslands, whereas the very low genetic diversity observed in the Pyrenees did not coincide with reduced species diversity.

A larger area of suitable habitats for siliceous alpine grasslands is found in the Alps, providing a straightforward explanation for the higher species pool and the increased plot-scale species richness observed in this region. By comparison, the Eastern European mountains (Carpathians and Balkans) exhibited 10 times less potential habitat than did the Alps. This could explain the lower diversity of current species assemblages in these mountains.

Using a modelling approach, we showed that the siliceous alpine grasslands of the Carpathian Mountains may have benefited from large and contiguous habitats during cooler periods, and that warmer periods in these areas triggered a much more severe habitat contraction compared to the Western European ranges. As discussed below, we hypothesize that these differing spatial dynamics over the Quaternary provide clues to explain the contrasting history of genotypes and species in these alpine grasslands.

Local determinants of diversity patterns

There have been contradictory hypotheses regarding the direct and local (i.e. at the community level) connection between genetic diversity of a focal species and the number of coexisting species. Variability in the competitive environment increases with species richness and this may favour the maintenance of several genotypes of a given species in a multispecies assemblage (Vavrek, 1998). Alternatively, a highly competitive pressure due to a large number of coexisting species may restrict the alpha niche of a focal species and be a source of stabilizing selection (Vellend & Geber, 2005). Here, we have reported large variations in both species and genetic diversity in the European grasslands dominated by *C. curvula*. Nevertheless, we found no relationship

between the two levels of diversity. This suggests that these local effects, though plausible, are not the main drivers of observed diversity patterns.

At the local scale, species richness may be determined by niche-based processes such as plant–plant interactions or availability of different microhabitats (Chave, Muller-Landau *et al.*, 2002; Lortie, Brooker *et al.*, 2004). For example, the relative cover of the dominant *C. curvula* may have contributed to reduce plot-scale species richness because of competitive effects. However, we did not find a significant effect of the abundance of *C. curvula* on species richness and evenness in the 29 investigated sites (data not shown). Similar results were obtained when analysing the larger floristic data set. Because we only considered a particular plant community with defined habitat requirements, the possible effect of environmental heterogeneity on the observed species diversity pattern is likely of limited importance. Of course, there are large climatic variations among the different mountain ranges investigated, and this clearly affects the regional abundance of the *C. curvula*-dominated grasslands (Choler & Michalet, 2002). One might expect that the drier alpine grasslands in the South European mountains under Mediterranean influence (Pyrenees and Balkans) exhibit higher microhetero-geneity that may favour increased species richness. But such a trend is not observed in the *C. curvula* grasslands (Fig. 3a).

Carex curvula grasslands are dominated by long-lived perennials, and *C. curvula* individuals may be surprisingly long-lived (Steinger *et al.* 1996). The grasslands recolonized high-elevation sites after the last ice retreat (around 15,000 years ago in the Alps, Frenzel *et al.* 1992). Though a significant influence of selection is certainly possible at this time scale, we assumed that speciation has not created large diversity differences since the Holocene.

Hence, it is apparent that none of these niche-based, local effects seem to primarily determine species and genetic diversity patterns in these alpine grasslands. We hypothesize that explanations

based on neutral processes operating at larger spatial and temporal scales are much more plausible.

Regional determinants of diversity patterns

The siliceous alpine belt of temperate European mountains can be viewed as a terrestrial system of habitat patches. Species–area relationships described in island biogeography are relevant for interpreting the distribution patterns of diversity within these alpine grasslands (MacArthur & Wilson, 1967). Here, we found that differences in regional species pools matched the differences in the extant area covered by these grasslands (Fig. 3). The local species richness is strongly related with the regional species pool (Zobel, 1997), and this provides a straightforward explanation for the low species richness observed in the Carpathian and Balkan sites. Glacial periods could lead to an expansion of suitable habitats for cold-adapted species but, surprisingly, few authors have examined the consequences of such an expansion for genetic and species diversity (Comes & Kadereit, 1998). In the Eastern European mountain ranges, our distribution model of potential habitat indicates that the current small patches of suitable habitats isolated from each other are the result of a marked contraction and fragmentation of a larger and more continuous habitat characterizing colder periods. These temporal changes in landscape structure are known to affect population and metapopulation persistence (Hanski & Ovaskainen, 2003). It is likely that an increased rate of species extinction in the Carpathians occurred during the current or previous interglacial periods, but the palaeobotanical evidence is still scarce (Obidowicz, 1996). Further work would be necessary to model species' extinction rates in alpine grasslands following post-glacial habitat contraction and fragmentation (Gurd, 2006).

On average, *C. curvula* individuals from the Carpathians exhibited the highest number of AFLP bands in any of the mountain ranges studied. This feature is even more pronounced when considering the within-individual AFLP diversity. For tree species, maximum allelic or AFLP band richness have been described as a possible feature of refugial areas (Comps, Gömöry *et al.*, 2001; Coart, Van Glabeke *et al.*, 2005; Magri, Vendramin *et al.*, 2006). The increase of favourable habitat in the Carpathians during cold periods (see above) likely corresponds to such a glacial refugium for *C. curvula* and other siliceous alpine species (Feurdean, Wohlfarth *et al.*, 2007). We hypothesized that the spatial configuration of the landscape at this time favoured gene flow among *C. curvula* populations and might have resulted in a large common genetic pool. After the ice retreat, each population may have started an upward colonization with a similar level of diversity. As these upward shifts were not representing long-distance migration events, the loss of within-population diversity may have been limited.

A contrasting scenario probably occurred in the Pyrenees. Here, the very low genetic diversity suggests the occurrence of a strong genetic bottleneck resulting from long-distance founder events. Additional data indicated that Pyrenean populations are nested within a South-Western Alps clade and share some region-specific AFLP bands with populations from the French Alps (Puşcaş *et al.*, 2008). A high level of endemism is described for the high-elevation flora of the Pyrenees, and this mountain range has often been considered to be an important glacial refugium because of its southern location within the European Alpine System (Küpfer, 1974). Our genetic data provide evidence that this was not the case for *C. curvula,* explaining the large discrepancy between genetic and species diversity found here.

CONCLUSION

The relationship between species diversity and genetic diversity in the European siliceous alpine grasslands dominated by *C. curvula* is primarily determined by large-scale temporal and spatial drivers. The lack of positive, or even negative, correlations between both levels of diversity is explainable by a contrasting history of genes of the dominant and the coexisting species in response to the spatial range dynamics induced by the alternate of glacial and interglacial periods. AFLP band richness remained preserved in the regions located near the main glacial refugia (Carpathians – Eastern Alps), as proposed for deciduous trees (Widmer & Lexer, 2001). However, sanctuaries for AFLP band richness do not appear to be sanctuaries for species richness. In the areas that experienced a striking habitat loss and fragmentation, subordinate species seem more prone to extinction than genes of the dominant.

Metapopulation persistence of genes and species in a fragmented landscape is highly dependent on the life-history traits of species. Further investigations should question how life-history traits may affect the response of alpine species to large-scale spatial and temporal range dynamics. Additionally, assessing the population genetics of a set of closely associated species, and not only the dominant, would certainly improve our understanding of the parallel effects of landscape dynamics on species and gene pools.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Appendix S1 A list of the monographs used for the synthesis on the floristic diversity of *C. curvula* dominated grasslands.

Appendix S2 Two examples of maps representing the potential range of the siliceous alpine belt in the Carpathians.

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