Broad-scale adaptive genetic variation in alpine plants is driven by temperature and precipitation

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Abstract

Identifying adaptive genetic variation is a challenging task, in particular in non-model species for which genomic information is still limited or absent. Here, we studied distribution patterns of amplified fragment length polymorphisms in response to environmental variation, in 13 alpine plant species consistently sampled across the entire European Alps. Multiple linear regressions were performed between AFLP allele frequencies per site as dependent variables and two categories of independent variables, namely Moran's eigenvector map variables (to account for spatial and unaccounted environmental variation, and historical demographic processes) and environmental variables. These associations allowed the identification of 153 loci of ecological relevance. Univariate regressions between allele frequency and each environmental factor further showed that loci of ecological relevance were mainly correlated with MEM. We found that precipitation and temperature were the best environmental predictors, whereas topographic factors were rarely involved in environmental associations. Climatic factors, subject to rapid variation as a result of the current global warming, are known to strongly influence the fate of alpine plants strongly. Our study shows, for the first time for a large number of species, that the same environmental variables are drivers of plant adaptation at the scale of a whole biome, here the European Alps.

Keywords: adaptation, climate change, environmental association analysis, genome scan, landscape genetics, loci of ecological relevance

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Introduction

Detecting adaptive genetic variation in response to environmental variation helps to better understand the potential of organisms for rapid evolutionary adaptation (Hoffmann & Willi 2008). However, identifying adaptive genetic variation is challenging, in particular in non-model species for which genomic information is still limited or absent (Stinchcombe & Hoekstra 2008).

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Nevertheless, technical advances in genomic screening for numerous molecular loci spread over the genomes of many individuals allow us to identify genetic variation linked to the environment even in non-model organisms. Such environmental association studies are based on the relationship of the frequencies of alleles at particular loci with the variation in particular environmental variables (Manel *et al.* 2010a). The underlying assumption is that natural selection along environmental gradients generates gradual changes in allele frequencies at loci physically linked to adaptive genes (Haldane 1948; Endler 1986; Schmidt *et al.* 2008). The distribution of alleles at loci of ecological relevance is

thus different from the distribution of alleles at neutral loci (Holderegger *et al.* 2010).

Patterns of genetic variation that seem to be caused by natural selection may in fact also result from historical demographic processes (Excoffier *et al.* 2009; Siol *et al.* 2010). First, isolation by distance may limit gene flow among populations over a large scale, and the frequency of neutral alleles will change simply as a result of genetic drift (Wright 1938). Second, contact and admixture zones, where populations that diverged in spatial isolation (e.g. glacial survival in different refugia) come into secondary contact, could confound adaptive genetic patterns (Endler 1977). Third, bottlenecks and inbreeding create patterns that mimic those of selective sweeps (Storz 2005). Searching for congruent patterns of adaptive loci across replicated regions represents one way of limiting the confounding effects of historical demographic and spatial processes, as it is unlikely that the latter produced similar genetic patterns at a given locus across independent environmental gradients (e.g. Poncet *et al.* 2010; Buehler *et al.* in, revision). In this study, we argue that finding similar patterns of correlation between loci and ecological factors in different species within the same vast study area constitutes another way of avoiding confounding effects, as it is unlikely that different species are subject to the same demographical processes.

Previous studies in plant species found distinct patterns of genetic variation in allozyme frequencies along environmental gradients (Allard *et al.* 1993; Linhart & Grant 1996; Prentice *et al.* 2000). For example Hirao & Kudo (2004) found a correlation between allozyme frequencies and flowering time in *Primula cuneifolia* along a snowmelt gradient. Shimono *et al.* (2009) detected morphological traits co-varying with allozyme frequencies in fellfield and snowbed populations of *Potentilla matsumurae*. Their results suggest that the timing of snowmelt causes a selective pressure that drives local adaptation in this alpine plant species (but see Stanton & Galen 1997). Recent technological advances in molecular markers have made larger genome scans feasible, more powerful and with high genomic resolution, facilitating the identification of loci that are potentially of ecological relevance (Manel *et al.* 2010a). For example Poncet *et al.* (2010) studied 825 amplified fragment length polymorphism (AFLP) loci at 208 locations across the European Alps in *Arabis alpina*. They detected four AFLP loci, common in two independent regions of the French and Swiss Alps, which are linked to mean annual temperature and/or precipitation. Recent studies at the whole genome level also confirmed the existence of loci under selection by climatic factors in the model plant *Arabidopsis thaliana* (Fournier-Level *et al.* 2011; Hancock *et al.* 2011a).

Here, we present patterns of AFLP allele distributions in response to environmental variation in 13 alpine plant species collected across the entire range of the European Alps following a stratified sampling design (Gugerli *et al.* 2008). Our objective was to (i) identify topographic and climatic factors potentially involved in the adaptation of alpine plants to their environment, to derive testable hypotheses about the mechanisms underlying patterns of adaptive genetic variation (Feder & Mitchell-Olds 2003). Once the important environmental factors were identified per species, we addressed the question of (ii) whether there are common patterns of genetic adaption to environmental variables across alpine plants. To date, published studies have largely focused on single species, preventing the detection of general patterns of adaptation to environmental factors (e.g. Jump & Penuelas 2005; Joost *et al.* 2007; Segelbacher *et al.* 2010; Cox *et al.* 2011). In contrast, multi-species studies, such as the one we provide here, make it possible to draw general conclusions about the environmental factors involved in the adaptive response of species.

Material and method

Plant collection and genotyping

The sampling and genotyping of the 13 alpine plant species studied were carried out according to the methods described in detail in Gugerli *et al.* (2008); Table 1, Fig. 1). All samples were collected within a single growing season (June–September 2004). Sampling was standardized across species by using a regular grid with a cell size of 20′ longitude by 12′ latitude across the whole area of the European Alps. Sampling was limited to those cells comprising area above 1500 m a.s.l., and only every second cell was considered. Within each grid cell considered, we arbitrarily chose one location per species based on known occurrences or as expected from species-specific habitat requirements, optimizing field work by searching for areas containing many species across just a few locations (Gugerli *et al.* 2008). The number of locations ranged from 74 locations for *Gentiana nivalis* to 137 for *Carex sempervirens*. Leaves were collected from three plants per sampling location. Despite the small number of specimens sampled per location, data were considered informative given the main objectives of our study, i.e. identifying broad-scale patterns of general adaptation. At the same time, the large number of sampling locations distributed over a large ecological and altitudinal range per species resulted in a broad variety of environmental conditions (Thiel-Egenter *et al.* 2009). All samples were genotyped using AFLPs (Vos *et al.* 1995) with three primer–enzyme combinations (for details, see Gugerli *et al.*

Table 1 Alpine plant species (with family) analysed at amplified fragment length polymorphism (AFLP) loci and tested for relations between AFLP allele frequencies and environmental variation. For each species, the laboratory responsible for AFLP genotyping is indicated. Number of sampling sites and individuals genotyped, Nei's (1973) gene diversity averaged over all sampling sites (± standard error, SE) and overall genetic differentiation (Φ_{ST}) are given for each study species. N_{tot}, total number of loci; N_{ler}, number of loci of ecological relevance; N_{MEM}, number of broad-scale Moran's eigenvector map (MEM) variables

Species	Sampling Sites	Individuals genotyped	Gene diversity	Φ_{ST}	N_{tot}	N_{ler}	N_{MEM}
(1) *Arabis alpina* L. (UJF)[*] [Brassicaceae]	129	385	0.061 ± 0.004	0.664	140	20	22
(2) *Campanula barbata* L. (UNE)[†] [Campanulaceae]	104	307	0.107 ± 0.004	0.385	114	13	16
(3) *Carex sempervirens* Vill. (WSL)[‡] [Cyperaceae]	137	408	0.083 ± 0.002	0.328	122	3	31
(4) *Dryas octopetala* L. (UJF) [Rosaceae]	124	370	0.123 ± 0.003	0.197	99	4	24
(5) *Gentiana nivalis* L. (UNE) [Gentianaceae]	74	218	0.079 ± 0.006	0.600	157	16	10
(6) *Geum montanum* L. (WSL) [Rosaceae]	122	363	0.091 ± 0.003	0.313	85	12	24
(7) *Gypsophila repens* L. (WSL) [Caryophyllaceae]	107	319	0.110 ± 0.003	0.238	94	2	22
(8) *Juncus trifidus* L. (WSL) [Juncaceae]	91	269	0.126 ± 0.005	0.289	86	8	14
(9) *Loiseleuria procumbens* (L.) Desv. (UJF) [Ericaceae]	90	270	0.188 ± 0.004	0.293	116	22	16
(10) *Phyteuma hemisphaericum* L. (UV)[§] [Campanulaceae]	76	225	0.112 ± 0.004	0.342	234	24	15
(11) *Rhododendron ferrugineum* L. (UJF) [Ericaceae]	126	377	0.135 ± 0.004	0.375	111	24	12
(12) *Saxifraga stellaris* L. (UV) [Saxifragaceae]	100	283	0.074 ± 0.003	0.428	70	2	22
(13) *Sesleria caerulea* (L.) Ard. (UCSB)[¶] [Poaceae]	113	265	0.201 ± 0.009	0.196	187	19	21

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2008). The primer–enzyme combinations were species specific. Thus, the AFLP loci amplified were clearly independent across species.

Nei's gene diversity (Nei 1973), which corresponds to the mean number of pairwise differences between individuals across AFLP loci per sampling site (Kosman 2003), was averaged across sampling sites per species using program ARLEQUIN 3.5.1.3 (Excoffier & Lischer 2010). Overall genetic differentiation per species was estimated with Φ_{ST} (Excoffier *et al.* 1992), assuming Hardy–Weinberg equilibrium at AFLP loci (Bonin *et al.* 2007) and testing significance based on 1000 bootstrap replicates in ARLEQUIN 3.5.1.3.

Environmental variables

Fourteen monthly and annual environmental variables related to temperature, precipitation, radiation and topography at 200 m resolution were extracted for each sampling location from published GIS topo-climatic layers from 1980 to 1989 (Zimmermann & Kienast 1999; Table 2, Fig. 1). These environmental variables, excluding topographic aspect, were transformed into quadratic orthogonal polynomials to account for non-linear relationships between AFLP allele frequencies and these variables (Legendre & Legendre 2012). Aspect was transformed into sin(aspect) and cos(aspect), rendering this variable appropriate for use in linear regressions.

Moran's eigenvector map variables

Moran's eigenvector map (MEM) variables were used as explanatory variables in the regressions, to account for purely spatial and for unmeasured environmental variables. They make it possible to separate and model the spatial patterns comprised in the variations in response data, which in this are AFLP allele frequencies. Moran's eigenvector map variables are the eigenvectors of a spatial weighting matrix calculated from the sites' geographical coordinates (Borcard & Legendre 2002; Dray *et al.* 2006). Moran's eigenvector map analysis produces uncorrelated spatial eigenfunctions used to dissect the spatial patterns of the studied variation across a range of spatial scales. The first few MEM variables with large Moran's *I* coefficients model broad-scale processes (e.g. genetic variation at large spatial scales such as phylogeographic patterns), whereas subsequent MEM variables with smaller Moran's *I* coefficients refer to the spatial autocorrelation generated by processes such as gene flow among subpopulations and genetic drift (Dray *et al.* 2006). As in our data set the number of sites varied among species, the number of MEM variables was species specific. For our analysis, we only used the broad-scale MEM variables, i.e. the first half of the MEM eigenfunctions with positive eigenvalues, which model broad-scale spatial variation (Manel *et al.* 2010b). MEM variables were computed
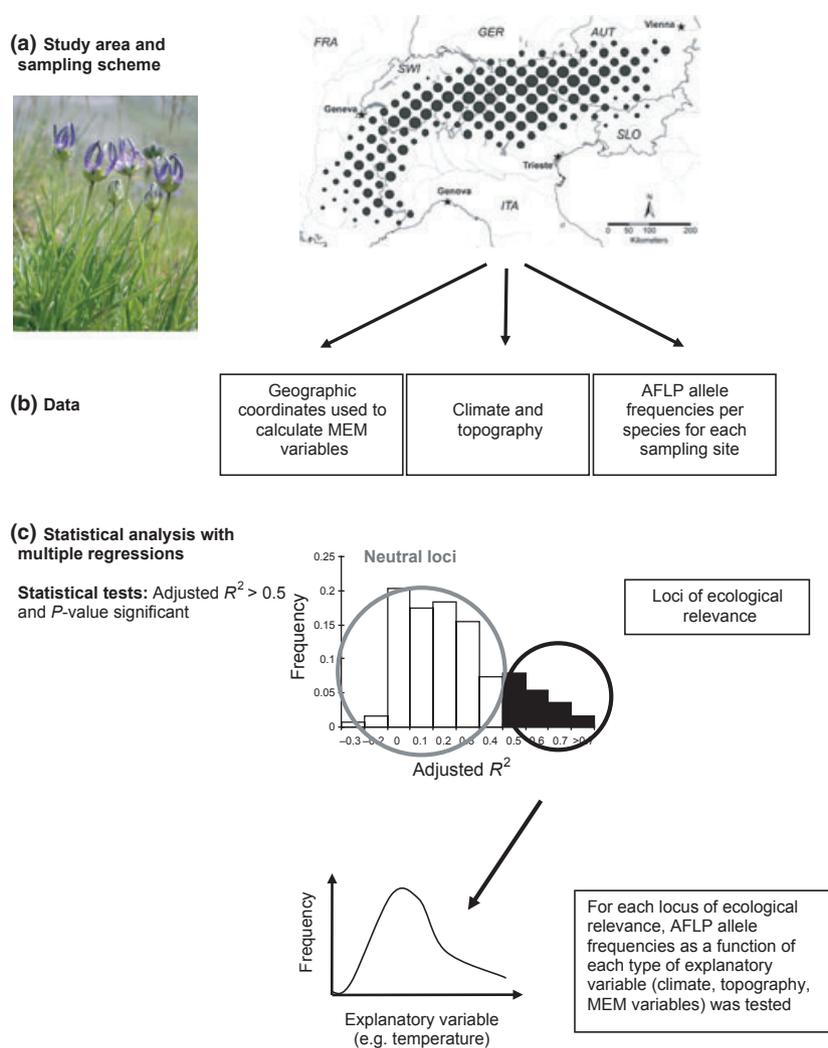
Fig. 1 Approach used in this study to detect loci of ecological relevance in 13 alpine plant species. (a) Map of the European Alps 9 showing the study range and sampling intensity (circle area proportional to number of species genotyped per grid cell). (AUT, Austria; FRA, France; GER, Germany; ITA, Italy; SLO, Slovenia; SWI, Switzerland). The number of sampling sites per species is given in Table 1. (b) Type of genetic, environmental and spatial data (MEM variables: Moran's eigenvector maps) used. (c) Schematic of the statistical analyses. The example given in (a) is *Phyteuma hemisphaericum*, one of the alpine plants studied.

using the 'PCNM' R package 2.12.2 (available at http://r-forge.r-project.org/R/?group_id=195). The computation of MEM variables is explained in detail in Borcard *et al.* 2011, (Chapter 7) and Legendre & Legendre (2012), Chapter 14.

Identification of loci of ecological relevance using multiple regression

Our study aims to find the general responses of AFLP allele frequencies to environmental variation across several species. However, the correlations among environmental variables were species specific. This meant we could not only select the environmental variables that were uncorrelated before performing single-species multiple regressions. We, therefore, used a two step-approach:

In the first step, (i), multiple linear regressions were performed between AFLP allele frequencies per site as dependent variables and MEM variables and all environmental variables as independent variables for each of the 13 studied species. This allowed us to identify loci of ecological relevance. In the second step, (ii), these loci putatively under selection were regressed against each of the environmental and MEM variables separately to identify particular variables in the environmental association. By doing so, we were able to assess the relative effects of spatial structure, climate and topography (Fig. 1), although it was not possible to separate these partly interdependent

Table 2 Topo-climatic variables used in this study

Yearly or seasonal climate layers (period 1980–1989)	(1) Annual mean of daily global radiation (horizon/terrain-corrected) [kJ/m²/day]
	(2) Mean annual precipitation sum [cm]
	(3) Summer seasonal precipitation: number of rain days from June to August [mm]
	(4) Spring seasonal precipitation: number of rain days from March to May [mm]
	(5) Annual degree days above 0 °C from daily climate maps [°C × days]
	(6) Number of days with maximum temperature below 0 C°
	(7) Annual mean of maximum daily temperatures [°C]
	(8) Number of days with minimum temperatures below 0 C°
	(9) Annual mean of minimum daily temperatures [°C]
Topography	(10) Slope [%]
	(11) Integrated topographic exposure map
	(12) Potential soil humidity*
	(13) Aspect [degree]
	(14) Altitude [m]

*Ratio of the upslope contributing region on the tangent of the slope angle (Beven & Kirby 1979).

factors fully. This approach is described in more detail below.

(i) For each species and each AFLP locus, the allele frequency of band presence (i.e. the frequency of a particular AFLP fragment) per site was regressed simultaneously on 14 environmental factors (Table 2), either untransformed or transformed, for a total of 28 environmental variables tested (i.e. including both linear and quadratic effects, cos and sin aspect), and on MEM variables. Part of the variation accounted for by MEM variables in regression analysis was also explained by the above 28 environmental factors, as the latter could be correlated with MEM variables. The remaining variation explained by MEM variables represented environmental variation that was not modelled by the environmental variables included in the study. We assumed that this broad-scale spatial variation could be partly related to environmental variables that had not been measured in our study, and partly to the historical dynamics of alpine plants (e.g. colonization routes during range contraction and expansion, survival in refugial areas, secondary contact after re-immigration, etc.; Alvarez *et al.* 2009). Note that in the multiple linear regressions, allele frequencies are discrete rather than continuous variables, with only four possible states (0, 0.33, 0.66 and 1) because only three individuals per site were taken into account.

The significance of the multiple regressions per locus against the environmental and MEM variables was corrected for multiple tests by the Holm correction (Wright 1992). Although the Holm procedure produces a correct experiment-wise error rate, it may still be seen as a liberal criterion because many loci with significant, but small amounts of explained variation will be retained. To make a conservative judgement on the importance of loci of ecological relevance, we therefore also required that a fixed proportion of the variation per

locus, measured by $R^2_{adj} = 0.5$ (i.e. 50%), was explained by the environmental variables and the broad-scaled MEM variables. The adjusted coefficient of determination, R^2_{adj} provides unbiased estimates of a response variable's variation accounted for in a linear model (i.e. explanatory power of variables in multiple regression) (Ohtani 2000).We only considered loci fulfilling both criteria (i.e. significance after accounting for multiple testing and $R^2_{adj} > 0.5$) as being of ecological relevance.

(ii) Because the explanatory variables were not uncorrelated, but species dependent (Fig. S1, Supporting information), allele frequencies at the identified loci of ecological relevance were then related with each environmental variable and each MEM variables separately in univariate regressions, to estimate the explanatory power provided by each predictor variable.

Results

For the present analysis, a total of 3963 individuals were genotyped for AFLP loci varying in number from 70 in *Saxifraga stellaris* to 234 in *Phyteuma hemisphaericum* (Table 1). This produced a total of 1615 AFLP loci analysed across 13 species. The reproducibility of the markers identified was checked with positive controls and 5–10% replicates from DNA isolation to selective polymerase chain reaction. We obtained mismatch error rates of <5% for all species (Gugerli *et al.* 2008), which represents a fairly low value for AFLPs (Bonin *et al.* 2007).

Nei's gene diversity across AFLP loci averaged across all sampling sites per species ranged between 0.061 for *Arabis alpina* and 0.201 for *Sesleria caerulea* (Table 1). All studied species showed significant overall genetic differentiation (Φ_{ST}). Inversely to Nei's gene diversity, *Sesleria caerulea* showed the lowest genetic differentiation (0.196), and *Arabis alpina* revealed the highest

genetic differentiation (0.664). These results show that there was ample genetic diversity within and genetic differentiation among the sampling sites in our study species, a prerequisite for the application of environmental association analysis.

The ranges of the environmental variables across the altitudes considered were broad, reflecting the variability of ecological conditions under which populations for all species were sampled in this study (Fig. S2, Supporting information). The number of broad-scaled MEM variables varied from 12 in *Geum montanum* to 24 in *Rhododendron ferrugineum* and *Phyteuma hemisphaericum* (Table 1).

We identified 153 loci (9%) that were significantly related to environmental and MEM variables across the 13 species studied (Table 1). The percentages of loci of ecological relevance varied among species and ranged from 2% in *Gypsophila repens* to 18% in *Rhododendron ferrugineum*. Details (i.e. P values and $R^2_{adj} \geq 0.5$) of loci identified as being of ecological relevance in multiple regressions are reported in Table S1 (Supporting information).

Univariate regressions showed that the broad-scaled MEM variables were involved in significant relations with allele frequencies in 149 of the 153 AFLP loci identified as being of ecological relevance (Table 3). They were detected as significant predictors for all loci of ecological relevance in six species, and as a major predictor in the seven other species (Tables 1 and 3). After accounting for broad-scale MEM variables, temperature and precipitation were the two major environmental factors related to AFLP allele frequencies at the identified loci of ecological relevance (Table 3). Summer seasonal precipitation was the major environmental factor, involved in 40 significant relations, followed by the number of days with minimum temperatures below 0 °C, involved in 36 significant relations (Table 3). Except for *Saxifraga stellaris*, for which only altitude was detected as a significant predictor, for all the other species either a precipitation or a temperature variable or both were involved when MEM variables were not the only relevant predictor variables (Table 3). We identified three types of species–environment interaction patterns according to these relations. In three species, precipitation was the major environmental factor affecting AFLP allele frequencies (*Campanula barbata*, *Carex sempervirens* and *Phyteuma hemisphaericum*); in five species, temperature played that role (*Arabis alpina*, *Gypsophila repens*, *Juncus trifidus*, *Loiseleuria procumbens* and *Rhododendron ferrugineum*) and, in four species, both precipitation and temperature were involved (*Dryas octopetala*, *Gentiana nivalis*, *Geum montanum* and *Sesleria caerulea*; Table 3). Topographic variables were only rarely involved in relevant relationships between AFLP allele frequencies and environmental variables (Table 3).

Discussion

Considering spatial structure caused by both unaccounted environmental variation and historical demographic processes, we found that environmental factors, mainly temperature and precipitation, influenced allele distributions at nearly 10% of the

Table 3 Number of significant univariate regressions per species and environmental or MEM variables. The names of the environmental variables (1–14) are shown in Table 2. Significance values were corrected for multiple tests. If both untransformed and transformed square variables of a particular environmental factor were simultaneously significant in a particular species, we considered only one significant relationship. The same rule was applied to MEM variables. For MEM variables, a AFLP marker associated with any number of the broad-scaled MEM variables was counted as a single relationship. Variables related to precipitations are highlighted by light grey shading and variables related to temperatures by dark grey shading

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	MEMs
Arabis alpina	0	1	11	0	1	12	12	13	13	0	0	0	0	3	17
Campanula barbata			5		1					1	1				13
Carex sempervirens			1												3
Dryas octopetala		1	1	1	1			1	1						4
Gentiana nivalis		7	1		0	2	2	1	1		1			1	12
Geum montanum		1	3	3	1			2	2						11
Gypsophila repens		1	1		1	1	1	1	1					1	2
Juncus trifidus			3	2		1	3	5	4						8
Loiseleuria procumbens				1				6	6						21
Phyteuma hemisphaericum		4	1	16	4										20
Rhododendron ferrugineum			7	1		3	6	7	7			1		1	20
Saxifraga stellaris														1	2
Sesleria caerulea		1		2	1	1					1				16
Total	0	9	40	27	10	20	24	36	35	1	3	1	0	7	149

AFLP loci tested across 13 alpine species from the European Alps. These loci of ecological relevance may be considered as either potentially adaptive or as linked to the genes or genomic regions under selection.

Population-based approaches of Bayesian geographical analysis can be used to test for correlations between allele frequencies and environmental variables, after correcting for background levels of population structure and differences in sample size (Yu *et al.* 2006; Hancock *et al.* 2008, 2010, 2011b). However, these approaches can only be applied to cases where a high number of individuals can be sampled in well-defined populations. In addition, they require independent genetic data to estimate population structure. As we only sampled three individuals per species per site, we could not apply these methods and, thus, we used instead the linear regression method used by Manel *et al.* (2010b). For instance, for most species considered to be relevant in conservation biology regarding climate change, it will neither be possible to sample large numbers of individuals from numerous populations nor to have an independent genetic data set available to test for genetic structure. To consider spatial demographic history, we used MEM variables as complementary explanatory variables. MEMs allowed us to model (i) pure large-scale spatial effects, (ii) environmental effects resulting from unmeasured environmental variables and (iii) spatial effects co-varying with measured environmental variables. However, one cannot fully disentangle these different effects from each other.

After considering spatial effects by MEM variables, the close relationship found between AFLP allele frequencies and temperature and/or precipitation across the 13 species studied (Table 3) strongly suggested that climatic factors are generally involved in affecting (putatively) adaptive genetic variation in alpine plants. This finding confirmed previous single-species studies on various plant species (e.g. Parisod & Bonvin 2008; Richardson *et al.* 2009; Cox *et al.* 2011). As we model the spatial effects of the historical dynamics of species using MEM variables and used AFLP loci that are considered being randomly distributed across the genome, it is unlikely that many of the loci identified were false positives (e.g. Richardson *et al.* 2009; Sork *et al.* 2010; Bierne *et al.* 2011). However, the occurrence of false positives cannot be completely ruled out, but should be rather low in this study.

Temperature and precipitation are well known to influence the survival of alpine plants strongly, although their effect may involve complex pathways (Körner 2003). Therefore, these environmental factors are likely to act as major drivers of selective responses in alpine plants (Boyer 1982; Chaves *et al.* 2003). In concordance with our results that found either or both of these two environmental factors to be important in different species, previous studies have shown the prominent role of temperature and precipitation in the general adaptation of plants (Skot *et al.* 2002; St Clair *et al.* 2005; Richardson *et al.* 2009). The importance of the above two environmental factors in the adaptation of alpine plants has also been found, at different spatial scales, in *Arabis alpina* (Manel *et al.* 2010b; Poncet *et al.* 2010). In *Boechera stricta*, a wild relative of *Arabidopsis thaliana*, Lee & Mitchell-Olds (2011) recently emphasized the role of ecological factors *versus* geographical distance in creating and maintaining adaptive genetic differentiation across a species' range. However, our study shows, for the first time, and for a large number of species, that the same environmental variables are drivers of plant adaptation at the scale of a whole biome, here European Alps. One major limitation of this study is that we only tested climatic and topographic factors and that we did not include soil properties, namely calcareous or siliceous bedrock, as environmental factors in this analysis. It is well known that soil properties affect the distribution of alpine plants (Alvarez *et al.* 2009) and are therefore also likely to play an important role in adaptive evolutionary responses (Körner 2003). However, data for soil variables are currently not available for the entire Alps at the spatial resolution needed for an environmental association analysis.

One contemporary, yet challenging question is whether adaptive evolution can keep pace with the rate and direction of environmental and climate changes induced by human activities (Lavergne *et al.* 2010). Several studies have shown that species have already shifted their geographic ranges in response to climate change (Walther *et al.* 2002; Frei *et al.* 2010), whereas the general potential of species to adapt to rapid environmental change is still being debated (Davis *et al.* 2005; Reusch & Wood 2007; Jay *et al.* in press). Our results identified temperature and precipitation as potential drivers of adaptation. Such information is highly useful in modelling future vegetation dynamics under climate change, which relies on scenarios of ecological change and respective responses of plant communities. As a consequence of the ever-increasing genomic data available as a result of technological progress (Shendure & Ji 2008), it might become possible not only to predict species distributions in response to climate change (Guisan & Thuiller 2005), but also to develop scenarios regarding the spatial distribution of adaptive genetic variation at the whole-genome level in response to changes in temperature and precipitation regimes as proposed by Fournier-Level *et al.* (2011) and Hancock *et al.* (2011a). Future steps will be to integrate the results on drivers of genetic adaptation into

bioclimatic models and to test the evolutionary and functional relevance of temperature and precipitation in alpine plants under experimental conditions.

The results presented here provide multi-species empirical evidence of genetic variation related to climatic variables. The correlation of temperature and precipitation to signals of adaptation in the alpine species studied here suggested that there should indeed be ample standing genetic variation available in alpine plants based on which adaptation could occur during the course of climate change.

Conclusions

Local adaptation is the only possible response that living organisms have to cope with climate change to avoid extinction, if their dispersal capacity and phenotypic plasticity are insufficient to keep pace with environmental change. So far, the scientific community has largely ignored the potential of adaptive genetic variation as a rapid response to environmental change. By combining genetic and topo-climatic data, we found that loci potentially linked to genes or genomic regions showing adaptive responses to climatic factors are present in alpine plant species. Our approach might allow researchers to assess general patterns of adaptive genetic response to environmental variation at the scale of whole biomes in virtually any group of organisms. This opens new perspectives for understanding the interplay between dispersal and adaptation in the evolutionary response of species to climate change.

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Data Accessibility

Sampling locations and AFLP data for all species: DRYAD entry doi:10.5061/dryad.f3rk4 (Meirmans *et al.* 2011a,b).

Supporting Information

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

Appendix S1 List of IntraBioDiv Consortium members.

Fig. S1 Principal component analysis of the environmental variables (as described in Table 2) for each studied species.

Fig. S2 Box plots of the variation in the environmental variables described in Table 2. Numbers along abscissas refer to species under study (Table 1).

Table S1 Adjusted R^2 and P-values of the significant multiple regressions per species and per locus.

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USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

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Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:
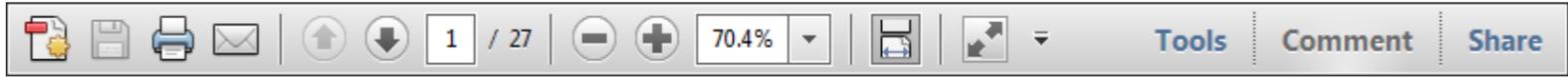
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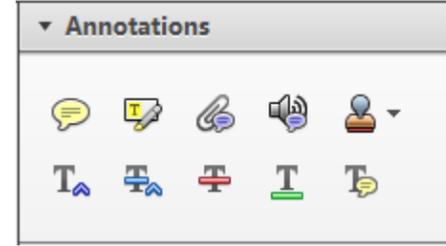
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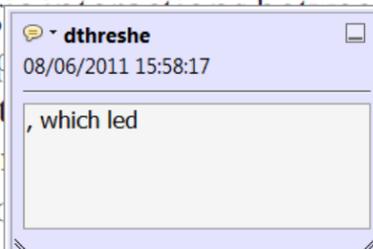
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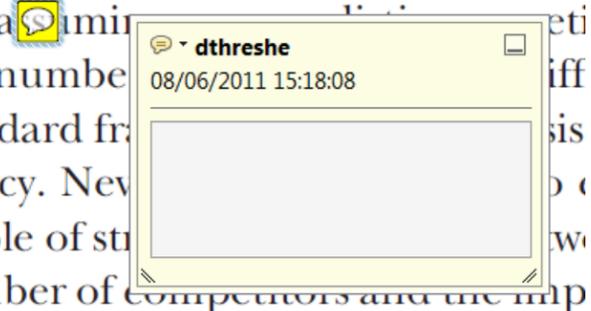### 4. Add sticky note Tool – for making notes at specific points in the text.

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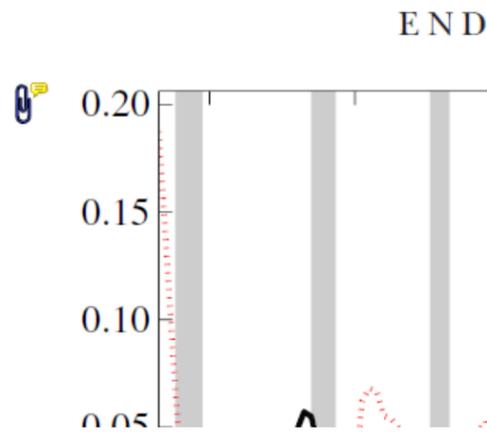
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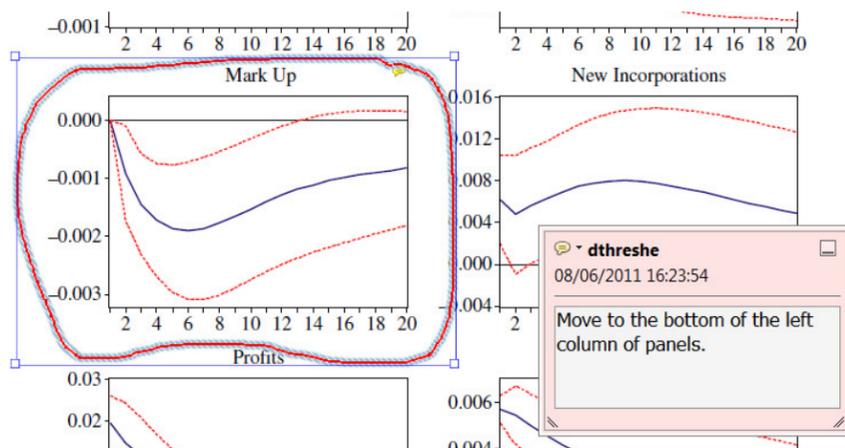
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