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Combined definition of seed transfer guidelines for ecological restoration in the French Pyrenees

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Abstract

Question: Can genetic tools combined with phytogeography help to define local plants and how geographically close the source population should be to the restoration site?
Location: Subalpine and alpine French Pyrenees.
Methods: The main phytogeographic boundaries in the French Pyrenees described by different authors were studied and this geographic pattern was compared with the results of genetic analysis for the four Pyrenean plants studied (*Trifolium alpinum*, *Festuca eskia*, *Festuca gautieri* and *Rumex scutatus*), based on random amplified polymorphic DNA (RAPD) marker analysis, unweighted pair-group method with arithmetic averages (UPGMA) analysis and Mantel correlograms comparing geographic and genetic distances.
Results: The genetic analysis allowed definition of two main evolutionarily significant units (ESUs) for the plants under study. Although the limit between the two zones was slightly variable according to the species considered, an eastern and a western ESU was consistently observed. This delineation was concordant with the main phytogeographic boundaries of the French Pyrenees.
Conclusion: RAPD markers and associated Mantel correlograms can be useful to draw ESUs for individual species when the sampling intensity is relatively dense, and similarities were revealed between species sharing the same distribution range. This delineation allowed integration of infraspecific plant variation in the management of natural resources for revegetation in the Pyrenees. Nevertheless, caution is needed for the establishment of seed pools in order to maximize genetic diversity in each of the pools during collection and production.

Keywords: Genetic distance; Genetic variation; Local provenance; Mantel correlogram; Native plant; Phytogeographic boundaries; RAPD markers; Seed collection strategy.

Nomenclature: Kerguélen, M. (1999).

Abbreviations: AMOVA = Analysis of molecular variance; RAPD = Random amplified polymorphic DNA; UPGMA = Unweighted Pair Group Method with Arithmetic mean.

Introduction

Revegetation practices: the need for native species

Unassisted recovery of vegetation in strongly disturbed areas is particularly long and difficult in extreme ecosystems, such as high altitude areas (Urbanska 1997; Muller et al. 1998; Peratoner 2006). With the constant increase in human activities, of which removal of the top soil is the primary threat to the ecosystem (Bradshaw 1997; Haselwandter 1997), revegetation above the timberline is becoming a common concern (Krautzer & Graiss 2006). In the French Pyrenees, machine grading affects more than 150 ha per year above 1 200 m a.s.l., generating the use of more than 40 t of seed mixtures per year (Malaval 2006).

Commercial herbaceous seed mixtures commonly used in alpine and subalpine revegetation are generally allochthonous, or from unknown geographic origin (Lumaret 1999). However, at these altitudes, they frequently fail to give durable results (Urbanska & Hasler 1992; Delarze 1994; Krautzer & Graiss 2006). This failure could be explained by a higher mortality and/or by a reduced vigour of the species at high elevation (Hufford & Mazer 2003; Krauss et al. 2005). In addition, the systematic use of non-native seeds takes its toll on biodiversity either through outbreeding depression (Fenster & Dudash 1994; McKay et al. 2005) or the introgression of genes that prove to be deleterious in this context (Rhymer & Simberloff 1996;

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Lumaret 1999; Keller et al. 2000; Sackville Hamilton 2001).

The European Bern Convention (19 September 1979) and the French law for the reinforcement of environment protection (no. 95-101, 2 February 1995), recommend the use of seeds of local provenance. Moreover, many scientists promote the reintroduction of native plants to degraded lands as an integral component of ecosystem restoration and biodiversity conservation (van Andel 1998; Lumaret 1999; Keller et al. 2000; Sackville Hamilton 2001; Burton & Burton 2002; Simberloff 2003; Krauss & Koch 2004; Krautzer & Wittmann 2005; Gustafson et al. 2005), although there are exceptions to this view (Wilkinson 2001). Species sourced locally can ensure technical success for revegetation, particularly at high altitude (Krautzer & Bohner 2002; Krautzer & Wittmann 2005).

What is a local plant?

In order to ensure conservation of flora and valorization in a sustainable way, it is crucial to define what a local plant is, and "to what extent a particular ecotype is local". This question implies delineation of seed transfer zones for each species. From a restoration point of view, these zones correspond to areas within which plant materials could be moved with minimal risk of maladaptation (as mentioned above) and minimal loss of biodiversity (Jones 2003).

In Europe, different approaches have been proposed to define seed transfer zones for native ecotype utilization or multiplication (Colomb et al. 2003; Krautzer et al. 2004; Aradottir & Johannsson 2006; Porqueddu & Maltoni 2006; Ševcíková et al. 2006). Those studies generally investigated transfer zones in a regional or national context and, when possible, in accordance with phytogeographical boundaries (Krautzer & Wittmann 2005; Vander Mijnsbrugge et al. 2005).

Phytogeographic boundaries in the French Pyrenees

Bridging from the Mediterranean Sea to the Atlantic Ocean, the Pyrenees encompass different climatic zones and geological substrates. From the calcareous west to the siliceous east, oceanic or continental conditions are dominant. From north (France) to south (Spain), humidity conditions evolve to a drought climate, influenced by Mediterranean conditions (Gruber 1980; Dupias & Rey 1985; Villar & Dendaletche 1994; Ozenda 2002). Owing to their climatic asymmetry, and the geological structure of the mountain range, the Pyrenees present a rich flora (around 3500 species and subspecies of vascular plants according to Dupias 1985) characterized by numerous endemic plants and plant associations, and a rich mosaic of vegetation types (Villar & Dendaletche 1994; Sesé et al. 1999). The combination of these factors makes the Pyrenees one of the European hotspots of plant diversity (Gómez et al. 2003).

The main floristic elements and the spatial organization of the vegetation and of the flora diversity have been emphasized by different authors. Gaussen & Leredde (1949) and Gruber (1978) highlighted an eastern, a central and a western floristic cluster. Later, Dupias & Rey (1985) defined 12 phytogeographical zones corresponding to the main watersheds of the French Pyrenean side. However, their biogeographical limits are not always precise and clear-cut as overlapping floristic gradients add complexity to the interpretation (Gruber 1978). Overall, five main boundaries can be defined as transition zones for plants, as illustrated in Fig. 1 (Gaussen 1924a, b, c, d; Gruber 1978).

Defining evolutionarily significant units (ESUs) with molecular tools

Genetic tools can be helpful in defining plant genetic structure and rules for infraspecific biodiversity conservation in restoration practises, even though there is no real consensus as to how to include genetic diversity in territory management (Moritz & Faith 1998; McKay et al. 2005). Neutral markers have been used to identify ESUs as well as management units for the conservation of continuous plant populations (Moritz 1999; Diniz-Filho & Telles 2002). Recently, Krauss & Koch (2004) demonstrated the usefulness of neutral genetic variation in combination with spatial autocorrelation analysis to delineate seed transfer zones in Australia.

In order to restore natural ecosystem services and to establish self-sustaining native populations, we studied the neutral genetic pattern of four Pyrenean herbaceous plants. For each species, we compared our data with phytogeographical delineations along the Pyrenean range. In the light of those elements, we propose a relevant delineation of transfer zones for seeds used in revegetation in the French Pyrenees. Finally, we discuss the possibility of comparing the genetic pattern obtained for each species in order to create, if possible, a general seed transfer model. We also propose practical rules for

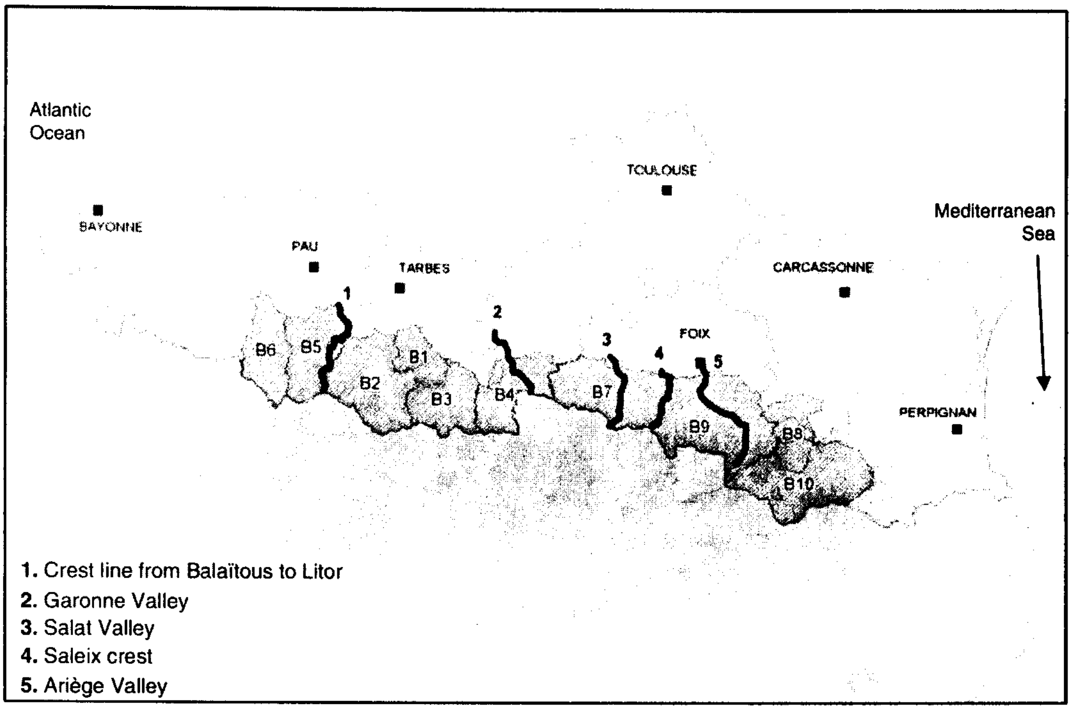

Fig. 1. Main phytogeographic boundaries (1-5) and location of the 10 watersheds (B1 to B10) defined for the sampling sites of plant populations in the Northern Pyrenees.

the conservation of plant infraspecific variability within seed production context.

Methods

Zone of interest

Straddling two countries and shading a principality (Andorra), the 100-km-wide chain of the Pyrenees extends over 400 km in a west-east direction from the Atlantic Ocean to the Mediterranean Sea (Gómez et al. 2003). Far less massive than the Alps, the Pyrenees do not present a real internal zone, but some of the deep valleys, isolated from the oceanic air masses by an orographic closure, can reach a degree of continentality (Gruber 1982; Izard 1988). In the eastern part of the chain, the Mediterranean influence is thwarted by intense periglacial processes (gelifluction, cryoturbation) that also affect plant distribution.

Species of concern

We focused our study on four common species widely distributed along the Pyrenees: *Trifolium alpinum* (Fabiaceae), *Festuca eskia* (Poaceae), *Festuca gautieri* (Poaceae), *Rumex scutatus* (Polygonaceae). These perennial species are considered key species for sub-alpine and alpine revegetation programs (Cassan et al. 2003; Malaval 2006); they present a strong colonization ability both by clonal division (stolons or tuft division) and seed dispersion. Their ecological and biological characteristics are presented in Table 1.

Sampling for genetic studies

The Pyrenean chain were divided into 10 watersheds (B1 to B10) in order to consider in the study the main topographic constraints of the mountainous region (i.e. river catchments); the edges of the two extreme regions are 242 km apart (Fig. 1). This outline also represents the most detailed delineation of phytogeographical areas as proposed by Dupias & Rey (1985). For each of the species, in each of the 10 watersheds, four populations were sampled representing four ecological contexts (variable ecological conditions and habitats of the species). In each population, five samples were collected in similar ecological conditions. A total of 200 individuals of each species were sampled (see the Supporting Information, Appendix S1), thus ensuring a continuous and dense coverage of the distribution range of the four species along the Northern Pyrenees.

Table 1. Biological and ecological characteristics of *Trifolium alpinum*, *Festuca eskia*, *Festuca gautieri* and *Rumex scutatus*. Sources: Nègre (1975), Kerguélen (1983) and Villar et al. (2001).

Species	Botanical family type	Gamy pollination dispersion	Chorology	Ecology infraspecific taxonomy
Trifolium alpinum	Fabaceae Perennial	Allogamous Entomogamous Epizoochorous	Southwest of Europe	Acidiphic grasslands and moving substrates from 1000 to 2850 m
Festuca eskia	Poaceae Perennial	Allogamous Anemogamous Epizoochorous	Pireneo-cantabric endemic	Acidiphic and oligotrophic subalpine grasslands, rocky zones and screes from 1700 to 2800 m Var. *orientalis* occurs in eastern Pyrenees cryoturbed soils and var. *eskia* in western Pyrenees biostatic environments
Festuca gautieri	Poaceae Perennial	Allogamous Anemogamous Epizoochorous	Endemic from the southwest European mountains	Xerophytic grasslands, cryoturbated substrates and mineral formations from 750 to 2950 m Calcareous soils for subsp. *scoparia* in central and western Pyrenees; acid soils for subsp. *gautieri* in eastern Pyrenees
Rumex scutatus	Polygonaceae Perennial	Allogamous Anemogamous Anemochorous	Orosiberian	Screes, mainly calcareous, moving rocky soils from 470 to 2700 m

Young green leaves were collected from individuals growing at least 5 m apart in order to avoid redundancy. In addition, five specimens from one population in the French Alps were included in the study of *T. alpinum* and *R. scutatus* (Table 1). Plant material was collected and stored in small bags with silica gel. Altitude (m) and geographic coordinates (WG 84) were recorded for each sampling site (see Appendix S1).

Genetic data and analysis tools

We used random amplified polymorphic DNA (RAPD) markers on a large number of individuals along the Pyrenees in order to identify the level and partitioning of genetic variation for each species, and the genetic distances among the populations. We considered that bands of equal fragment size were homologous. The intensity of the bands was not interpreted as a difference. Each RAPD marker was assumed to correspond to a locus with two alleles. Presence or absence of the band was recorded in a square matrix. unweighted pair-group method with arithmetic averages (UPGMA) cluster analyses applied on Nei's distances (Nei 1972) between populations were used to search for a biogeographic pattern using the programs POPULATIONS (Langella 2000) or TFPGA (Miller 1997), respectively. The variation in RAPD patterns was investigated with analyses of molecular variance (AMOVA) based on the pairwise squared Euclidean distances between all pairs of RAPD phenotypes using ARLEQUIN ver 2.000 (Schneider et al. 2000). The AMOVA allowed to evaluate among population and among watershed differentiation and to calculate variance components and their significance levels for variation among watersheds, among populations within watersheds and within populations. Further details on the method and analyses are presented in Lauga et al. (2009).

In order to test for a correlation between pairwise population genetic distances (Nei 1978) and geographical distances (in km), Mantel tests (Mantel 1967) were performed using the IBD program (Bohonak 2002). A Mantel test was also carried out with distance classes following the procedure of Oden & Sokal (1986) and Stehlik (2002). Numerous spatial autocorrelation analyses were performed on each data set in order to highlight a potential genetic structure for each species. For all species, a large number (13) of distance classes were chosen with a minimum of 34 (one exception 28 for one class of *R. scutatus*) pairwise comparisons: $1 \leq d < 16$, $16 \leq d < 25$, $25 \leq d < 35$, $35 \leq d < 47$, $47 \leq d < 60$, $60 \leq d < 75$, $75 \leq d < 95$, $95 \leq d < 120$, $120 \leq d < 140$, $140 \leq d < 156$, $156 \leq d < 175$, $175 \leq d < 205$, $205 \leq d < 250$ km. This division allowed us to obtain the most regular distribution of number of pairwise comparisons in each class of distance for all the species: an average of 45-60 pairwise comparisons according to each species in each distance class. The same distance classes were used for all the species, as we aimed to compare and generalize the results obtained for the different species.

The first x-axis intercept of the correlogram is widely considered to approximate the diameter of a "patch" size (Diniz-Filho & Telles 2002; Escudero et al. 2003), and this distance can be considered to represent a genetic unit for conservation or management (Diniz-Filho & Telles 2002). Another method uses the point at which the correlogram goes from significantly ($P \leq 0.05$) positive to non-significant as the diameter of a patch size (Krauss & Koch 2004). In the present study, we compared the two methods to approximate the diameter of such "patches" along an entire mountain range.

Results

Genetic diversity and relationship between populations

All four species displayed relatively high levels of genetic variation, with 20 polymorphic markers for *T. alpinum*, 14 for *F. eskia*, 48 for *F. gautieri* and 29 for *R. scutatus*. As shown by the AMOVA, most of the genetic variation (from 72% to 96%) was found within populations (Tables 2 and 3), rather than among populations or among watersheds.

As a result of our intensive sampling, we were able to detect important similarities in the pattern of the diversity of the four species studied, through UPGMA analysis of Nei unbiased genetic distances between all pairwise groupings of the populations (Table 3 and Fig. 2):

- an isolated eastern cluster spread over two to four watersheds,
- one or two western clusters,
- a geographic limit between eastern and western clusters situated in or around B7 watershed (between Garonne Valley and Ariège Valley).

However, significant differences also occurred between species, (Fig. 2):

- *T. alpinum* did not present a transversal cluster of populations, whereas the three other species did, and showed an opposition between western and eastern groups of populations. This suggested that the gene flow between eastern and western populations of the Northern Pyrenees was at least restricted or entirely lacking, whereas genetically homogenized populations through pollen or seed dispersal were found within each geographic region. The boundary between the two groups is between Garonne Valley and Salat Valley.
- The spatial structure of populations of *R. scutatus* was not so apparent and its populations were weakly differentiated ($F_{st} = 0.045$). Moreover, the lack of structure at this scale and sampling intensity was concordant with the absence of a

Table 2. Summary of main random amplified polymorphic DNA (RAPD) elements and genetic results for each of the four Pyrenean species studied.

Species	Number of samples	Number of markers scored	Genetic variation (AMOVA)			Genetic diversity (Shannon index)		Genetic differentiation (F_{st})
			Within population (%)	Among population within watershed (%)	Among watershed (%)	Population	Watershed	Global F_{st}
Trifolium alpinum	205	20	76.4	9.7	13.3	0.065–0.302	0.169–0.340	0.39
Festuca eskia	189	14	75.15	8.5	16.31	0.047–0.394	0.304–0.414	0.248
Festuca gautieri	101	48	99.32	0.069	0	0.053–0.389	0.2105–0.389	0.00676
Rumex scutatus	180	29	95.52	4.02	0.46	0.084–0.311	0.141–0.365	0.045

Table 3. Summary of main UPGMA elements (derived from random amplified polymorphic DNA data) and results for each of the four Pyrenean species studied.

Species	Number of populations	Number of clusters	Western clusters	Eastern clusters	Occurrence of a transversal cluster	Zone of transition
Trifolium alpinum	41	2	B1 to B6	B7 to B10	0	Between Garonne Valley and Salat Valley (B4-B7)
Festuca eskia	40	3	B1 to B7	B8, B9 and B10	1	Around Saleix crest (B7-B9)
Festuca gautieri	35	3	B1 to B7	B9 and B10	1	Between Salat Valley and Saleix crest (B7)
Rumex scutatus	36	4	B1 to B6	B7 to B10	1	Between Garonne Valley and Salat Valley (B4-B7)

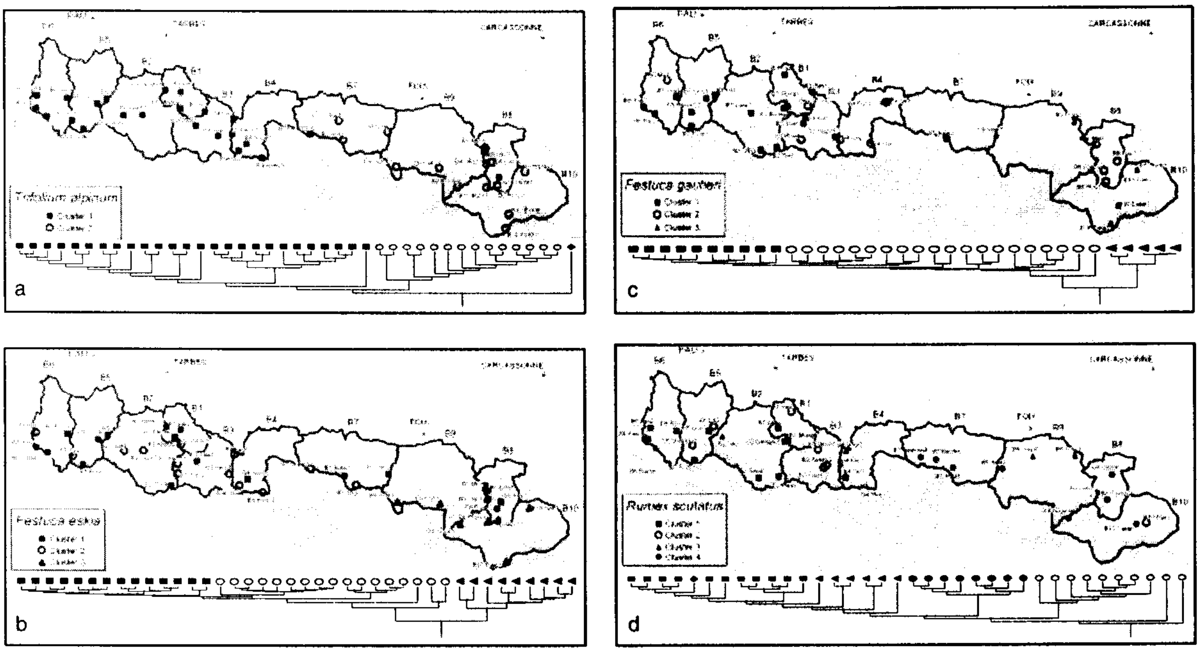

Fig. 2. Geographical locations and UPGMA clustering (derived from random amplified polymorphic DNA data) for populations of (a) *Trifolium alpinum*, (b) *Festuca eskia* (c) *Festuca gautieri* and (d) *Rumex scutatus*. Populations are denoted by the watershed (B1 to B10) followed by the name of the localities plus a number. For each species, populations belonging to a UPGMA clusters were reported on the map and visualized by a symbol (closed squares, populations belonging to the first cluster for UPGMA results; open circles, populations belonging to the second cluster; closed triangle, populations belonging to the third cluster; full circles, populations belonging to the fourth cluster; diamonds, populations from the Alps).

relationship between genetic and geographic distances (Mantel test performed upon all populations: $r = 0.1298$, $P = 0.064$).

- For the three other species, the Mantel test performed on all populations revealed an isolation by distance ($r = 0.3971$, for *T. alpinum*, $r = 0.4359$, for *F. eskia* and $r = 0.4603$, for *F. gautieri*; $P \leq 0.0010$ for all three species). This indicated that genetic distance increased with geographical distance among populations.

Correlation between genetic and geographic distances

Figure 3 shows the correlation between the genetic and geographic distances for the different populations. We show here the correlograms that best detected significant genetic structure within the sampling intensity used.

Mantel correlograms generated for *F. eskia*, *F. gautieri* and *T. alpinum* showed three important results:

- a positive correlation between geographic and genetic distances up to a distance of 75-113 km, identified both by the first x-axis intercept (respectively, 95, 85 and 113 km) and by the distance where the correlogram goes from significant to nonsignificant (respectively, 75, 75 and 95 km);
- a patch size for the correlation between genetic and geographic distances concordant with the length of the clusters observed in UPGMA analysis;
- globally similar patterns for the three species, with similar "patch" size diameters: 75-113 km.

Conversely, the correlograms confirmed the contrasting results between *R. scutatus* and the other species. For *R. scutatus* there was an absence of significantly positive values for distance classes 1-6 (Fig. 3). This was concordant with the lack of evidence for a genetic structure in the UPGMA analysis of *R. scutatus*. Indeed, the correlogram specified that there was no detectable genetic structure at this scale and sampling intensity.

Discussion

The UPGMA analysis and Mantel test correlograms were useful to determine geographic limits for homogeneous groups of populations for each species studied and the corresponding patch size diameter. Such contrasting results – the weakly structured genetic pattern of *R. scutatus* on one side and, on the other side, the obvious pattern of neutral

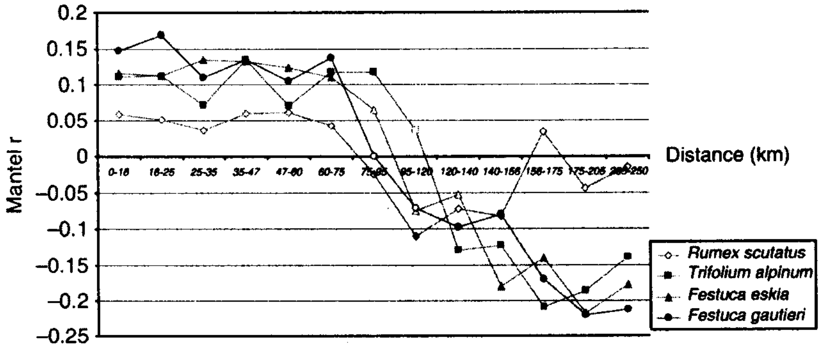

Fig. 3. Mantel's *r* correlograms showing the genetic correlation coefficient *r* as a function of distance, for *Trifolium alpinum*, *Rumex scutatus*, *Festuca eskia* and *Festuca gautieri* in the French Pyrenees. Closed shapes represent autocorrelation coefficients of the Mantel statistic that are significantly different from their expected value ($P \leq 0.05$).

genetic diversity for the three other species studied – might be partially explained by the biological structure and life history of the species. Multiple diversification patterns are indeed promoted by disparate biological characteristics (notably phytogenetic relationships, breeding systems, dispersal mechanisms and ecological requirements), together with geological complexity, quaternary climatic oscillations, pre-Holocene genetic structure, and other factors, including random processes (Vargas 2003). The west-east contrast in *F. eskia*, *F. gautieri* and *T. alpinum* may be explained by palaeoclimatic episodes of the Quaternary involving altitudinal species migration from two distinct peripheral glacial refugia (Vargas 2003). However, this hypothesis does not apply to *R. scutatus*. In addition, it cannot be excluded that the extant pattern of neutral genetic diversity might reflect an adaptive divergence owing to two relatively contrasting western and eastern ecosystems (Lauga et al. 2009). This could be particularly relevant for the *Festuca* species, for which infraspecific taxonomy is associated with ecological differences between eastern and western Pyrenees (see Table 1).

Ultimately, differences between species could also be related to differences in reproduction, pollination and dispersal. With a tendency to vegetative multiplication, *T. alpinum* might have limited its dispersal around mother plants. In contrast, the weakly structured genetic pattern of *R. scutatus* could reflect its high dispersal capacity (anemogamous and anemochorous species).

Implications of RAPD and correlogram results for establishing seed transfer zones in the French Pyrenees

Spatial genetic variation is highly scale-dependent and thus conclusions about spatial autocorrelation are related to the scale and the intensity of sampling (Krauss et al. 2005). We assume that the high sampling intensity used in this study yields a robust delineation of seed transfer zones.

In the four cases studied, the results of Mantel correlograms were clearly correlated with the shape of the neutral diversity pattern along the French side of the Pyrenees, supporting the discriminating power of genetic tools to distinguish the main zones of differentiation. For three of the four species studied (*Festuca* species and *T. alpinum*), genetic and spatial autocorrelation analyses were generally concordant, leading to a possible definition for two main seed transfer zones along the Pyrenees. In the case of *R. scutatus*, we can also take in account differences between eastern and western populations as a precautionary principle. Hence, a general frontier can be defined in B7 watershed between the Garonne Valley and Ariège Valley. The general shapes of the seed transfer zones were concordant with phytogeographical and biogeographical delineations (Gaussen 1924b, c; Gruber 1978) and the climatic transition zones along the Pyrenees. However, the phytogeographical limit between Atlantic and central floristic elements (phytogeographical delineation 1) was not demonstrated by our molecular analysis.

Interestingly, although this point was not highlighted in the original work, the studies of *Trollius europaeus* (Desprès et al. 2002) and *Anthyllis montana* (Kropf et al. 2002) exhibited, as in *T. alpinum* and *Festuca species*, an eastern/western biogeographic pattern along the Pyrenean massif.

In view of our results, the process for native seed production for the Pyrenees can be easily managed and controlled, considering the two main seed transfer zones. It will provide a reasonable compromise between managing costs and maintaining the structure of neutral genetic diversity.

Retaining the highest possible genetic variability in the seed mixtures

Defining ESUs based on genetic characteristics may be considered as the first step in outlining seed transfer zones. In the case of severe disturbances, such as topsoil removal, high levels of genetic variation may facilitate the adaptation of plants to novel or stressful environments, as well as long-term persistence (Lesica & Allendorf 1999; Burton & Burton 2002). Therefore, for each of the zones defined by the previous analyses, and in addition to the high level of neutral variation within populations, we also need to consider the ecological heterogeneity of the species habitats providing adaptive genetic resources.

Recent studies have shown that ecological provenance is more important than geographical provenance for successful plant establishment (Montalvo & Ellstrand 2000; Vitt & Havens 2004; Smith et al. 2005; Bischoff et al. 2006; Raabová et al. 2007), although the relationship between the geographic and ecological scales of local adaptation has been poorly investigated (McKay et al. 2005). Our goal will be to favour a sufficiently high level of adaptive genetic variation to ensure successful revegetation. However, it is equally important to avoid an excessive level of genetic variation in order to prevent negative consequences for population fitness. Thus, within each ESU, we propose to use a mixture of genotypes from ecologically distinct populations, all collected in mountainous zones (above 1000 m a.s.l.). In practice, when native species are produced by the seed industry, financial and technical restrictions can emerge, leading to a loss of genetic diversity in the seed pools (Smith et al. 2007). In order to limit this loss, it is necessary to compromise. Such a compromise aiming at conserving genetic identity and maximizing the genetic adaptation of seed pools has been previously reported as a founding principle for ecological restoration (Burton & Burton 2002; Jones 2003). For example, the primary Restoration Gene Pool (Jones 2003) includes only material from the target site plus adjacent areas that are genetically connected to the site via gene flow, that is, the metapopulation (Antonovics et al. 1994).

However, the question of pertinence and representativeness of the pools is still subject to debate. Do we have to collect seeds solely in the populations that we have sampled and consider strictly to which cluster they belong? How many sample sites are necessary in order to represent the diversity of the watershed, and how many are needed for the whole seed transfer zone? As the level of intrapopulation diversity was relatively high for all the species studied, we can assume that more than three collection zones in the populations can preserve most of this diversity.

In addition, a strong inadvertent directional selection could occur during cultivation (Knapp & Rice 1994; Burton & Burton 2002; Havens et al. 2004). To reduce the potential for accidental selection during seed production, it is important to harvest from the entire planted population, preferably as often as possible. It is also very important to produce the seed as close as possible to the site of restoration (McKay et al. 2005).

In conclusion, we propose simple rules in the context of Pyrenean seed multiplication:

- a minimum number of three populations should be collected for each species and in each seed transfer zone delineated by the neutral genetic pattern outlined above;
- each of the three populations should be collected in contrasting ecological conditions as defined, in particular, by elevation, exposure, natural habitat and bedrock;
- the collection of samples for each of the three or more populations should be evenly distributed throughout the zone;
- to avoid genetic drift during seed production, newly collected seeds from *in situ* populations should be regularly introduced into the seed pool.

Our results defined two seed transfer zones on the French side of the Pyrenees. Although they form a consistent entity, they do not take into account the Spanish side where the four species studied also occur. Since the southern side is subjected to a different climatic influence, further investigations may reveal another spatial organization of plant populations, and broaden our understanding of revegetation and flora conservation.

We also face the challenge of generalizing existing results and drawing guidelines for the use and production of seed pools for species as yet unanalysed in the context of restoration. Since our results show particular similarities, we could use the most restrictive case in order to extrapolate genetic information about new species introduced into the program. Our study has demonstrated the suitability of the RAPD technique to generate data on genetic structure among plant populations quickly and accurately, and further studies may refine our hypothesis for the delineation of seed transfer zones in the Pyrenees.

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References

van Andel, J. 1998. Intraspecific variability in the context of ecological restoration projects. *Perspectives in Plant Ecology, Evolution and Systematics* 1: 221–237.

Antonovics, J., Thrall, P., Jarosz, A. & Stratton, D. 1994. Ecological genetics of metapopulations: the *Silene-Ustilago* plant–pathogen system. In: Real, L.A. (ed.) *Ecological genetics*. pp. 146–170. Princeton University Press, Princeton, NJ, US.

Aradottir, A.L. & Johannsson, M.H. 2006. Ecological restoration with native species in Iceland. In: Krautzer, B. & Hacker, E. (eds.) *Proceedings of the conference soil-bioengineering: ecological restoration with native plants and seed material*. pp. 175–180. Raumberg-Gumpenstein, AT.

Bischoff, A., Crémieux, L., Šmilauerová, M., Lawson, C.S., Mortimer, S.R., Doležal, J., Lanta, V., Edwards, A.R., Brook, A.J., Macel, M., Lepš, J., Steinger, T. & Müller-Schärer, H. 2006. Detecting local adaptation in widespread grassland species – the importance of scale and local plant community. *Journal of Ecology* 94: 1130–1142.

Bohonak, A.J. 2002. IBD (isolation by distance): a program for analyses of isolation by distance. *Journal of Heredity* 93: 153–154.

Bradshaw, A.D. 1997. The importance of soil ecology in restoration science. In: Urbanska, K.M., Webb, N.R. & Edwards, P.J. (eds.) *Restoration ecology and sustainable development*. pp. 33–64. Cambridge University Press, Cambridge, UK.

Burton, P.J. & Burton, C.M. 2002. Promoting genetic diversity in the production of large quantities of native plant seed. *Ecological Restoration* 20: 117–123.

Cassan, S., Cambecèdes, J. & Largier, G. 2003. Revégétaliser autrement en montagne pyrénéenne. *Acta Botanica Barcinonensia* 49: 393–400.

Colomb, P., Becker, D. & Peeters, A. 2003. Le programme 'Ecotype': récolte et production de semences de plantes sauvages d'origine contrôlée pour la restauration de sites. *Les Naturalistes Belges* 84: 121–128.

Delarze, R. 1994. Dynamique de la végétation sur les pistes ensemencées de Crans-Montana (Valais, Suisse). Effets de l'altitude. *Botanica Helvetica* 104: 3–16.

Després, L., Loriot, S. & Gaudeul, M. 2002. Geographic pattern of genetic variation in the European globeflower *Trollius europaeus* L. (Ranunculaceae) inferred from amplified fragment length polymorphism markers. *Molecular Ecology* 11: 2337–2347.

Diniz-Filho, J.A.F. & Telles, M.P.D.C. 2002. Spatial autocorrelation analysis and the identification of operational units for conservation in continuous populations. *Conservation Biology* 16: 924–935.

Dupias, G. 1985. *Végétation des Pyrénées. Notice détaillée de la partie pyrénéenne de la carte de la végétation de la France au 1/200.000^e*. CNRS, Toulouse, FR.

Dupias, G. & Rey, P. 1985. In: Centre d'Ecologie des Ressources Renouvelables. (ed.) *Document pour un zonage des régions phyto-écologiques*. CNRS, Toulouse, 40 p.

Escudero, A., Iriondo, J.M. & Torres, M.E. 2003. Spatial analysis of genetic diversity as a tool for plant conservation. *Biological Conservation* 113: 351–365.

Fenster, C.B. & Dudash, M.R. 1994. Genetic considerations for plant population restoration and conservation. In: Bowles, M.L. & Whelan, C.J. (eds.) *Restoration of endangered species: conceptual issues, planning and implementation*. pp. 34–62. Cambridge University Press, Cambridge, UK.

Gaussen, H. 1924a. Note sur l'importance de la vallée de l'Ariège comme limite. *Bulletin de la Société Botanique de France* 71: 26–30.

Gaussen, H. 1924b. Note sur l'importance du chaînon du port de Saleix comme limite. *Bulletin de la Société Botanique de France* 71: 34–39.

Gaussen, H. 1924c. Note sur l'importance de la région du Salat comme limite. *Bulletin de la Société Botanique de France* 71: 42–44.

Gaussen, H. 1924d. Note sur l'importance de la vallée de la Garonne comme limite. *Bulletin de la Société Botanique de France* 71: 47–63.

Gaussen, H. & Leredde, C. 1949. Les endémiques pyrénéo-cantabriques dans la région centrale des Pyrénées. *Bulletin de la Société Botanique de France* 96: 57–83.

Gómez, D., Sesé, J.A. & Villar, L. 2003. The vegetation of the Alpine Zone in the Pyrenees. In: Nagy, L., Grabherr, G., Körner, Ch. & Thompson, D.B.A. (eds.) *Alpine biodiversity in Europe*. pp. 85–92. [Ecological Studies no.167]. Springer-Verlag, Berlin, DE.

Gruber, M. 1978. *La végétation des Pyrénées ariégeoises et catalanes occidentales. Thèse de l'Université d'Aix-Marseille III soutenue le 28 avril 1978.* Faculté des sciences et techniques de Saint-Jérôme, Marseille, FR.

Gruber, M. 1980. Etages et séries de végétation de la chaîne pyrénéenne. *Ecologia Mediterranea* 5: 147–174.

Gruber, M. 1982. Degré de continentalité de quelques vallées pyrénéennes; relations avec la végétation. *Ecologia mediterranea* 8: 57–68.

Gustafson, D.J., Gibson, D.J. & Nickrent, D.L. 2005. Using local seeds in prairie restoration-data support the paradigm. *Native Plants Journal* 6: 25–28.

Haselwandter, K. 1997. Soil micro-organisms, mycorrhiza, and restoration ecology. In: Urbanska, K.M., Webb, N.R. & Edwards, P.J. (eds.) *Restoration ecology and sustainable development*. pp. 65–80. Cambridge University Press, Cambridge, UK.

Havens, K., Guerrant, E.O., Maunder, M. & Vitt, P. 2004. Guidelines for *ex situ* conservation collection management: minimizing risks. Appendix 3. In: Guerrant, E.O., Havens, K. & Maunder, M. (eds.) *Ex situ plant conservation – supporting species survival in the wild*. pp. 454–473. Society for Ecological Restoration International. Center for Plant Conservation, Island Press, Washington, DC, US.

Hufford, M.K. & Mazer, S.J. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology and Evolution* 18: 147–155.

Izard, M. 1988. Sur la continentalité dans les Pyrénées et son impact sur la végétation. In: Instituto de Estudios Altoaragoneses. (ed.) *Homenaje a Pedro Montserrat*. pp. 597–602. [Monografias del instituto Pirenaico de Ecologia no. 4]. Instituto Pirenaico de Ecología, Zaragoza, ES.

Jones, T.A. 2003. The restoration gene pool concept: beyond the native versus non-native debate. *Restoration Ecology* 11: 281–290.

Keller, M., Kollmann, J. & Edwards, P.J. 2000. Genetic introgression from distant provenances reduces fitness in local weed populations. *Journal of Applied Ecology* 37: 647–659.

Kerguélen, M. 1999, modifié Bock, B. 2005. *Index synonymique de la flore de France, Tela Botanica - BDNFF - Version n°4.02 du 27 avril 2005*. Available at http://www.telabotanica.org/page:liste_projets?id_projet = 1&act = documents&id_repertoire = 87.

Kerguélen, M. 1983. Les Graminées de France au travers de '*Flora Europaea*' et de la 'Flore du CNRS'. *Lejeunia* 110: 1–79.

Knapp, E.E. & Rice, K.J. 1994. Starting from seed: genetic issues in using native grasses for restoration. *Restoration & Management Notes* 12: 40–45.

Krauss, S.L. & Koch, J.M. 2004. Rapid genetic delineation of provenance for plant community restoration. *Journal of Applied Ecology* 41: 1162–1173.

Krauss, S.L., Koch, J.M. & Vlahos, S. 2005. A novel approach for the rapid genetic delineation of provenance for minesite revegetation. *Ecological Management & Restoration* 6: 153–155.

Krautzer, B. & Bohner, A. 2002. Restoration of high altitudes grassland with indigenous seed mixtures. In: Durand, J.L., Emile, J.C., Huyghe, C. & Lemaire, G. (eds.) *Multi-function grasslands, quality forages, animal products and landscapes, Proceedings of the 14th symposium of the European Grassland Federation, May 27–30, La Rochelle, France*. pp. 380–381. Raumberg Gumpenstein, AT.

Krautzer, B. & Graiss, W. 2006. Restoration with site-specific seed mixtures. From theory to practical realisation. In: Krautzer, B. & Hacker, E. (eds.) *Proceedings of the conference soil-bioengineering: ecological restoration with native plants and seed material*. pp. 107–112. Raumberg-Gumpenstein, AT.

Krautzer, B. & Wittmann, H. 2005. Restoration of alpine ecosystems. In: van Andel, J. & Aronson, J. (eds.) *Restoration ecology: the new frontier, chapter 15*. pp. 208–220. Blackwell, Oxford, UK.

Krautzer, B., Peratoner, G. & Bozzo, F. 2004. *Site-specific grasses and herbs, seed production and use for restoration of mountain environments. [Plant Production and Protection Series, no. 32]*. Food and Agriculture Organization of the United Nations, Rome, IT.

Kropf, M., Kadereit, J.W. & Comes, H.P. 2002. Late Quaternary distributional stasis in the submediterranean mountain plant *Anthyllis montana* L. (Fabaceae) inferred from ITS sequences and amplified fragment length polymorphism markers. *Molecular Ecology* 11: 447–463.

Langella, O. 2000. *Population ver 1.2.28*. CNRS, Paris, FR.

Lauga, B., Malaval, S., Largier, G. & Regnault-Roger, C. 2009. Two lineages of *Trifolium alpinum* (Fabaceae) in the Pyrenees: evidence from random amplified polymorphic DNA (RAPD) markers. *Acta Botanica Gallica* 156: 317–330.

Lesica, P. & Allendorf, F.W. 1999. Ecological genetics and the restoration of plant communities: mix or match? *Restoration Ecology* 7: 42–50.

Lumaret, R. 1999. Exemples de problèmes génétiques liés à l'introduction d'espèces non locales. In: Association Française des Ingénieurs écologues. (ed.) *L'approvisionnement en espèces végétales locales dans les aménagements: Quel(s) enjeu(x) pour la diversité végétale?* pp. 53–56. Journée technique de l'Association Française des Ingénieurs Ecologues, Versailles, FR.

Malaval, S. 2006. Revegetation with native species in the French Pyrenees Mountains. In: Krautzer, B. & Hacker, E. (eds.) *Proceedings of the conference soil-bioengineering: ecological restoration with native plants and seed material*. pp. 197–200. Raumberg-Gumpenstein, AT.

Mantel, N. 1967. The detection of disease clustering and generalized regression approach. *Cancer Research* 27: 209–220.

McKay, J.K., Christian, C.E., Harrison, S. & Rice, K.J. 2005. 'How Local Is Local?' – a review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology* 13: 432–440.

Miller, M.P. 1997. *Tools for population genetic analysis (TFPGA)*. Department of Biological Sciences, Northern Arizona University, AZ, US.

Montalvo, A.M. & Ellstrand, N.C. 2000. Transplantation of the subshrub *Lotus scoparius*: testing the home-site advantage hypothesis. *Conservation Biology* 14: 1034–1045.

Moritz, C. 1999. Conservation units and translocations: strategies for Conserving Evolutionary Processes. *Hereditas* 130: 217–228.

Moritz, C. & Faith, D.P. 1998. Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology* 7: 419–429.

Muller, S., Dutoit, T., Alard, D. & Grévilliot, F. 1998. Restoration and rehabilitation of species-rich grassland ecosystems in France: a review. *Restoration Ecology* 6: 94–101.

Nègre, R. 1975. Observations morphologiques sur les gentianes du groupe *alpina-acaulis*, sur *Festuca paniculata* et *F. eskia* en Pyrénées. *Candollea* 30: 301–321.

Nei, M. 1972. Genetic distance between populations. *American Naturalist* 106: 283–292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.

Oden, N.L. & Sokal, R.R. 1986. Directional autocorrelation: an extension of spatial correlograms to two dimensions. *Systematic Zoology* 35: 608–617.

Ozenda, P. 2002. *Perspectives pour une géobiologie des montagnes. Collection Biologie*. Presses Polytechniques et Universitaires Romandes, Lausanne, CH.

Peratoner, G. 2006. Extreme sites and their restoration requirements in Arctic-Alpine climatic regions. In: Krautzer, B. & Hacker, E. (eds.) *Proceedings of the conference soil-bioengineering: ecological restoration with native plants and seed material*. pp. 37–42. Raumberg-Gumpenstein, AT.

Porqueddu, C. & Maltoni, S. 2006. Esperienze e prospettive di recupero ambientale conspecie erbacee in Italia. In: Krautzer, B. & Hacker, E. (eds.) *Proceedings of the conference soil-bioengineering: ecological restoration with native plants and seed material*. pp. 219–226. Raumberg-Gumpenstein, AT.

Raabová, J., Münzbergová, Z. & Fischer, M. 2007. Ecological rather than geographic or genetic distance affects local adaptation of the rare perennial herb. *Aster amellus*. *Biological Conservation* 139: 348–357.

Rhymer, J.M. & Simberloff, D. 1996. Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics* 27: 83–109.

Sackville Hamilton, N.R. 2001. Is local provenance important in habitat creation? A reply. *Journal of Applied Ecology* 38: 1374–1376.

Schneider, S., Kuffer, J., Rossli, D. & Excoffier, L. 2000. *ARLEQUIN*. Genetics and Biometry Laboratory, University of Geneva, Geneva, CH.

Sesé, J.A., Ferrández, J.V. & Villar, L. 1999. La flora alpina de los Pirineos. Un patrimonio singular. In: Villar, L. (ed.) *Espacios Naturales Protegidos del Pirineo. Ecología y cartografía*. pp. 57–76. Consejo de Protección de la Naturaleza de Aragón, Zaragoza, ES.

Ševcíková, M., Šrámek, P., Jongepierová, I. & Zimková, M. 2006. Working with site specific seeds and vegetation in Czech Republic and Slovakia, experiences and prospects. In: Krautzer, B. & Hacker, E. (eds.) *Proceedings of the conference Soil-bioengineering: Ecological Restoration with native plants and seed material*. pp. 201–206. Raumberg-Gumpenstein, AT.

Simberloff, D. 2003. Confronting introduced species: a form of xenophobia? *Biological Invasions* 5: 179–192.

Smith, B.M., Diaz, A., Winder, L. & Daniels, R. 2005. The effect of provenance on the establishment and performance of *Lotus corniculatus* L. in a re-creation environment. *Biological Conservation* 125: 37–46.

Smith, S.L., Sher, A.A. & Grant, T.A. III. 2007. Genetic diversity in restoration materials and the impacts of seed collection in Colorado's restoration plant production industry. *Restoration Ecology* 15: 369–374.

Stehlik, I. 2002. Glacial history of the alpine herb *Rumex nivalis* (Polygonaceae): a comparison of common phylogeographic methods with nested clade analysis. *American Journal of Botany* 89: 2007–2016.

Urbanska, K.M. 1997. Restoration ecology research above timberline: colonization of safety islands on a machine-graded alpine ski run. *Biodiversity and Conservation* 6: 1655–1670.

Urbanska, K.M. & Hasler, A.R. 1992. Ecologically compatible revegetation above the timberline: a model and its application in the field. In: Hassell, W.G., Nordstrom, S.K., Keammerer, W.R. & Todd, J. (eds.) *Proceedings: high altitude revegetation workshop no. 10*. pp. 247–253 [Colorado Water Resources Research Institute Information Series 71]. Colorado Water Resources Research Institute, Fort Collins, CO, US.

Vander Mijnsbrugge, K., Cox, K. & van Slycken, J. 2005. Conservation approaches for autochthonous woody plants in Flanders. *Silvae genetica* 54: 4–5.

Vargas, P. 2003. Molecular evidence for multiple diversification patterns of alpine plants in Mediterranean Europe. *Taxon* 52: 463–476.

Villar, L. & Dendaletche, C. 1994. Pyrenees. France, Spain and Andorra. In: Heywood, V.H., David, S.D. & Hamilton, A.C. (eds.) *Centers of plant diversity. A guide and strategy for their conservation*. pp. 61–64. WWF and IUCN, Cambridge, UK.

Villar, L., Sesé, J.A. & Ferrández, J.V. 2001. *Atlas de la Flora del Pirineo Aragonés Vol. II*. Instituto de Estudios Altoaragoneses, Consejo de Protección de la Naturaleza de Aragón, Huesca, ES.

Vitt, P. & Havens, K. 2004. Integrating quantitative genetics into *ex situ* conservation and restoration practices. In: Guerrant, E.O., Havens, K. & Maunder, M. (eds.) *Ex situ plant conservation - supporting species survival in the wild, chapter 14*. pp. 286–304. Society for Ecological Restoration International, Center for Plant Conservation, Island Press, Washington DC, US.

Wilkinson, D.M. 2001. Is local provenance important in habitat creation? *Journal of Applied Ecology* 38: 1371–1373.

Supporting Information

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

Appendix S1. Population location, geographical coordinates (WG 84) and elevation (m), of *Trifolium alpinum*, *Festuca eskia*, *Festuca gautieri* and *Rumex scutatus*, within the 10 French Pyrenean regions defined for the study.

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