Genetic introgression as a potential to widen a species' niche: Insights from alpine *Carex curvula*

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Understanding what causes the decreasing abundance of species at the margins of their distributions along environmental gradients has drawn considerable interest, especially because of the recent need to predict shifts in species distribution patterns in response to climatic changes. Here, we address the ecological range limit problem by focusing on the sedge, *Carex curvula*, a dominant plant of high-elevation grasslands in Europe, for which two ecologically differentiated but crosscompatible taxa have been described in the Alps. Our study heuristically combines an extensive phytoecological survey of alpine plant communities to set the niche attributes of each taxon and a population genetic study to assess the multilocus genotypes of 177 individuals sampled in typical and marginal habitats. We found that ecological variation strongly correlates with genetic differentiation. Our data strongly suggest that ecologically marginal populations of each taxon are mainly composed of individuals with genotypes resulting from introgressive hybridization. Conversely, no hybrids were found in typical habitats, even though the two taxa were close enough to crossbreed. Thus, our results indicate that genotype integrity is maintained in optimal habitats, whereas introgressed individuals are favored in marginal habitats. We conclude that gene flow between closely related taxa might be an important, although underestimated, mechanism shaping species distribution along gradients.

alpine flora | evolutionary ecology | introgressive hybridization | local adaptation | niche theory

The search for general mechanisms driving species performance along ecogeographical gradients has received considerable attention from both ecologists and evolutionary biologists (1). Understanding what shapes a species' niche is considered of primary interest in part because of the potential to predict dynamic changes in natural communities from the knowledge of species' ecological requirements (2). Current distribution patterns of species are influenced by both adaptation to environmental conditions and historical events (3). Along an ecological gradient, populations occurring at the tail of a species' distribution curve have been called marginal populations (4), and many studies have linked the lower fitness of these marginal populations with resource depletion, physical stress (5), or competitive exclusion (6).

Another perspective of the ecological range limit problem is to look at mechanisms responsible for the maintenance of populations far from their ecological optima. Clinal variation and its presumable adaptive value were among the first proposed mechanisms to account for broadened ecological amplitude (7, 8). Methods for assessing molecular diversity at the genome level offer new opportunities to unravel genotype–phenotype relationships along a niche width (9). Unfortunately, few studies have considered niche separation along environmental gradients and population genetic structure.

Alpine floras offer excellent opportunities to address the evolutionary processes of local adaptation and ecological speciation because of the steepness of the environmental gradients leading to a high turnover in species composition with relatively small changes in the landscape (10). Correlations among plant distributions and environmental variables have been studied on many mountain ranges and have provided numerous examples of ecologically differentiated taxa (11).

Here, we focus on the high-elevation sedge *Carex curvula*, a dominant sedge of most alpine grasslands in the European Alps. This species is particularly well suited for ecological genetic study, because morphological and ecological variation has been addressed in several regional studies (see, for example, refs. 12 and 13), leading to the recognition of two edaphically differentiated taxa, currently treated as subspecies in most floras (14): *C. curvula* subsp. *curvula* (hereafter *Cc*) and *C. curvula* subsp. *rosae* (hereafter *Cr*).

Our main objectives were to test whether (*i*) morphological and ecological variation is correlated with genetic differentiation, and (*ii*) ecologically marginal populations of each taxon exhibit particular multilocus genotypes when compared to populations of optimal habitats. Niche optima and niche width were derived from a direct gradient analysis of species–environment relationships. Genetic relatedness between individuals was inferred from amplified fragment length polymorphism (AFLP) markers. These markers have proven highly informative in studies of plant population genetic structure and history (15, 16).

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Methods

Study Species. *C. curvula* is a clonal, long-lived species (17) forming dense tussocks in alpine grasslands (Fig. 1*A* and *B*) (18). The species is protogynous, wind-pollinated, and primarily outcrossing. The heavy seeds have limited dispersal capacity. Seventy years ago, two taxa were described (19), mainly based on transverse leaf-section variations (Fig. 1*A* and *B*). More recently, several regional studies have pointed to different ecological characteristics in the two taxa (12, 13, 20). *Cc* forms extensive swards in the central and eastern part of the European Alps (Fig. 1*A*) and has been considered as a keystone species of acidic alpine grasslands (21). *Cr* is mainly found in the southwestern Alps (Fig. 1*B*) and occurs generally on base-rich substrates. *Cc* and *Cr* populations found within a radius of a few kilometers, i.e., close enough to interbreed, were considered as sympatric even if the spatial distribution of the two taxa does not overlap at a finer grain. Morphologically intermediate individuals have been observed in a few localities, suggesting crossability between *Cc* and *Cr* (22). Furthermore, these intermediates are fertile (22). Experimental crosses between *Cc* and *Cr* have never been attempted.

Gradient Analysis and Ordination-Defined Niche Attributes. Multivariate techniques are particularly well designed to investigate niche separation between species along environmental gradients

Abbreviations: *Cc*, *C. curvula* subsp. *curvula*; *Cr*, *C. curvula* subsp. *rosae*; AFLP, amplified fragment length polymorphism; MCA, multivariate component analysis; *Ccm*, marginal *Cc* populations; *Crm*, marginal *Cr* populations.

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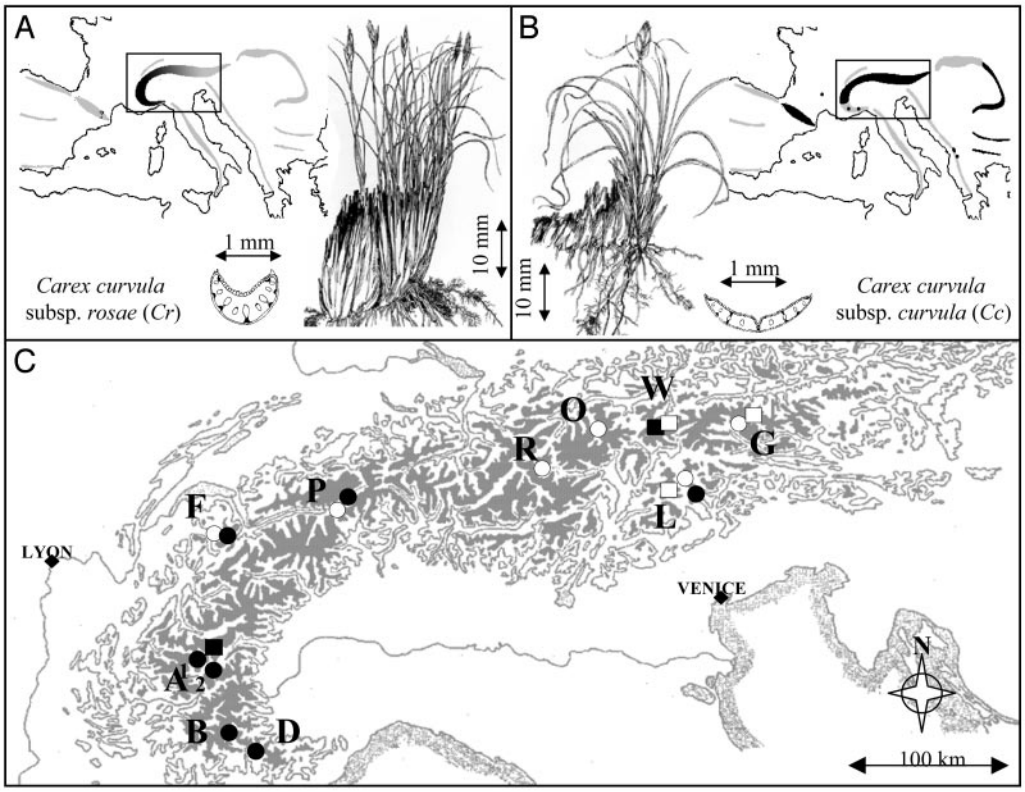

Fig. 1. (*A* and *B*) Overall distribution range in Europe, growth habit, and transverse leaf sections of *Cr* and *Cc*. Drawings are adapted from ref. 18. Distribution of *Cc* or *Cr* is shown in black. (*C*) Location of the populations studied with AFLP in the Alps. Shaded areas, >1,000 m. *Crm* and *Ccm* indicate marginal populations of *Cr* and *Cc*, respectively. ○, *Cc*; ●, *Cr*; □, *Ccm*; ■, *Crm*.

(23). The main objective of our large-scale gradient analysis was to define for each taxon an *n*-dimensional hypervolume corresponding to its realized niche as outlined by Hutchinson (24) and to discriminate between optimal and marginal habitats. Our data set comprised 1,300 vegetation relevés of alpine grasslands in the Alps. The locations of the relevés were chosen so that different mountain ranges, altitudes, topographic positions, and substrates were sampled in a roughly stratified approach. We relied on several regional studies as well as unpublished data (see *Supporting Bibliography*, which is published as supporting information on the PNAS web site, for a detailed list of references). Data for each relevé include a species list and six local-scale environmental variables: elevation (meters above sea level); relative south aspect (sine of aspect, from +1-north to −1-south, with flat coded 0); slope inclination (in degrees); acidity of bedrock (dominant bedrocks include hard calcareous rocks, shales, metamorphic rocks, and granites); mesotopographical landform (an index ranging from convexity to concavity at the decameter scale); and physical disturbance estimated as the proportion of bare ground. Details of the methodology are given elsewhere (20). The floristic and environmental data were analyzed by using principal correspondence analysis. A coinertia analysis of the two tables was then carried out according to the methods described in ref. 25. Relevés for which no environmental data were available (280 of 1,300) were projected as supplementary cases in the coinertia ordination space. Rare species (frequency <5%) were removed before analyses. All multivariate analyses were carried out by using the ADE-4 software package (26).

Population Sampling for Genetic Analyses. Eighteen populations of *C. curvula* were sampled over the Alps (Fig. 1*C* and Table 1). An allopatric population of *Cc* from the Pyrenees was also included. In a few localities, we deliberately sampled populations for which we had strong presumptions of habitat marginality because of the floristic assemblage and the geomorphological features of the sites. The marginality of these populations was confirmed later in the multivariate analysis. Based on transverse leaf sections and habit, all sampled individuals, including those from marginal habitats, were unambiguously ascribed to one or the other taxon. In July 1999, young green leaves of 8–10 individuals, separated by 5–10 m, were collected for each population. Tissues were stored in silica gel desiccation beads until DNA extraction. In each sampling site, floristic assemblage and local-scale environmental variables were documented and included in the niche analysis.

AFLP Protocol and Multivariate Genotype Analysis. DNA extraction was performed with the DNeasy Plant Mini Kit (Qiagen, Chatsworth, CA), according to the manufacturer's protocol, by using 20 mg of dried leaf material. DNA concentration was determined by fluorimetry with the PicoGreen dsDNA quantification kit (Molecular Probes). AFLP analysis was carried out on a total of 177 individuals following the protocol described by Vos *et al.* (27) and modified by Gaudeul *et al.* (28). Selective PCR amplification was performed by using three different primer combinations. Electrophoresis was run for 6 h on an automated sequencer ABI 377 (Perkin–Elmer). Size labels were included in each sample. AFLP patterns were visualized with GENESCAN ANALYSIS 3.1 (Perkin–Elmer). We scored 261 unambiguous polymorphic peaks with a size ranging from 200 to 500 bp. The presence/absence data from the 177 AFLP phenotypes were ordinated by using multivariate component analysis (MCA) (29).

Table 1. Location of sampled populations for genetic studies

Population	Locality, moutain range, country	*N*	Longitude (east)	Latitude (north)	Elevation, m
Cc F	Flaine, Savoie Alps, France	9	6°26′	46°01′	2,450
Cc G	Grossglockner, Hohe Tauern, Austria	10	12°51′	47°04′	2,500
Cc L	Pala Santa, Dolomites, Italy	9	11°31′	46°21′	2,300
Cc O	Obergurgl, Ötztal Alps, Austria	8	11°01′	46°31′	2,400
Cc P	Furkapass, Lepontic Alps, Switzerland	8	8°35′	46°34′	2,420
Cc R	Berninapass, Rhetic Alps, Switzerland	10	9°54′	46°23′	2,300
Cc Y	Pic du Canigou, Pyrenees, France	10	2°27′	42°31′	2,550
Ccm G	Edelweissspitze, Hohe Tauern, Austria	10	12°51′	47°08′	2,560
Ccm L	Valsorda, Dolomites, Italy	9	11°35′	46°21′	2,500
Ccm W	Hühnerspiel, Zillertal Alps, Italy	9	11°19′	46°58′	2,650
Cr A1	Col du Galibier, Dauphiné Alps, France	10	6°18′	45°05′	2,750
Cr A2	Col du Galibier, Dauphiné Alps, France	10	6°18′	45°05′	2,750
Cr B	Col de la Cayolle, Maritime Alps, France	9	6°45′	44°16′	2,550
Cr D	Col de la Bonette, Maritime Alps, France	8	6°51′	44°19′	2,800
Cr F	Flaine, Savoie Alps, France	9	6°26′	46°01′	2,450
Cr L	Latemar, Dolomites, Italy	10	11°32′	46°22′	2,450
Cr P	Nufenenpass, Lepontic Alps, Switzerland	10	8°34′	46°25′	2,450
Crm A	Valloire, Dauphiné Alps, France	10	6°15′	45°14′	2,680
Crm W	Weissspitze, Zillertal Alps, Italy	9	11°20′	46°58′	2,600

N, number of sampled individuals. *Ccm* and *Crm*, ecologically marginal populations found in intermediate habitats between *Cc* and *Cr* optimal habitats (see Fig. 2).

Based on the frequencies of AFLP bands, we calculated between population genetic distances according to the formula given by Nei (30). We also calculated allele frequencies and *F* statistics under the random-mating hypothesis ($F_{is} = 0$), using the ARLEQUIN software package (31). A Mantel test was used to estimate the correlation between the matrices of geographic and genetic distances [1,000 permutations; ADE-4 software package (26)].

Results

The results of the coinertia analysis indicate that our set of environmental variables explained 41% and 33% of the floristic table variance along axes 1 and 2, respectively. The costructure between the two tables was highly significant, as indicated by a Monte–Carlo permutation test (1,000 seeds, $P < 0.0001$). The first axis of variation is indicative of a temperature gradient driven by elevation and aspect and, to a lesser extent, of a substrate acidity gradient. The second axis of variation corresponds primarily to mesoscale geomorphological gradient varying from concave little-disturbed to convex highly disturbed sites (Fig. 2*D*). The results revealed clear niche separation between *Cc* and *Cr*, which was especially marked along the second coinertia axis (Fig. 2 *A* and *B*). Furthermore, *Cc* preferentially occurs on siliceous rocks, whereas *Cr* covers a wider range along the bedrock acidity gradient. The distance between the mean habitat of one taxon and the position of a sampling population was taken as an indicator of population marginality. Marginal *Cc* populations (*Ccm*) for AFLP analyses occurred on base-rich substrates with a higher level of disturbance (Fig. 2*C*, G, L, W), whereas marginal *Cr* populations (*Crm*) occurred in concave, acidic, and low-disturbed places (Fig. 2*C*, A, W).

The analysis of AFLP phenotypes revealed strong dissimilarity between *Cc* and *Cr* (Fig. 3). The first MCA axis explaining 15.7% of the total variance clearly differentiates *Cc* and *Cr* individuals (Fig. 3*A*), suggesting that genetic differentiation is primarily related to habitat preference and not to geographical location. The second MCA axis accounts for 4.5% of the total variance and splits the *Cc* populations between the Pyrenees and the Alps. *Cc* or *Cr* populations found in optimal habitats form two core groups, within each of which there are no significant differences along the first MCA axis (Fig. 3*A*). This strong genetic relatedness among populations of each core group is confirmed by low F_{st} values ($F_{st} = 0.13$ for *Cc*, $= 0.09$ for *Cr*). Conversely, genetic differentiation between *Cc* and *Cr* populations is much higher with a mean F_{st} value of 0.35.

Along the first MCA axis, the positions of four marginal populations (*Ccm* L, *Ccm* W, *Crm* A, and *Crm* W) differ significantly from the scores of the corresponding core group and are shifted toward the other core group (Fig. 3*B*). AFLP markers occurring in >80% of individuals from one core group and in <5% of individuals from the other core group were considered as diagnostic markers. On the one hand, we found that AFLP phenotypes of marginal individuals of one taxon contain diagnostic markers from the other one (Table 2); on the other hand, we did not find any specific markers for *Ccm* or *Crm* populations.

Fig. 4 shows the relationships between Nei's genetic distance (30) and geography. Within each core group, the matrix of between-population pairwise genetic distances was significantly correlated with the corresponding matrix of geographical distances (Mantel test: $r = 0.79$, $P = 0.02$ for *Cc*; $r = 0.58$, $P = 0.03$ for *Cr*). The same significant correlation was found with genetic distances based on F_{st}. Conversely, when genetic distances were calculated between *Cc* and *Cr* core-group populations, we found no relationship with geographical distances (Mantel test: $r = 0.08$, $P = 0.59$), indicating that the genome integrity of each taxon is maintained even if *Cc* and *Cr* populations are very close (<1 km as in locality F).

Discussion

A Single Origin for *Cc* and *Cr*. In the Alps, morphological and ecological variation in *C. curvula* populations strongly correlates with genetic differentiation. Genetic relatedness between *Cc* and *Cr* is independent of geographical distance. For example, *Cc* populations from the Alps are more closely related to the allopatric Pyrenean population than to sympatric *Cr* populations. The same is reflected by the F_{st} values that are low within each taxon but high among them. Thus, our results are strongly indicative of a single event of divergence between *Cc* and *Cr* in the Alps.

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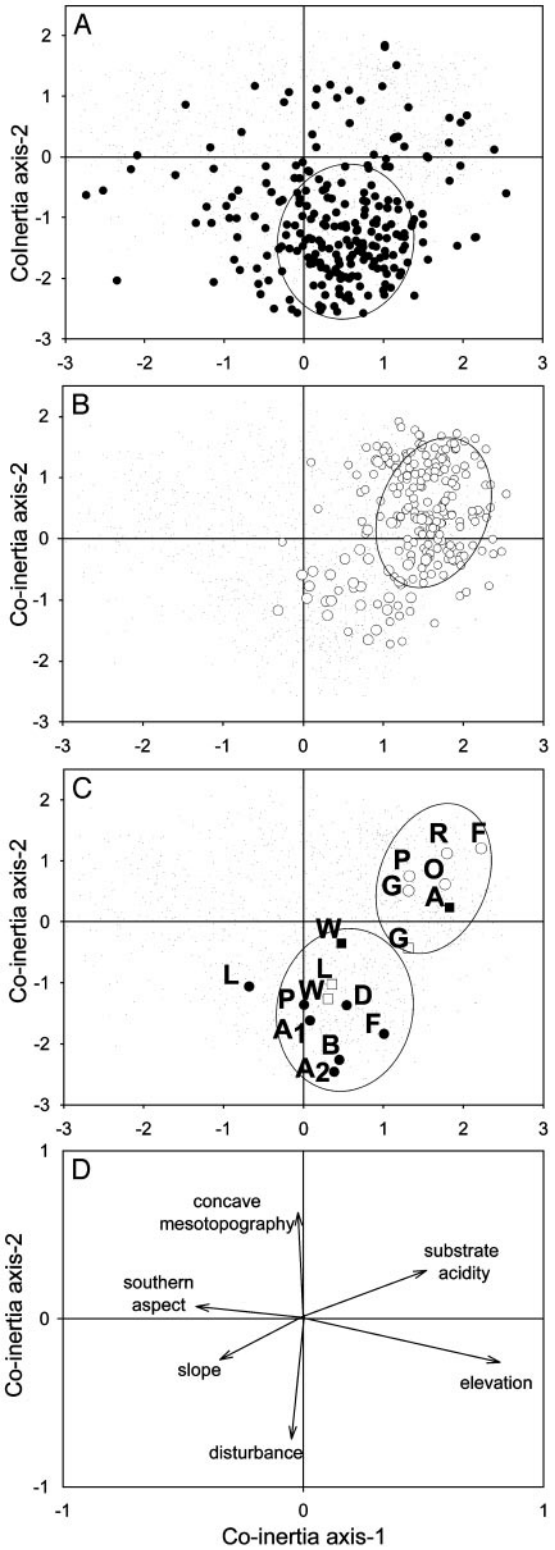

Fig. 2. Coinertia analysis of species–environment relationships in alpine grasslands of the Alps. (*A–C*) Display of all 1,300 vegetation relevés along the first two axes of variation with superimposition of relevés containing *Cr* (*A*) and *Cc* (*B*) or relevés corresponding to the sampling sites of AFLP investigated populations (*C*). Ellipses were drawn to include 80% of *Cc* or *Cr* relevés. The ellipse is centered on the mean of the ordination scores; its width and height are given by the variances, and its slope is given by the covariance of ordination scores. (*D*) Display of the environmental variables along the first two axes of variation. Original sources for these data are available in *Supporting Bibliography*. Symbols are as in Fig. 1.

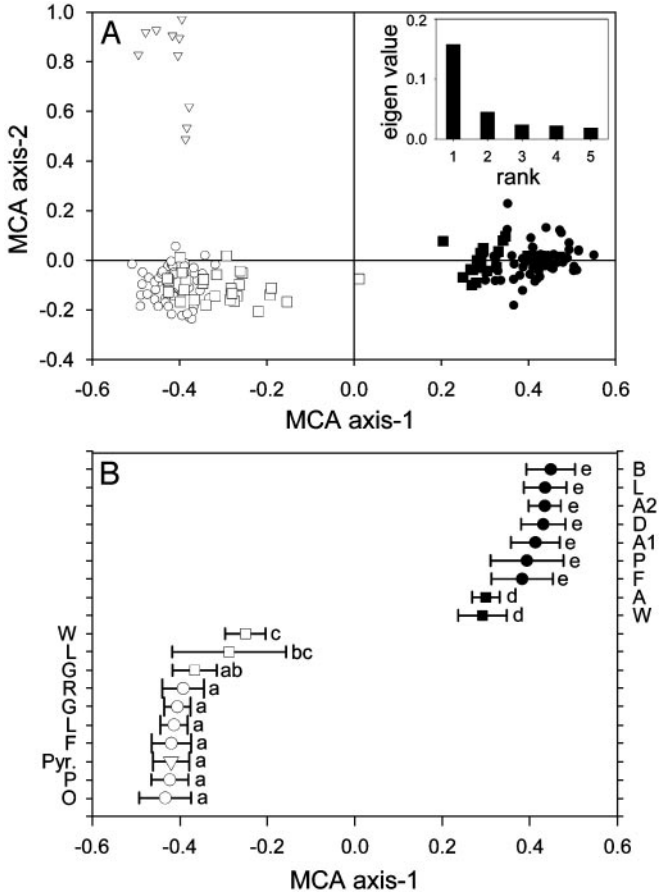

Fig. 3. MCA of the AFLP phenotypes. (*A*) Display of the 177 individuals AFLP phenotypes along the first two axes of MCA that accounted for 16.3% and 4.5% of the total variance. Eigenvalues are shown (*Inset*). (*B*) Population score (mean and SE) along the first MCA axis. A one-way ANOVA followed by a post hoc Tukey test was run to test for differences among populations. Different letters indicate significant differences at $P < 0.05$. ▽, *Cc* from the Pyrenees; other symbols are as in Fig. 1.

The gradient analyses show that the current niche separation between *Cc* and *Cr* embraces differential response curves along multiple environmental gradients, hence suggesting that several physiological and morphological adaptations were crucial in the evolutionary history of each taxon. It is presently unknown whether the use of different niches by mostly sympatric populations triggered the separation of the two lineages, or whether the two taxa largely evolved allopatrically.

In alpine landscapes, strong limitation of gene flow causing genetic differentiation has been attributed either to geographical isolation, with much emphasis given to habitat fragmentation induced by Pleistocene glaciations (32, 33), or to reproductive isolation along environmental gradients, including elevational (34, 35), snowmelt (36), or edaphic (37) gradients. Cross-pollination between *Cc* and *Cr* is likely because (*i*) the species is a widespread dominant plant of high-elevation grasslands exhibiting long-distance wind pollen dispersal, and (*ii*) the two taxa are sympatric over large areas of the Alps.

In our view, two possible explanations for the maintenance of strong genetic differentiation between sympatric *Cc* and *Cr* populations are possible. First, habitat specialization along a mesotopographical gradient would reduce gene flow via pollen, because unequal snow-cover duration between concave and convex sites induces phenological shifts in flowering time. Such a premating reproductive isolation along the snowmelt gradient

Table 2. Distribution of *Cc* and *Cr* diagnostic AFLP markers

AFLP markers		Type of individuals			
		Cc (n = 54)	*Cr* (n = 66)	*Ccm* (n = 28)	*Crm* (n = 19)
Cc diagnostic markers (n = 14)	Mean number of individuals per marker (±SE)	45.5 (6.5)	2.8 (2.6)	21.7 (5.6)	2.1 (1.7)
	Frequency, %	84.3	4.4	77.5	**10.5**
Cr diagnostic markers (n = 33)	Mean number of individuals per marker (±SE)	2.5 (2.9)	54.2 (10.2)	5.8 (3.6)	15.8 (6.7)
	Frequency, %	4.6	86.0	**20.7**	79.0

The Pyrenean population is not included. AFLP markers were considered as diagnostic markers for one taxon if present in >80% of individuals of that taxon and in <5% of individuals of the other taxon. Numbers in bold refer to the AFLP phenotypes of ecologically marginal populations.

has been documented for populations of the alpine buttercup (38). Second, lower hybrid fitness in *Cc* or *Cr* typical habitats would reinforce premating isolation. Indeed, selection against hybrids is expected to increase rapidly with increasing difference in ecological traits among populations (39).

Genetic Introgression. AFLP phenotypes of ecologically marginal populations (*Ccm*, *Crm*) might result from genetic introgression between *Cc* and *Cr*, because individuals from these populations exhibit a higher percentage of diagnostic markers from the opposite taxon. Alternatively, these AFLP phenotypes could result from adaptive polymorphism within the gene pool of each taxon. We consider the first hypothesis as the most parsimonious. First, morphologically intermediate hybrids have been described (13), suggesting the possibility of gene flow between *Cc* and *Cr*. Second, the most striking "nontypical" AFLP phenotypes and ecologically marginal populations are in areas where *Cc* and *Cr* are sympatric. The Dolomites mountain range (populations L, Fig. 1) is probably the best example in this regard (13). To our knowledge, the existence of marginal populations is confined to the western and middle parts of the Alps, i.e., in regions where *Cr* and *Cc* grow sympatrically.

In Fig. 3, the location of the AFLP phenotypes in marginal populations provides convincing support that initial hybridization was followed by repeated backcrosses with one parental taxon preferentially, i.e., introgressive hybridization (40, 41). This is consistent with the fact that individuals of these populations are phenotypically very similar to one or the other parental type. As a whole-genome fingerprinting technique, AFLP markers are particularly helpful in detecting such gene exchange among taxonomically related taxa (42). It has long been postulated that hybridization and introgression should be sources of variation and ultimately of new species (41, 43); recent molecular data largely support this view (44, 45). Hybridization among distinct evolutionary lineages in secondary contact zones is a recurrent topic of phylogeographical studies (46). However, these studies often fail to relate patterns of genetic variation with niche attributes. By contrast, our study addresses genetic structure in an explicit ecological context, allowing us to state that the stabilized introgressants occur in ecologically intermediate habitats. Reinforcement and introgression are held as two counteracting forces during the sympatric phase of the ecological speciation process, the former enhancing genetic divergence, and the latter having the potential to erase it (47). In light of our results, we propose that the balance between these two mechanisms is strongly habitat-dependent, with an overwhelming impact of stabilizing selection in typical habitats and a superiority of introgressed individuals in intermediate habitats.

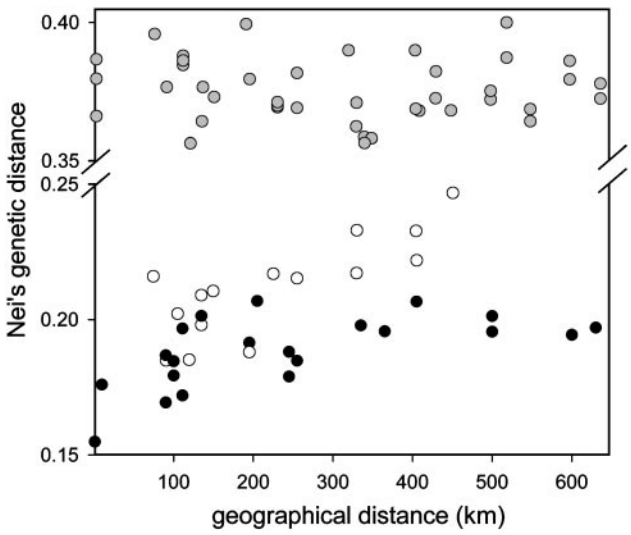

Fig. 4. The relation between geographical distance and genetic distance for pairs of populations of the *Cc* core group (open circles), *Cr* core group (filled circles), and between *Cc* and *Cr* core-group populations (shaded circles). The Pyrenean population is not included.

Implications for Niche Theory. Although niche separation between *Cc* and *Cr* has a single origin, initial divergence was probably followed by multiple events of secondary gene flow in areas of sympatry. We hypothesize that such introgressive hybridization created new avenues for the persistence of populations in marginal habitats. Interestingly, similar conclusions have been drawn in studies of invasive species (43, 48, 49). In the last case, an evolutionary lineage with new ecological requirements and adaptations may originate from genetic introgression between two alien species or between an alien and a native species (50), leading to dynamic shifts in niche distribution.

To our knowledge, there has been no attempt to incorporate the type of interdependence among taxa described here in current theories of plant community organization. That species tend to be distributed independently of one another in "continua" along environmental gradients is a fundamental tenet of the individualistic perspective of plant community organization (51), and current models used to predict plant response to global warming largely conform to this dominant paradigm. However, these approaches are questionable, because they largely ignore (*i*) how genotypic diversity along niche width might alter a species' response and (*ii*) how interdependence among species might also be affected by climate change (52, 53). Our findings suggest that genetic introgression between related taxa is an important process shaping species distribution along gradients and, as such, probably deserves greater attention when attempting to predict niche shift under global change.

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