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Diversity of Rich Fen Vegetation and Related Plant Specialists in Mountain Refugia of the Iberian Peninsula

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Abstract In temperate mountains, fens have been reported as relict habitats subject to geographical fragmentation and broad climatic gradients, but few studies have analyzed the influence of these factors on plant diversity. Here we investigate the effect of isolation on the vegetation diversity of rich fens (*Caricion davallianae*) in the mountains of the Iberian Peninsula, the distribution limit of these habitats in south-western Europe. We used plot-based vegetation data from the Pyrenees and the Cantabrian mountain range to evaluate their regional species-pool, occurrence of specialists, beta-diversity and the effect of geo-climatic variables on their species-richness and species-composition. We found a lower ratio of rare specialists in the Pyrenees than in the Cantabrian range, but similar estimates in the species pools, total species-richness per plot and beta-diversity. The isolation of the two mountain regions resulted in different species assemblages best predicted by summer precipitation and bedrock types, showing region-based differences in the response of vegetation and plant specialists to the environment. The tighter correlation between local climate and diversity estimates in the Cantabrian range suggests relict character of rich fens in that region, where climatic conditions have restricted local distribution of formerly more widely distributed specialists. Although there is no relevant evidence of vegetation impoverishment in that region, historical isolation has probably resulted in the existence of fragmentary plant communities. We conclude that fen vegetation may experience long-time persistence in climatically sub-optimal mountain refugia, but related plant specialists may be sensitive to climatic changes and subject to the extinction of local populations.

Keywords Alpha-diversity · Beta-diversity · Cantabrian range · Diversity · Fen · Paleorefugia · Pyrenees · Vegetation composition · Wetlands

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Plant nomenclature Castroviejo et al. (1986–2011); Casas et al. (2006)

Introduction

In temperate regions, bogs and fens are naturally subject to rarity and fragmentation (Tiner 2003; van Diggelen et al. 2006). The consequences of such factors in plant and vegetation diversity have been studied at landscape scales (Peintinger et al. 2003; Hooftman et al. 2004; Nekola 2004) but their implication for broad-scale patterns has hardly been assessed. Rich fens, which are geographically widely dispersed but may be locally extremely rare because of their need for particular geo-edaphic conditions can be used to study the effect of geographic fragmentation (Grootjans et al. 2006). As a result of the climatic changes that have occurred during the last 15,000 years, rich fens have been suggested as relict habitats subject to spatial isolation in temperate Europe and North America (Amon et al. 2002; McClellan et al. 2003; Hájek et al. 2009). In concordance with the biogeographical concept of relict species (Lomolino et al. 2006), the interpretation of relict plant communities assumes the existence of remnants of once widespread vegetation, which are currently isolated in optimal or suboptimal refugia (Zimmermann et al. 2009). However, the evolutionary processes behind relictualism have been mainly assessed at species level (Habel et al. 2009) and less attention has been paid to the relict status of plant communities. In the case of rich fens, a high ratio of rare and endangered species has been detected whose current distribution is the result of past climatic oscillations and adaptation to regional ecological gradients (Virolainen et al. 1998; Bedford and Godwin 2003; Johnson and Steingraeber 2003; Flinn et al. 2008), but there is a lack of information about the possible effects of historical isolation in the vegetation of these habitats.

In Europe, the vegetation of rich fens is mainly represented by the phytosociological alliance *Caricion davallianae* Klika 1934, comprising a well-defined group of plant communities requiring a base-rich water supply (Ellenberg 1988; Wheeler and Proctor 2000; Hájek et al. 2006). Although similar vegetation is found in most European countries (generally denoted as alkaline fens, small-sedge fens, brown-moss fens, etc.), the diversity center of these habitats is commonly referred to the Alps because of the higher diversity of characteristic species and higher beta-diversity of vegetation (Ellenberg 1988). Accordingly, other mountain systems of central and southern Europe are recognized as important plant areas for rich fens (Dierssen and Dierssen 1985; Hájková et al 2006; Bergamini et al. 2009; Sekulová et al. 2011). In marginal mountains outside the massif of the Alps, the combination of geographical isolation and the adaptation to local conditions can be reflected in regional differences on plant and vegetation diversity. Isolation of plant populations is a recognized effect of past climatic oscillations, with an important effect on genetic differentiation and potential declining in peripheral mountains (Csergö et al. 2009; Kramp et al. 2009; Cires et al. 2011). At vegetation level, long-term isolation and regional climatic changes could have resulted in a certain impoverishment of plant communities, with a possible decrease in alfa- and beta-diversity. Isolation could also explain the existence of “fragmentary” plant communities in the sense of Braun-Blanquet (1932), i.e., assemblages with a reduced number of characteristic species and differentiation of species composition that provides an incomplete picture of the original vegetation. Although the occurrence of relict fen species has been described in suitable refugia of central Europe (Zimmermann et al. 2009; Hájek et al. 2011) there is lack of evidence on the possible effects of isolation on relict fens of southern-European mountain ranges. Such information could improve our understanding of rich-fen vegetation and help us to prevent the loss of their biodiversity.

In this paper, we study the vegetation diversity of rich fens along the Cantabrian and Pyrenean mountain ranges (Iberian Peninsula) that represent the south-western distribution limit of this vegetation in Europe. These mountains have been traditionally considered a refuge for base-rich fens in western Europe (Braun-Blanquet 1948; Rivas-Martínez et al. 1984; Casanovas 1991). In particular, the Cantabrian mountains have a smaller range size and a stronger Mediterranean influence than the Pyrenees, and therefore a decrease of characteristic species and vegetation diversity could be expected. However, data on species diversity and distribution for the Cantabro-Pyrenean rich fens are confined to local studies, and there is an important gap in the comprehensive knowledge of these habitats in the Iberian Peninsula. Our main aim is to evaluate possible syndromes of relictualism in this vegetation, studying the response of plant communities to long-term geographical isolation. Specifically, we ask *i*) which differences in regional species pool, beta-diversity and plot species-richness exist between two marginal, isolated mountain regions? *ii*) Do ecological specialists have a distinct response to broad or regional ecological gradients? *iii*) Are patterns of diversity influenced by geographical isolation and local adaptation of species?

Methods

Study Area

The Cantabro-Pyrenean mountains represent the western limit of the European Mountain System, and a transitional zone between Eurosiberian and Mediterranean regions in the Iberian Peninsula. Although the Cantabrian and the Pyrenean ranges share Alpine orogeny, there are relevant geological and bioclimatic differences between the two regions, which are physically separated by lowland areas covered by Tertiary depositions (Vasco-Cantabrian depression). In the context of European biogeography (Roekaerts 2002) the Cantabrian range is part of the Atlantic region, while the Pyrenees form the western border of the Alpine region (Fig. **1**). Strong differences between the two mountain ranges have also been identified in the biogeographic and bioclimatic context of the Iberian Peninsula (Rivas-Martínez et al. 2002). The northern lowlands of the Cantabrian range are strongly influenced by the Temperate Atlantic climate, while the central axis and the southern exposures show a sub-Mediterranean influence (Rivas-Martínez 2007). The Pyrenees, however, are higher in altitude, more extended and complex, including continental nuclei, clearly alpine landscapes and a lower Mediterranean influence than the Cantabrian Mountains. Furthermore, plant assemblages in the Pyrenees are much closer to those of the Alpine chain (despite vicariance and endemism), and the influence of central European flora is lower in the Cantabrian range.

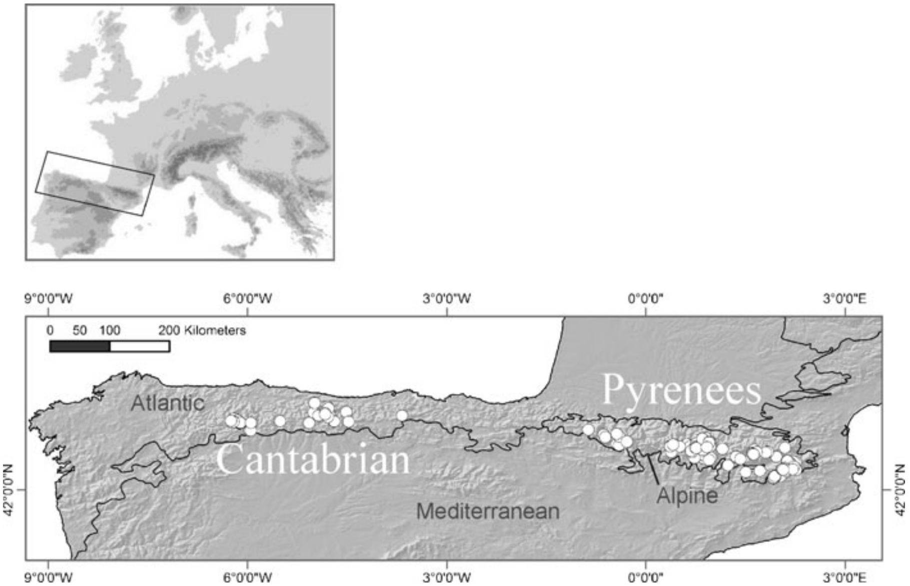

Fig. 1 Distribution of the fen sites investigated in the Cantabrian and Pyrenean mountains, and classification of the study area according to the main European biogeographic regions. The upper map shows the location of the study area in the context of the European Mountain System

Vegetation Data

As an initial step to this study, we performed a systematic review of published and unpublished sources about rich fen vegetation in the Cantabro-Pyrenean mountains, including more than 500 plot-based samples (relevés) originally assigned to base-rich fens and related vegetation. Original data were compiled from the literature, the Spanish Vegetation Database SIVIM (Font et al. 2010) and unpublished data derived from local vegetation databases (provided by the Atlantic Botanic Garden/University of Oviedo, the University of Basque Country and the French Conservatoire National Midi-Pyrénéés) together with our own data sampled during the last five years in unsurveyed areas. We used a numerical classification of the compiled database (Jiménez-Alfaro et al. 2011) to select those samples assigned to the *Caricion davallianae* plant communities in the study area, excluding those more related to nutrient-rich communities (fen grasslands associated to *Molinetalia*) spring vegetation (*Cratoneurion commutati*) and arctic-alpine communities (*Caricion maritimae*), the latter being exclusively represented in a few Pyrenean locations (Benito Alonso 2003).

Only samples containing cover data for vascular plants and bryophytes were considered, and georeferenced using digital maps and the original information on geographical position (except data already sampled with the assistance of GPS), removing data with low accuracy (precision >1 km). For the sake of comparability, we selected only samples with plot sizes between 4 and 40 m^2 and taken above 1,000 m of altitude, because these were the criteria followed by most authors. To ecologically homogenize the data to be analyzed, we re-sampled the database according to the presence of at least three plant specialists of the studied vegetation in the

Cantabro-Pyrenean mountains. In this way we removed 5 % of the samples (considered as transitional vegetation types possibly sampled under different criteria) allowing a relatively high homogeneity and a minimal number of plant specialists to be compared within the dataset. The list of ecological specialists was created according to the characteristic species defined by phytosociological literature focused on *Caricion davallianae* in the study area (Braun-Blanquet 1948; Rivas-Martínez et al. 1984, 2002; Casanovas 1991). A total of 189 samples were finally selected for further analyses, covering most of the localities known to support rich fen sites in the Iberian Peninsula.

Environmental Data

We defined four geo-climatic variables to be used as predictors of broad scale patterns in the vegetation data set. Altitude (variable 1) was obtained from a digital elevation model for the Iberian Peninsula at 200 m resolution, using the geographical coordinates provided by original information of samples or the geo-referencing process. Coordinates were also used to extract climatic data from the WORDCLIM model at 1-km resolution (Hijmans et al. 2005) assigning an estimated value of summer precipitation (variable 2) and mean annual temperature (variable 3) to each sample. We selected these variables because they are the main drivers of vegetation worldwide (Prentice 1992) and we assume they best reflect main climatic gradients in the study area (although other temperature and precipitation variables were closely correlated to them). Finally, we used a variable reflecting geo-hydrological bedrock types in the study area, as a surrogate of mineralization and pH in different lithological conditions. For this purpose, we used the hydro-geological map of Europe (Gilbrich 2000) to classify the samples as coming from *i*) fissured aquifers (including karst) or *ii*) insignificant aquifer. According to the geological maps of the study area (which cannot be used because they do not reflect superficial layers) this classification correlates with the presence/absence of limestone bedrock, and therefore we refer to it as limestone (variable 4), which was used as a categorical variable with 1 and 0 values. Spatial data and variables were all performed using ArcGIS 9.2 (ESRI Inc., Redlands CA, USA).

Statistical Analyses

We used the statistical tools implemented in JUICE 7.0 software (Tichý 2002) to calculate the regional species pool (total number of species per region) and mean species-richness per plot (for all species and for the ecological specialists only) in the Cantabrian and Pyrenean mountain ranges. To compare species pool sizes of vascular plants and bryophytes in the two regions as a function of sampling effort, we used sample-based rarefaction curves based on statistical methods (Colwell et al. 2004) implemented in JUICE. We finally estimated the relative (percentage) frequency (RF) of specialist species normalized from 0 to 1, as the division between the number of occurrences for a given species (n_i) and the total samples (N) of regional subsets ($RF=n_i/N$). To assess possible similarities in the patterns of rarity of specialist plants, we used the Pearson correlation to analyze the relationship between relative frequencies of these species in the two regions.

To analyze the correlation between the explanatory variables and the species-richness measured for each sample, we used generalized linear models (GLM) with a Poisson error distribution and a logarithmic link function (R software, R 2.10.0) as a method to explain the non-linear distribution of species-richness counts. When more than one variable was detected as significant, we used a stepwise variable selection based on the Akaike information criterion (AIC) to create the best model. To compare the possible effect of geography and the specialist species pool in the analyses, GLM was calculated for *i*) all data, *ii*) subsets of the Cantabrian and the Pyrenean regions and *iii*) all the species and the subset of specialists. Because these analyses could be biased by the effect of plot size (Chytrý 2001), we conducted a first GLM using the sampled area as the unique variable. We found a significant effect of plot size when analyzing total species richness of all dataset ($\mathrm{Chi}^2=5.959$; $P=0.015$) and the Pyrenees subset ($\mathrm{Chi}^2=4.852$; $P=0.028$). However, subsets of Cantabrian data and specialists were clearly not affected by this factor – P values (Chi^2)=0.88 (0.02); 0.41 (0.68); 0.12 (2.39) and 0.38 (0.76). This is probably caused by the lower range of plot size found in the Cantabrian data (where the maximum area was 30 m^2 instead of 40 m^2) and the small minimal areas needed for sampling representative plant communities in this vegetation (estimated in <2 m^2 for Pyrenean fens, Casanovas 1991). To provide comparable analyses for all the subsets, we removed outlying samples (>30 m^2) of the two datasets affected by plot size, which resulted in relatively unbiased data containing >90 % of original samples. Although other standardization methods could be employed (for example, using Area/log(size) relationships) previous analysis showed that the transformation of fewer specialists could provide unrealistic estimates and therefore biased results.

The variability of species composition between the two studied regions was compared in a synoptic table including the diagnostic species according to the Phi coefficient (Tichý and Chytrý 2006). We used the length of gradient (first axis) measured by detrended correspondence analysis (DCA) and the mean Sørensen dissimilarity of the samples to estimate beta-diversity (Anderson et al. 2006). Sørensen dissimilarity was calculated through JUICE using presence/absence data and a bootstrap procedure with 500 iterations. DCA was also used to assess the relationship between species composition and explanatory variables, and to generate ordination plots for the interpretation of data variability and the weight of indicator species. The relative influence of the environmental variables on species composition was modelled through canonical correspondence analysis (CCA) using a forward selection and Montecarlo test with 999 permutations (Lepš and Šmilauer 2003). Gradient analysis was performed with CANOCO 4.5 software (Biometris, Wageningen, NL) using a square-root transformation of the cover data and downweighting of rare species. To compare the possible effect of geography and specialist species pool in the analyses, both GLM and CCA were calculated for *i*) all data, *ii*) subsets of the Cantabrian and the Pyrenean regions and *iii*) all the species and the subset of specialists.

Results

A total number of 320 species of vascular plants (*vp*) and bryophytes (*br*) were sampled in the 189 plots analyzed in this study. Total species-richness was clearly different for the two geographic areas, with more species in the Pyrenees (N=257; Nvp=211; Nbr=46) than in the Cantabrian range (N=185; Nvp=151; Nbr=34). However, rarefaction curves (Fig. 2) showed similar rates in the accumulation of species-richness when increasing the number of plots, indicating high similarity of species pools at the same sampling effort between the two regions. The number of plots sampled especially influenced the number of vascular plants, whereas the number of bryophytes was less dependent on this factor. The two mountain regions, however, shared the vast majority of fen specialists, with 19 out of the 20 specialists identified in the whole data set inhabiting each region (Table 1). The only species completely absent in the Cantabrian rich fens was *Pinguicula vulgaris*, while *Blysmus compressus* was the only one not present in the Pyrenees data set. The ratio of rare specialists (species with low or very low values of *RF*), however, was higher in the Cantabrian range than in the Pyrenees. Correlation between the relative frequency of specialists between both data sets was not significant (Pearson coefficient=0.388; P= 0.091) although marginally significant at P values between 0.05 and 0.1. Main differences were caused by some species (e.g., *Tofieldia calyculata*, *Primula farinosa*) relatively frequent in the Pyrenees but extremely rare in the Cantabrian region.

Mean species-richness by plot was very similar in the Cantabrian (23.36±7.60) and the Pyrenean (23.92±6.27) subsets, but mean richness of specialists was significantly higher (Mann–Whitney test; U=2499; $P<0.01$) in the Pyrenees (6.79±2.11) than in the Cantabrian range (5.83±1.98). For the whole data set, the environmental variables were not correlated with the total species-richness, but a significant relationship was found between the number of plant specialists and altitude, presence of limestone and annual temperature (Table 2) from which only the latter two variables were included in the stepwise model selection (Specialists = 2.1865 – 0.0337 × Temperature – 0.12375 × Limestone). Analysis of the two geographical subsets showed a different effect of the environmental variables. Number of specialists increased with increasing

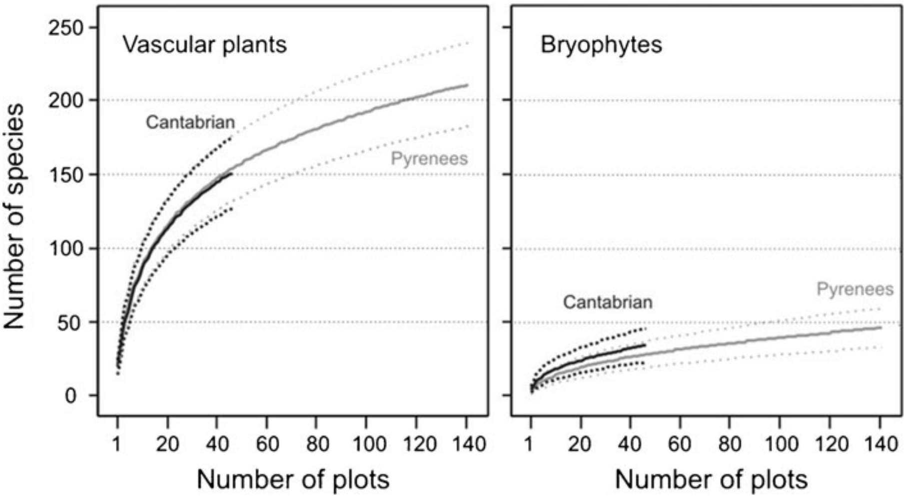

Fig. 2 Rarefaction curves showing the cumulative number of vascular plants and bryophytes with increasing the number of plots sampled in rich-fens of the Cantabrian (black lines) and the Pyrenean (grey lines) mountain ranges. Solid lines show the estimated species-richness while dashed lines show their 95 % confidence intervals

Table 1 Total number of occurrences (N) and relative frequency (*RF*) of specialist species of rich fens in the Cantabrian (n=48) and Pyrenean (n=141) mountain regions. (*br*) indicates bryophyte species

	Cantabrian		Pyrenees	
Species	*N*	*RF*	*N*	*RF*
Aneura pinguis (*br*)	10	0.21	5	0.04
Blysmus compressus	12	0.25	0	0
Campylium stellatum (*br*)	11	0.23	79	0.56
Carex davalliana	20	0.42	111	0.79
Carex lepidocarpa	47	0.98	105	0.74
Eleocharis quinqueflora	12	0.25	28	0.2
Equisetum variegatum	10	0.21	3	0.02
Eriophorum latifolium	21	0.44	53	0.38
Juncus alpino-articulatus	11	0.23	49	0.35
Parnassia palustris	40	0.83	112	0.79
Pedicularis mixta	21	0.44	23	0.16
Pinguicula grandiflora	39	0.81	33	0.23
Pinguicula vulgaris	0	0	63	0.45
Potentilla fruticosa	2	0.04	3	0.02
Primula farinosa	1	0.02	66	0.47
Selaginella selaginoides	15	0.31	80	0.57
Scorpidium cossoni (*br*)	6	0.13	61	0.43
Swertia perennis	9	0.19	45	0.32
Tofieldia calyculata	1	0.02	74	0.52
Triglochin palustris	8	0.17	30	0.21

altitude and decreasing annual temperature in the Cantabrian range, although the model selection included only the first variable (Specialists = − 2.24728 + 0.00559 × Altitude). In the Pyrenean subset, only the Altitude was correlated with total species richness, but the variables had no significant effect on the number of plant specialists.

According to the phi-coefficient, the number of species with significant fidelity to the regions studied was rather similar (Table 3). However, the number of specialist species with high diagnostic value was higher in the Pyrenees. Gradient analysis (Fig. 3) showed a correlation between the geographic distribution of samples and the environmental variables. The length of gradient on the first DCA axis for the whole dataset was 3.13 s.d. (standard deviation) units, which was reduced when using the Pyrenean (2.67 s.d. units) and the Cantabrian (2.36 s.d. units) subsets. Sørensen dissimilarity of the whole data set was 0.69 (±0.01) showing no differences between the Pyrenean (0.66±0.01) and Cantabrian (0.66±0.01) subsets. The variability on plot species composition extracted by DCA (Fig. 3) showed that the main gradient (Axis 1) along the two mountain ranges is correlated to the presence of limestone bedrock (Kendall's Tau=−0.497; $P<0.001$) and with summer precipitation (Kendall's Tau=0.325; $P<0.001$). The second DCA axis is mainly correlated with altitude (Kendall's Tau=0.441; $P<0.001$) and mean annual temperature (Kendall's Tau=−0.224; $P<0.001$).

Table 2 Results of individual GLM analysis (Poisson distribution, log-link) performed to test the effect of the environmental variables on the species richness sampled by plot of rich fens in the Iberian Peninsula. Positive (+) and negative (−) signs reflect the effect of significant variables on species richness (** – $P<0.01$; n.s. – not significant). Calculations are based on different geographical areas (All data, Cantabrian, Pyrenees), all species (Total) and only specialists (Spec)

	All data		Cantabrian		Pyrenees	
	Total	Spec	Total	Spec	Total	Spec
Altitude	n.s.	(+)**	n.s.	(+)**	(−)**	n.s.
Limestone Presence	n.s.	(+)**	n.s.	n.s.	n.s.	n.s.
Annual Temperature	n.s.	(−)**	n.s.	(−)**	n.s.	n.s.
Summer Precipitation	n.s.	n.s.	(−)**	n.s.	n.s.	n.s.

According to the CCA results, the total variance accounted for by the environmental factors was between 8 % and 22 % (Table 4) with higher values for the Cantabrian subset. Altitude, presence of limestone, and summer precipitation were the factors accounting for most of the variability in species composition. However, differences in the relative weight of the variables between all species and the subset of plant specialists were found both for the whole data and the geographical subsets. In the Cantabrian range, annual temperature was the main variable affecting total species composition, with a less significant effect of altitude, while specialist species were mainly influenced by limestone and altitude. In the Pyrenees, the two subsets were equally influenced by altitude, presence of limestone and summer precipitation.

Discussion

Main Patterns of Vegetation Diversity

The estimates of species pools, total species-richness by plot, beta-diversity and the number of plant specialists by region do not support the hypothesis of vegetation impoverishment, suggesting a certain persistence capacity of fen vegetation in the study area. Although we found a higher species pool in the Pyrenees than in the Cantabrian range, these differences may be attributed to the different number of regional sample size, influencing the number of vascular plants rather than bryophytes as reflected by rarefaction curves. Accordingly, regional species-richness seems not to provide differences in beta-diversity of the two datasets, and the high number of species occurring only in the Pyrenees is not reflected by higher variability on species composition. As described by Pärtel et al. (2001) this effect is probably caused by a similar ratio of rare species in the two data sets. Although differences in beta-diversity within mires have been attributed mainly to strong ecological factors such as soil composition and water level (Økland 1990; Flinn et al. 2008) or management (Bergamini et al. 2009) these factors are likely not to affect the analyzed vegetation, because of its ecological homogeneity and the similarity of historical management in the two regions studied.

Gradient analysis revealed that the main factors accounting for differences in species composition of the two mountain regions are the summer precipitation and

Table 3 Shortened synoptic table of species percentage in the rich fen samples of the Cantabrian and Pyrenean mountain regions. Only species with a phi-coefficient of association (ϕ)>0.3 are included (* – ϕ>0.3; ** – ϕ>0.4; *** – ϕ>0.5). Calculations are standardized to equal size only for species with a significant diagnostic value at 0.01 level (Fisher's exact test)

	Cantabrian	Pyrenees
Number of samples	43	114
Pinguicula grandiflora	83***	25
Crepis paludosa	55***	6
Philonotis calcarea	40**	5
Geum rivale	30**	.
Blysmus compressus	26*	.
Agrostis stolonifera	30*	3
Mentha longifolia	30*	4
Carex lepidocarpa	98*	75
Palustriella decipiens	21*	1
Palustriella commutata	45*	15
Pedicularis mixta	45*	16
Caltha palustris	60*	29
Genista hispanica subsp. *occidentalis*	17*	.
Centaurium somedanum	17*	.
Tofieldia calyculata	2	52***
Pinguicula vulgaris	.	44***
Primula farinosa	2	46***
Bartsia alpina	.	41**
Succisa pratensis	13	56**
Potentilla erecta	28	69**
Selinum pyrenaeum	.	29*
Carex davalliana	43	78*
Scorpidium cossonii	13	43*
Eriophorum angustifolium	.	20*
Campylium stellatum	23	56*
Carex paniculata	.	18*
Molinia caerulea	17	46*
Pedicularis pyrenaica	.	17*

the presence of limestone. Precipitation regime is a fundamental factor explaining the bioclimatic similarity of the Pyrenees to the Alpine chain and its biogeographic description within the Iberian Peninsula (Fillat 1984; Rivas-Martínez 2007) but the influence of this factor in fen vegetation has not been demonstrated before. The influence of limestone, however, agrees with the presence of calcareous substrata along the Iberian Peninsula, where this factor has an important effect on plant diversity (Lobo et al. 2001). Although rich fens are predominantly found on limestone in the two regions, these substrata are very extended in the Cantabrian sites

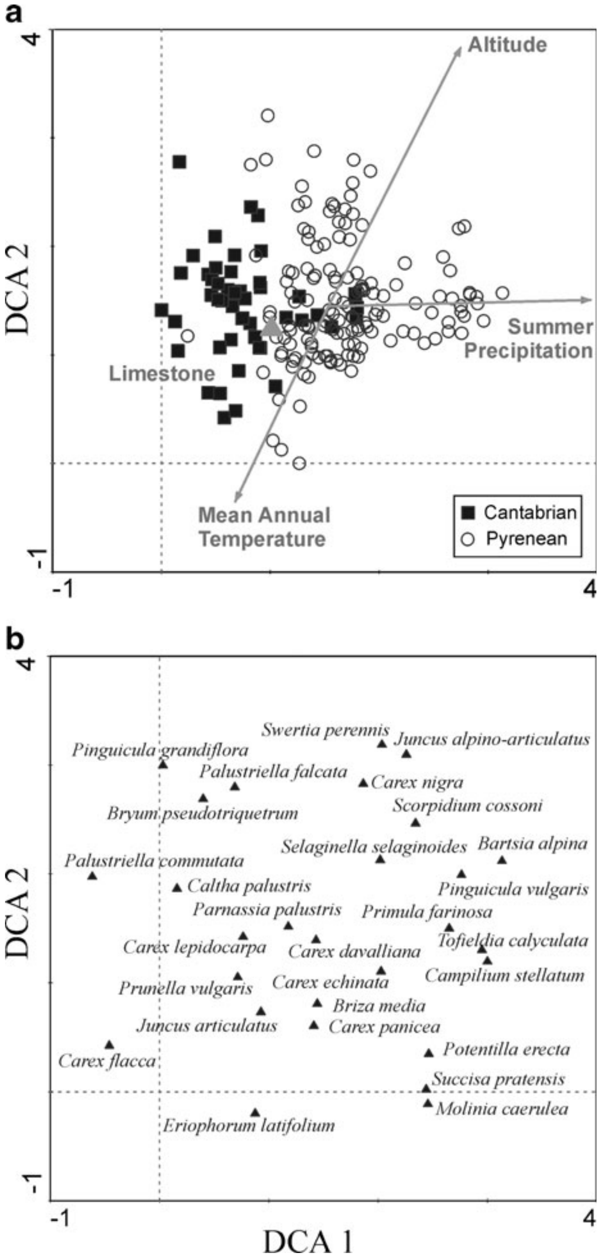

Fig. 3 DCA ordination plots (first and second axis) obtained from the vegetation data of rich fens analyzed in this study. Sample-environment biplot **a** shows the distribution of sites according to the two mountain regions compared (Cantabrian and Pyrenees), together with the environmental variables passively projected on the diagram. Species plot **b** includes the species with higher weight (>15) along the gradients

analyzed here, whereas in the Pyrenees they are concentrated in the central and western parts of the range – the eastern sector being mainly formed by slate and granite. This explains the high diagnostic value of *Tofieldia calyculata*, *Pinguicula vulgaris* or *Primula farinosa* in the Pyrenees (Table 3), species less strictly related to carbonates in the study area (Casanovas 1991). Although we did not have pH measurements for the whole data set, a partial analysis of the plots provided with these data (results not shown) gave a significant correlation between the bedrock

Table 4 Relationships between species-composition of rich fens and the explanatory variables, analyzed by canonical correspondence analysis (CCA). Numbers in parentheses reflect the order of inclusion of significant variables by forward selection (** – $P<0.01$; * – $P<0.05$; n.s. – not significant). The CCA parameters shown are: variation of species composition (Total inertia), sum of all canonical eigenvalues (Explained variation) and percentage of total inertia (% explained). Calculations are based on different geographical areas (All data, Cantabrian, Pyrenees), all species (Total) and only specialists (Spec)

	All data		Cantabrian		Pyrenees	
	Total	Spec	Total	Spec	Total	Spec
Altitude	(1)**	(2)**	(4)*	(2)**	(1)**	(1)**
Limestone presence	(3)**	(3)**	(2)**	(1)**	(2)**	(2)**
Annual Temperature	(4)*	n.s.	(1)**	n.s.	n.s.	n.s.
Summer Precipitation	(2)**	(1)**	(3)**	(3)*	(3)**	(3)**
(Total inertia)	4.44	2.03	3.72	1.95	3.85	1.66
(Explained variation)	0.41	0.23	0.61	0.43	0.33	0.15
(% explained)	9.23	11.3	16.4	22.1	8.6	9.0

types and the pH, i.e., a clear geochemical determinism on fen vegetation. This finding suggests a lithological differentiation between calcareous fens along the study area, according to the main floristic groups of rich fens recognized in Europe (Hájek et al. 2006): calcareous fens (here found on limestone) and extremely rich fens (on granite). Besides precipitation and bedrock, CCA results show the importance of altitude in explaining the variability in species composition, a variable that is generally supposed to affect fen diversity in mountainous regions (Hájek et al. 2008; Sekulová et al. 2011).

Despite possible limitations of data from the vegetation databases for the calculation of diversity estimates (Lájer 2007), our results seem to be consistent enough to interpret general patterns in the vegetation studied. The natural rarity and ecological uniqueness of rich fens in the study area could favor the representativeness of the data collected under preferential sampling (Roleček et al. 2007; Michalcová et al. 2011) while the high estimates of beta-diversity in the two datasets could prevent the effect of (slightly) different plot sizes in ordination (Otýpková and Chytrý 2006). However, there are still potential limitations for the estimation of absolute species-richness when using phytosociological data (Lepš and Šmilauer 2007) and therefore further interpretations of such results should be conducted with care.

Distribution of Plant Specialists

The list of characteristic species that we defined as specialists is similar to the species similarly considered in other regions (Hájek et al. 2006; Bergamini et al. 2009). In contrast with the expected lower suitability of the Cantabrian range to support rich fen specialists, we found almost the same number of these species in the two mountain regions. However, the lower number of specialists by plot and their higher rarity could indicate less suitable conditions for rich fens in that region, supporting the existence of "fragmentary" plant communities (sensu Braun-Blanquet 1932) as a possible result of previous local extinctions of specialists. The Pyrenees support many sites suitable for rich fens, and therefore the absolute frequency of plant specialists is generally high. We, however, found low correlation in the relative frequency of species, suggesting that the patterns of rarity of specialist plants are specific to each geographical area. The absence of *Pinguicula vulgaris* in the data of Cantabrian rich fens is probably caused by its different ecological behaviour in the two regions, given that this species occurs only on acid fens in the Cantabrian range, but may inhabit rich fens in the Pyrenees. A similar difference has been described for *Eriophorum* species in different regions of Central and Western Europe (Hájková et al. 2008) indicating local adaptations of fen species. Nevertheless, the absence of *Blysmus compressus* in the Pyrenees is difficult to explain, as this species is clearly related to calcareous fens in Europe (Ellenberg 1988). A possible explanation for this could be related to a distinct biogeographical history of rich fens in the two mountain regions, although the ultimate causes affecting past distribution of this vegetation in the Iberian Peninsula are still unknown.

The significant effect of altitude on the number of plant specialists by plot in the Cantabrian subset (but not in that of the Pyrenees) may reflect the existence of more ecological restrictions in that region. This could explain the extreme rarity of *Primula farinosa* and *Tofieldia calyculata* in the Cantabrian range, species whose distribution is restricted to the European Mountain System and cold environments of Central Europe. Besides the combined effect of climate and altitude, the presence of limestone explained a higher species-richness of plant specialists along the two mountain regions. The influence of bedrock type in calcareous water supplies has been recognized as one of the main factors related to plant communities of the *Caricion davallianae* in Europe (Grootjans et al. 2006; Topić and Stančić 2006). As indicated before, limestone-rich substrata are dominant in the Cantabrian range but less frequent in the Eastern Pyrenees, explaining the effect of this factor along the whole study area. However, the presence of limestone *versus* granite bedrocks seems not to be correlated with the presence/absence of fen specialists, and most species may occur in both conditions. This finding suggests that the bedrock factor could have a major effect at community level as evidenced by the differences in species composition.

Diversity of Relict Fen Vegetation

Our results show a relatively high similarity of alpha- and beta-diversity in rich fens of two isolated mountain regions, but different patterns in the species assemblages and the ecological responses of vegetation and plant specialists to geo-climatic gradients. As commonly found in the vegetation of mountain regions (Nagy et al. 2003), the differentiation of species composition along geographical gradients has been recognized in European wetlands (Hájek et al. 2008). However, the influence of historical rather than ecological factors to determine the existence of relict vegetation in fens is difficult to assess, and a possible combination of both aspects should be considered (Horsák et al. 2007). In our case study, fen vegetation seems to be adapted in a different way to the regional conditions found in two nearby but isolated mountain regions, as an evident effect of geographic fragmentation. Nevertheless, the fact that species composition and specialist occurrence in the Cantabrian subset were more influenced by local climatic gradients than in the Pyrenean one may reflect a narrower ecological niche of this vegetation in the first region. This may explain the character of paleo-refugia (Nekola 1999) of particular Cantabrian biotopes, where rich fens probably represent remnants of a wider distribution in the past. This hypothesis would explain *i*) the higher absolute rarity of the Cantabrian fens and related plant specialists and *ii*) the high number of locally endangered plants that have been identified in the rich fens of this region (e.g., *Swertia perennis*, *Triglochin palustris*) including virtual extinction of local micro-endemic populations such as *Juncus balticus* subsp. *cantabricus* and *Salix hastata* subsp. *picoeuropeana* (Nava and Fernández Casado 2004; Robinson et al. 2008). Although Pyrenean rich fens may also be considered relict at a European scale, this region supports a lower number of critically endangered species in the studied habitats, and local populations of the vicariant *Juncus balticus* (subsp. *pyrenaeus*) and *Salix hastata* (subsp. *hastatella*) are not subject to extinction syndromes.

We suggest that Cantabrian rich fens constitute relict plant communities surviving in sub-optimal refugia. The lack of paleo-ecological evidence makes it difficult to assess the period and ecological conditions in which particular species respond to relictualism (Hájek et al. 2011) so further studies are needed to understand such patterns in the Iberian fens. Despite this, the similarities in diversity estimates found in the regions studied suggest a high capability of persistence in the vegetation of rich fens and related plant specialists in response to long-term isolation, which could be unexpected given the marginal position and isolation of the Cantabrian range. This contrasts with the difference in the response of calcareous fens to isolation between the inner and outer Carpathians (Horsák et al. 2007) resulting in different species pools of plant specialists. Such divergences may be explained by historical connections of the Cantabro-Pyrenean flora, reflected in the existence of endemic species shared by rich fens in the two regions (e.g., *Pedicularis mixta*, *Leontodon duboisii*). However, molecular studies (Vargas 2003; Peredo et al. 2009) suggest a different post-glacial development and evolution of plants in these two mountain regions, indicating that connectivity of Cantabro-Pyrenean fens could have been high only before or during glaciations. In any case, our study gives evidences of shifts in rich fen vegetation and related plant specialists to high-altitude refugia, suggesting a certain sensitivity of fens to climate warming in marginal mountains of Europe. Although the effect of climate warming in wetlands has been scarcely studied (Felton 2009), we highlight that relict fens from southern European mountains could be sensitive to climate changes, and monitoring programs for these habitats should be focused on specialist species living in potential mountain refugia.

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