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BIODIVERSITY RESEARCH

Allozyme diversity and geographic variation in the widespread coastal sedge, *Carex arenaria*

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Abstract. Allozyme electrophoresis was used to investigate the structure of genetic variation in the rhizomatous coastal sedge, Carex arenaria, throughout its European range — from the SW Iberian peninsula to the Baltic region. Material was sampled from 77 sites in five geographic regions. Nine of the 13 investigated loci were polymorphic in the total material and there were interregional differences in the number of polymorphic loci per site and the percentage of variable sites. In the Scandinavia/Baltic region only 61% of the sites contained at least one locus with more than one allele, whereas all the British and SW Iberian sites were variable. There was a general tendency for the regional frequencies of the less common alleles at individual loci to decline from SW to NE. The mean (over loci and sites) within-site gene diversity ($\bar{H}_{site}$) was 0.064 (in calculations based on the number of observed multilocus allozyme genotypes within each sampling site). Although there was considerable variation between geographically adjacent sites, within-site diversity showed a general decrease from SW to NE in Europe. There were significant differences in within-region gene diversity (H_{reg}) for the four most variable loci between the five regions. H_{reg} generally decreased from SW to NE Europe and most loci showed the highest diversity in the SW Iberian peninsula and the Bay of Biscay regions. The mean (over loci) gene diversity in the total material (H_{tot}) was 0.070 and the levels of diversity in Carex arenaria are substantially lower than is usual in rhizomatous sedges. The within-site, between-site and between-regional components of the total diversity were 92.4%, 2.5% and 5.1%, respectively. The low levels of overall gene diversity in C. arenaria and the successive decrease in diversity from SW to NE are interpreted in terms of the species' history of post-glacial spread into northern Europe. Despite the overall northwards decrease in diversity, the widespread occurrence of less common alleles and the lack of regional deviations from Hardy–Weinberg genotype frequency expectations suggest that C. arenaria is not predominantly self-fertilized.

Key words. Cyperaceae, genotypic diversity, geographic variation, isozymes.

INTRODUCTION

Climatic changes during the Quaternary have led not only to repeated, dramatic changes in species distributions, but have also had an impact on the structure of genetic variation within many species (Critchfield, 1984). Theory predicts that genetic variation will be lost as a result of drift in small and isolated refugial populations. In addition, a cumulative loss of genetic variation, resulting from repeated founder effects, is expected during the serial expansion of a species' range into previously glaciated areas (e.g. Sage & Wolff, 1986; Hewitt, 1996; Soltis *et al.*, 1997; Le Corre & Kremer, 1998). Species that survived glaciations

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in several separate refugia are likely to show inter-regional differences in, for example, morphological characters or molecular markers that reflect stochastic or selective divergence between the refugial isolates (Hewitt, 1996). In contrast, species that survived in only one refugial area are expected to show little racial differentiation and more gradual patterns of clinal variation.

There are relatively few studies of genetic variation that cover the full distributional ranges of widespread European or North American plant species. However, spatially extensive geographical studies often reveal patterns of genetic differentiation or a structuring of gene diversity that can be interpreted in terms of postglacial immigration history. For example, the European oaks *Quercus robur* and *Q. petraea* show a large-scale pattern of variation in chloroplast DNA races that suggests divergence in separate southern refugia during glacial periods (Ferris *et al.*, 1993, 1998). The large-scale E–W racial differentiation in *Silene latifolia* is also consistent with a scenario of northward immigration into Europe from separate geographical isolates (Prentice, 1986). The similar N–S geographical patterns of cpDNA differentiation in six Pacific north-west plant species are interpreted in terms of patterns of refugial survival and postglacial recolonization (Soltis *et al.*, 1997). Northern populations of the North American *Pinus contorta* have fewer alleles per locus than populations further to the south, near to the species' glacial refugia (Cwynar & MacDonald, 1987).

Carex arenaria is a widespread rhizomatous sedge that forms extensive clonal populations in sandy, coastal habitats in western and northern Europe — from the Atlantic coast of SW Spain to the northern Baltic coasts (cf. Noble, 1982; Hultén & Fries, 1986). Despite its wide present-day geographical distribution, the dependence of *C. arenaria* on open, sandy habitats suggests that the species' postglacial colonization of northern Europe is likely to have been channelled along a narrow coastal corridor. In the present study, we investigate the structure of allozyme variation in *C. arenaria* throughout the species' native range. We also explore the extent to which the pattern of allozyme differentiation and the geographical levels of gene diversity can be interpreted in terms of immigration scenarios, dispersal and breeding system.

MATERIALS AND METHODS

The species

The sand sedge, *Carex arenaria* L., has an Atlantic–Baltic distribution in western Europe (Hultén & Fries, 1986) and is locally common along sandy beaches from SW Spain and northwards and eastwards to S Finland, Estonia and Sweden. Inland populations occur in several regions, particularly in the Netherlands and eastwards to northern Poland and southern Sweden. The absence of *C. arenaria* from many coastal dune systems at the southern end of its distributional range (B.O. Jonsson, personal observations), suggests that the summer availability of soil water limits the species' occurrence in the south as the climate becomes more Mediterranean. In its sites in southern Spain, *C. arenaria* is confined to patches with a high groundwater table and is absent from drier, but otherwise suitable, surrounding habitats (B.O. Jonsson, personal observation). Outside Europe, the species is found (as a presumed introduction) in a few isolated sites along the coast of Virginia (eastern USA) and in Brazil (Mackenzie, 1931; Hegnauer in Tidema, 1981; cf. Hultén & Fries, 1986).

Carex arenaria is characteristically found on sandy soils and in open, early successional plant communities, but populations may also occur in open forests (cf. Noble, 1982). *Carex arenaria* reproduces both vegetatively and sexually. Preliminary crossing experiments indicate that the species is self-compatible (B.O. Jonsson, unpublished). Although large numbers of viable seeds are produced each season, seedling establishment in natural populations is extremely rare (Huiskes, 1977; see also Tidmarsh, 1939; Noble, 1982). *Carex arenaria* has robust rhizomes with long internodes and few branches. The apical shoots of the rhizomes extend in a linear fashion. In less dense populations, long rows of shoots in the sand indicate the structure of the extensive underground rhizome system and demonstrate the great potential for spatial extension of individual genets by vegetative growth. Tidmarsh (1939) showed that clone (genet) extension in British populations ranged from less than 50 cm to up to 4 m per year. Long-distance dispersal of clones may occur through the establishment of detached rhizomes, which are naturally transported by water (B.O. Jonsson & H.C. Prentice,

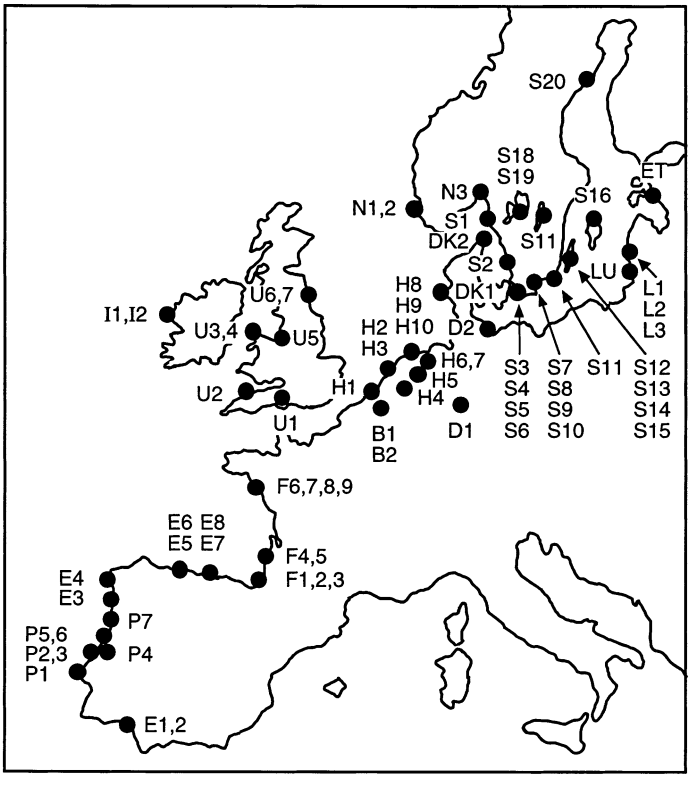

Fig. 1 Study sites of *Carex arenaria* L. in five regions of Europe (cf. Appendix 1). Sampling covered most of the species' distributional range. Each dot indicates the approximate position of one to four sites. Sites represented by the same dot are separated by distances of between 1 and 50 km. Regions are SW Iberian Peninsula (sites E1–E4, P1–P7), Bay of Biscay (E5–E8, F1–F9), British Isles (U1–U7, I1–I2), North Sea Mainland (B1–B2, H1–H10, D1, DK1) and Scandinavia/Baltic (D2, DK2, N1–N3, S1–S20, LU, L1–L3, ET).

personal observations), or accidentally transferred to new sites in sand transports or in ships' ballast. Locally, *C. arenaria* has also been planted as a soil-stabilizer within sand-dune systems or in areas of blown sand (Lindal, 1947; P. Janiesch, personal communication 1997).

Sampling, sites and regions

Live material of *Carex arenaria* was collected from 77 sites throughout the species' distributional range in Europe (Fig. 1; Appendix 1). The sites included a variety of coastal and inland habitats. The sampling strategy was designed to give an overall picture of variation in allele frequencies throughout the species range. Priority was given to achieving a good geographic coverage and sampling a large number of sites rather than to sampling large numbers of individuals per site (cf. Brown & Briggs, 1991). The minimum distance between neighbouring sites was 1 km.

Within sites, we aimed to collect 10–20 samples (vegetative ramets) at 20 m intervals along a transect. However, at some sites, the sampling design had to be slightly modified to fit the local conditions. If the distribution of *C. arenaria* was patchy or fragmented, the distance between some of the samples was more than 20 m. In spatially restricted populations, where a single linear transect gave less than 10 samples, more than one transect or an S-shaped transect were used. In some small populations, and in populations sampled during a pilot study, samples

were taken with a distance of 10 m between adjacent samples.

Each sample consisted of the rooted, apical portion of a single rhizome with three to five ramets (shoots). The sampled material was planted in a greenhouse for the production of young leaf tissue for enzyme extraction.

Enzyme electrophoresis

Tissue from the bases of one young and one fully developed leaf per sample was used to produce extracts for electrophoresis. Leaves from individual samples were crushed, together with fine sand, in an extraction buffer consisting of 200 mM Tris, 52.6 mM sodium metabisulphite, 2.63 mM EDTA, 2 mM $MgCl_2$, 14.3 mM di-mercaptoethanol and 455 mM PVP (adjusted to pH 7.5 with HCl) in a chilled mortar. Chromatography paper wicks (3 × 9 mm; Whatman no. 3) soaked with the leaf extracts were stored for 0–12 months at –80 °C prior to electrophoresis. Horizontal gels (225 × 150 × 8 mm) were prepared from 9.5% Sigma (S4501) starch for a lithium–borate/Tris–citrate buffer system (Prentice & Giles, 1993) and 11.4% Reppin PSG 1000 starch for a morpholine–citrate buffer system (system no. 2 in Soltis & Soltis, 1990).

Ten enzyme systems were screened for variation. Aspartate aminotranferase (AAT; E.C. 2.6.1.1), formate dehydrogenase (FDH; E.C. 1.2.1.2), phosphoglucomutase (PGM; E.C. 5.4.2.2) and triosephosphate isomerase (TPI; E.C. 5.3.1.1) were well-resolved with the lithium–borate/Tris–citrate buffer system (pH 8.4/8.1, 5 h, 60 mA). Isocitrate dehydrogenase (IDH; E.C. 1.1.1.42), malate dehydrogenase (MDH; E.C. 1.1.1.37), phosphogluconate dehydrogenase (PGD; E.C. 1.1.1.44), phosphoglucoisomerase (PGI; E.C. 5.3.1.9), shikimate dehydrogenase (SKD; E.C. 1.1.1.25) and uridine 5′diphosphoglucose pyrophosphorylase (UGP; E.C. 2.7.7.9) gave better resolution with the morpholine–citrate buffer (pH 6.1, 60 mA, 5 h). Staining procedures followed (with minor modifications) Manchenko (1994) for Soltis & Soltis (1990) for the other enzyme systems.

The 10 enzyme systems were interpreted in terms of 15 putative loci. Two of the interpreted loci (Fdh-1 and Ugp-1) had high levels of missing data and were not included in the statistical analyses. Of the 13 loci that were included in the analyses, nine were polymorphic and four were monomorphic. The 13 loci had, together, 27 alleles in the investigated material. Alleles and loci for each enzyme system were numbered in order of descending anodal mobility. The enzyme system AAT showed two loci (Aat-2 and Aat-3) with good activity. Some of the alleles at these two loci had overlapping mobilities. A third, more anodal, AAT locus was not interpreted. Aat-2 was monomorphic in all but one site (where allele 2 was present in one sample), while Aat-3 had four alleles. FDH showed one monomorphic locus (Fdh-1). IDH had one locus (Idh-1) which was monomorphic for allele 1 in most sites. Two rare Idh-1 alleles (alleles 2 and 3) were each found in one site. For the enzyme system MDH, comparisons with other *Carex* species which have two to three polymorphic MDH loci (Jonsson *et al.*, 1996; B.O. Jonsson & H.C. Prentice, in prep.) suggest that the three monomorphic MDH bands in *C. arenaria* represent three monomorphic loci. PGD had one polymorphic locus (Pgd-1) with one common allele and one rare allele that was present in one sample. One PGI locus (Pgi-2, with one common and two rare alleles) gave good resolution, while a more anodal locus was not reliably interpretable. PGM showed one locus (Pgm-1) with two alleles. At two Spanish sites, Pgm-1 heterozygotes (with alleles 1 and 2) showed consistently unbalanced band staining intensities, suggesting different dosages of the two alleles. The ‘dosage heterozygote’ was present at site E2 (in all samples, which probably belonged to one genet) and at site E6 (in one sample). Despite having high chromosome numbers, *Carex* species usually show diploid patterns of variation at allozyme loci (e.g. Waterway, 1990; Ford *et al.*, 1991; Hedrén & Prentice, 1996; Jonsson *et al.*, 1996) and crossing experiments need to be carried out to investigate the genetic background of the Pgm-1 dosage effect. In the calculations of allelic variation, the ‘dosage heterozygote’ was treated as a 1 : 1 heterozygote (with one copy of each allele). SKD had one polymorphic locus (Skd-1) with one common allele and two rare alleles. TPI had one monomorphic locus (Tpi-1) and one polymorphic locus (Tpi-2) with three alleles. Alleles 1 and 1s at Tpi-2 had similar mobilities and could not be

reliably distinguished from each other on most gels. These two alleles were pooled in the calculations on allelic variation but were used to discriminate between genets within site S2. In UGP, the (monomeric) Ugp-1 locus had two alleles while the (dimeric) Ugp-2 locus had four alleles. At Ugp-2, alleles 1 and 1s had similar mobilities and alleles 2f and 2 had similar mobilities. These pairs of alleles were treated as single alleles in the analyses of allelic variation. All four alleles were used to discriminate between clones in the sites within which they could be clearly separated.

Analyses of allozyme variation

One set of analyses was carried out on the data from all the sampled ramets ('ramet-level' analyses) and another set of analyses was carried out on the basis of the minimum number of genets (given by the number of multilocus allozyme genotypes) present in each study site ('genotype-level' analyses). Most of the presented results are based on the genotype-level but ramet-level data are presented for comparison in some of the analyses. Samples with missing data at any locus were excluded from the analyses.

The analyses were based on 13 putative loci. Most of the estimates of allozyme variation are based on within-region data from five geographical regions (SW Iberian Peninsula, Bay of Biscay, British Isles, North Sea Mainland, Scandinavia/Baltic; Fig. 1; Appendix 1). The regions are separated either by a physical barrier (the sea) or by stretches of coast without samples. Allozyme data from sites were pooled to provide the regional data. The data from the separate sites were used (without pooling) in the calculation of the percentage of variable sites within regions, the median number of polymorphic loci per site in each region, within-site allele frequencies, in the partitioning of gene diversity (between sites, regions and total material) and in a cluster analysis.

The numbers of polymorphic loci and the numbers of alleles at individual loci were counted in each site. The percentage of variable sites (sites with more than one allele at one locus at least) for each of the loci Tpi-2, Pgm-1, Ugp-2 and Aat-3 and the median number of polymorphic loci per site were calculated for each region (sites with sample sizes of less than eight were excluded from these estimates). Differences in the median number of polymorphic loci between regions were tested for with Kruskal–Wallis ANOVA (Kruskal & Wallis, 1952) using the program STATISTICA (Statsoft, 1994). The percentage of polymorphic loci (P) and the mean number of alleles per locus (A) were calculated within regions and in the total material: (1) on the basis of all alleles and (2) using the '99% criterion' for polymorphism. The number of rare alleles (with genotype-level allele frequencies < 0.01 in the total material) was counted within each region.

Nei's (1973) gene diversity statistic, H, was used to estimate gene diversity. Unbiased H-values (cf. Prentice & White, 1988) and standard errors for H were obtained for each locus using a jack-knifing procedure (Sokal & Rohlf, 1981). The jack-knifed pseudovalues for H were used in analyses of variance. A one-way ANOVA was used to test for interregional differences in levels of within-region gene diversity (H_{reg}) at each locus and a two-way ANOVA was used to test for interregional and interlocus differences in H_{reg}.

The hierarchical partitioning of gene diversity was analysed with Nei's (1973) G statistics. For each locus, the total diversity is given by H_{tot}, the mean within-site diversity is by $\bar{H}_{site}$ and the mean within-region diversity by $\bar{H}_{reg}$. The within-site component of diversity is given by $\bar{H}_{site}/H_{tot}$, the between-site component of diversity by $G_{site.reg}$ ($G_{site.reg} = \bar{H}_{reg} - \bar{H}_{site})/H_{tot}$ and the between-region component of diversity by $G_{reg.tot}$ ($G_{reg.tot} = (H_{tot} - \bar{H}_{reg})/H_{tot}$).

The number of multilocus genotypes present in each site was used as a minimum estimate of the number of clones (genets) in the sites. The low numbers of different genotypes within each site meant that the 'expected' cell values were too low (< 1) to allow testing for significant differences between observed genotype frequencies and those expected under Hardy–Weinberg equilibrium within sites. Deviations from expected genotype frequencies at individual loci were tested for using a χ^2 test on the regional level (with site data pooled within regions and using the genotype-level dataset). When necessary, rare alleles were pooled to obtain expected cell values of 1 or more.

The pattern of between-site differentiation in allele frequencies was investigated in a cluster

analysis based on Rogers' genetic distances (nine polymorphic loci, 76 sites) and Ward's (1963) method of cluster analysis. Site S19 lacked data for the Skd-1 locus and was not included in the cluster analysis.

Most statistics were calculated using the program MULE written by Richard J. White.

RESULTS

Geographic variation in allozyme polymorphism and numbers of alleles

Within-site variation

Most sites showed variation (had more than one allele) at one to five loci (Appendix 1). However, 18% of the sites were monomorphic at all 13 loci. The monomorphic sites were found mainly in the two north-eastern regions (North Sea Mainland and Scandinavia/Baltic). Within these two regions, the nonvariable sites (20 of 44 sites) were scattered among the variable sites, apart from in the province of Skåne, in southern Sweden, where there was a concentration of monomorphic sites. In Skåne, seven of the eight sites were monomorphic at all allozyme loci (Appendix 1).

There were clear inter-regional differences in the number of polymorphic loci per site and in the percentage of variable sites, with the highest levels of variation being found in the two south-western regions (SW Iberian Peninsula and the Bay of Biscay) and in the British Isles. The median number of polymorphic loci per site was two in the two south-western regions and in the British Isles, compared with one in the two north-eastern regions (based on data from sites with ≥ eight samples; Table 1). The between-region differences in the median number of polymorphic loci per site were statistically significant ($P = 0.002$, Kruskal–Wallis ANOVA) and there was no significant difference in the within-site sample sizes between regions ($P = 0.155$, Kruskal–Wallis ANOVA). The median numbers of samples per site in the five regions (for sites with ≥ eight samples) were, from SW to NE, 15.5, 10, 10, 11 and 10. The percentage of variable sites was lowest in the Scandinavia/Baltic region (61%) and highest in the SW Iberian Peninsula and in the British Isles, where all sites with sample sizes of eight or more contained at least one locus with more than one allele (Table 1). The decline in variation along the SW–NE transect in Europe is also illustrated by the percentage of variable sites at the loci Tpi-2, Pgm-1, Ugp-2 and Aat-3 (Table 1).

Within-region variation

The regional percentages of polymorphic loci (P) and mean numbers of alleles per locus (A), both declined from SW to NE in Europe. When A and P were calculated after the exclusion of rare

Table 1 Allozyme variation in *Carex arenaria* L. within five European regions. Variation is measured as the median number of polymorphic loci (loci with more than one allele) per sampling site, the percentage of variable sampling sites (sites with more than one allele present at least at one locus) and as the percentage of variable sites for the loci Tpi-2, Pgm-1, Ugp-2 and Aat-3, respectively (percentages are not given for the other five loci that were variable only at one or two sites in Europe). The differences in the median number of polymorphic sites between regions are statistically significant (Kruskal–Wallis ANOVA, $P = 0.0023$). The number of polymorphic loci at each sampling site is given in Appendix 1

Region/total sample	No.† sites	Median no. polym. loci/site	Variable sites (%)	Tpi-2 (%)	Pgm-1 (%)	Ugp-2 (%)	Aat-3 (%)
SW Iberian Peninsula	8	2	100	82	50	38	62
Bay of Biscay	11	2	91	62	36	27	55
British Isles	8	2	100	88	38	50	12
North Sea Mainland	10	1	70	29	40	20	0
Scandinavia/Baltic	18	1	61	37	17	22	0
Total	55	1	82	64	33	29	22

† Sites with sample sizes of less than eight ramets are not included in the statistics.

Table 2 Allelic variation in *Carex arenaria* L. in Europe. P (percentage polymorphic loci) and A (mean number of alleles per locus) are calculated both on the basis of all alleles and on the basis of the 99% criterion (where a locus was regarded as monomorphic if the most common allele had a frequency of > 0.99 in the total material). H (gene diversity)† and H_O (observed heterozygosity) calculations are based on all alleles and are means over the 13 studied loci. The statistics are presented for each of the five geographic regions included in the study as well as for the total material. Sample sizes are given by NR for the ramet level calculations (where all sampled ramets are included) and by NG for the genotype-level calculations (based on the minimum number of genets in the sites, with the ramets with the same multilocus genotypes pooled and treated as a single sample at each site)

	99% criterion		Including rare alleles		Ramet-level			Genotype-level		
	P	A	P	A	H†	H_O	NR	H†	H_O	NG
SW Iberian Peninsula	31	1.38	38	1.46	0.090	0.094	132	0.093	0.086	34
Bay of Biscay	31	1.38	38	1.46	0.099	0.063	154	0.101	0.080	39
British Isles	31	1.31	38	1.38	0.055	0.038	80	0.075	0.058	29
North Sea Mainland	23	1.23	23	1.23	0.020	0.020	140	0.037	0.036	28
Scandinavia/Baltic	23	1.23	61	1.61	0.015	0.015	255	0.038	0.038	57
Mean over regions	28	1.32	44	1.46	0.050	0.042	152	0.066	0.058	37
Total material	31	1.46	69	2.08	0.056	0.042	761	0.070	0.058	187

† H_{reg}, within each of the five regions; $\bar{H}_{reg}$ for mean over regions; H_{tot} in the total material.

alleles (using the '99% criterion'), within-region values for P ranged from 23% to 31% and values for A from 1.23 to 1.38 (Table 2). However, if P and A were estimated on the basis of all alleles (including rare alleles) most variation was found in the Scandinavia/Baltic region in the NE ($P = 61\%$, A = 1.61, compared with $P = 38\%$, A = 1.46 in both the SW Iberian Peninsula and the Bay of Biscay regions; Table 2).

Ten alleles were rare (with genotype-level allele frequencies below 0.01 in the total material) and these rare alleles were always found in heterozygotes (together with the commonest allele at the particular locus). The highest number of rare alleles was found in the Scandinavia/Baltic region, that contained five rare alleles which were not found in the other regions. The other four regions, together, contained five other rare alleles.

At Idh-1, two rare alleles were present in two adjacent sites (on Öland) in the Scandinavia/Baltic region (site S13 where allele 3 was present in one sample and site S14 where allele 2 was present in three samples). The locus Pgd-1 had one rare allele in one sample from the Bay of Biscay (site F5). Two rare alleles were present at Pgi-2: allele 1f in one sample from the SW Iberian Peninsula region (site P5); and allele 2 in one sample from the Scandinavia/Baltic region (site ET). Skd-1 had two rare alleles: allele 1f in the British Isles (two samples in site U4); and allele 2 in the Scandinavia/Baltic region (one sample in site S14). Aat-2 also had a rare allele (allele 2) in one sample from the Scandinavia/Baltic region (site S13). At Aat-3, allele 2f was found in the SW Iberian Peninsula region (one sample in site P5) and allele 3 occurred in both the Bay of Biscay (all samples in site E7 and one sample at site F2) and in the British Isles (one sample in site U7).

Geographic variation in allele frequencies

Total variation and variation between sites

The most common allele at each of the nine polymorphic loci had a high frequency in the total European material (frequencies ranging from 0.780 for Tpi-2 to 0.997 for Pgd-1 and Aat-1; genotype-level data).

Even though local sites were often monomorphic for the commonest allele at each of the four most variable loci (Tpi-2, Pgm-1, Ugp-2 and Aat-3), the second most common allele at each of these loci was widespread within Europe. The mosaic pattern of variation in allele frequencies between

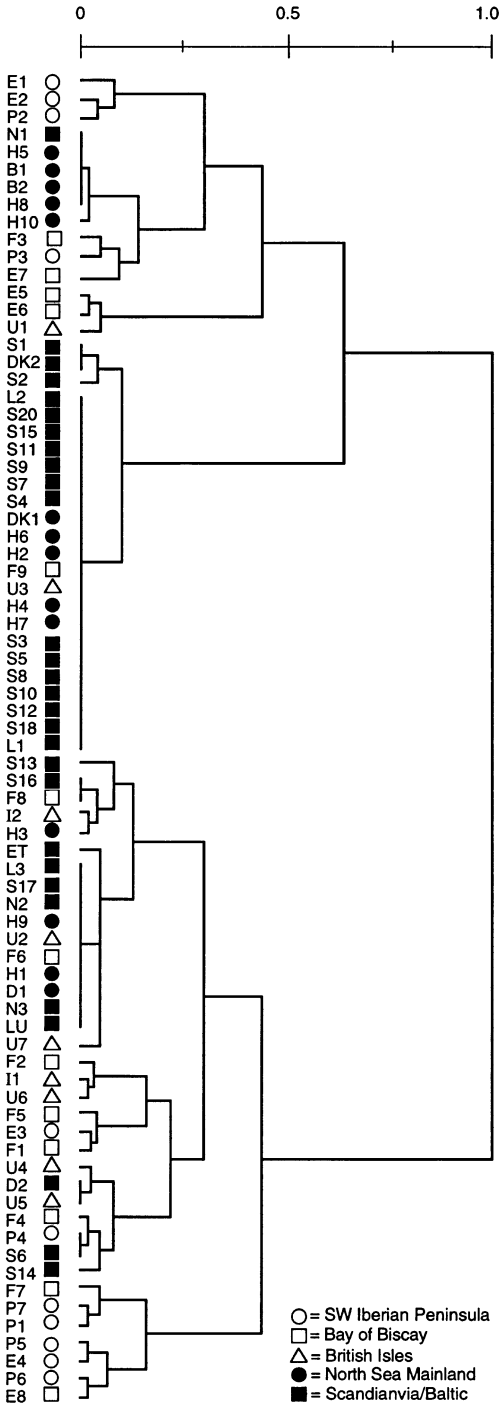

Fig. 2 Cluster analysis of 76 *Carex arenaria* L. sites (site codes in Appendix 1) belonging to five European regions. The dendrogram was produced using Ward's method of cluster analysis and Rogers' genetic distance, and is based on the allozyme frequencies (at nine polymorphic loci) calculated on the basis of the different multilocus genotypes (minimum number of genets) that were represented in each site. Scale = semipartial *R*-squared.

sites is reflected in the cluster analysis (Fig. 2). There is no clear geographical pattern in the clustering of sites. Sites from one region often cluster with sites from other regions and the two major branches in the dendrogram both contain sites from all five geographic regions.

Variation between regions

If the allele frequency data for individual loci are analysed on the regional level (with the site data pooled within regions), the decline in frequency of the less common alleles from SW to NE in Europe is seen more clearly than in the site-level data (Fig. 3). For three of the four most variable loci (Tpi-2, Pgm-1 and Aat-3), the highest within-region frequencies of the second most common allele were found in the SW Iberian Peninsula and in the Bay of Biscay. At the locus Ugp-2, however, the highest frequencies for the second most common allele were found in the Bay of Biscay and in the British Isles (Fig. 3).

Ramet-level and genotype-level gene diversity

The mean (over loci and sites) within-site gene diversity ($\bar{H}_{site}$) was 0.064 in the genotype-level calculations but considerably lower ($\bar{H}_{site} = 0.035$) in the ramet-level calculations. Within-site diversity (for both ramet- and genet-levels) generally decreased from SW to NE in Europe but showed, at the same time, large differences between geographically adjacent sites, both in the mean H_{site} values over all 13 loci, and for individual H_{site} values at each of the most variable loci (Tpi-2, Pgm-1, Ugp-2 and Aat-3).

The mean (over loci and regions) within-region gene diversity ($\bar{H}_{reg}$) was 0.066 in the genotype-level calculations and somewhat lower ($\bar{H}_{reg} = 0.050$) in the ramet-level calculations (Table 2). At each of the four most diverse loci (Tpi-2, Pgm-1, Ugp-2 and Aat-3 which, together,

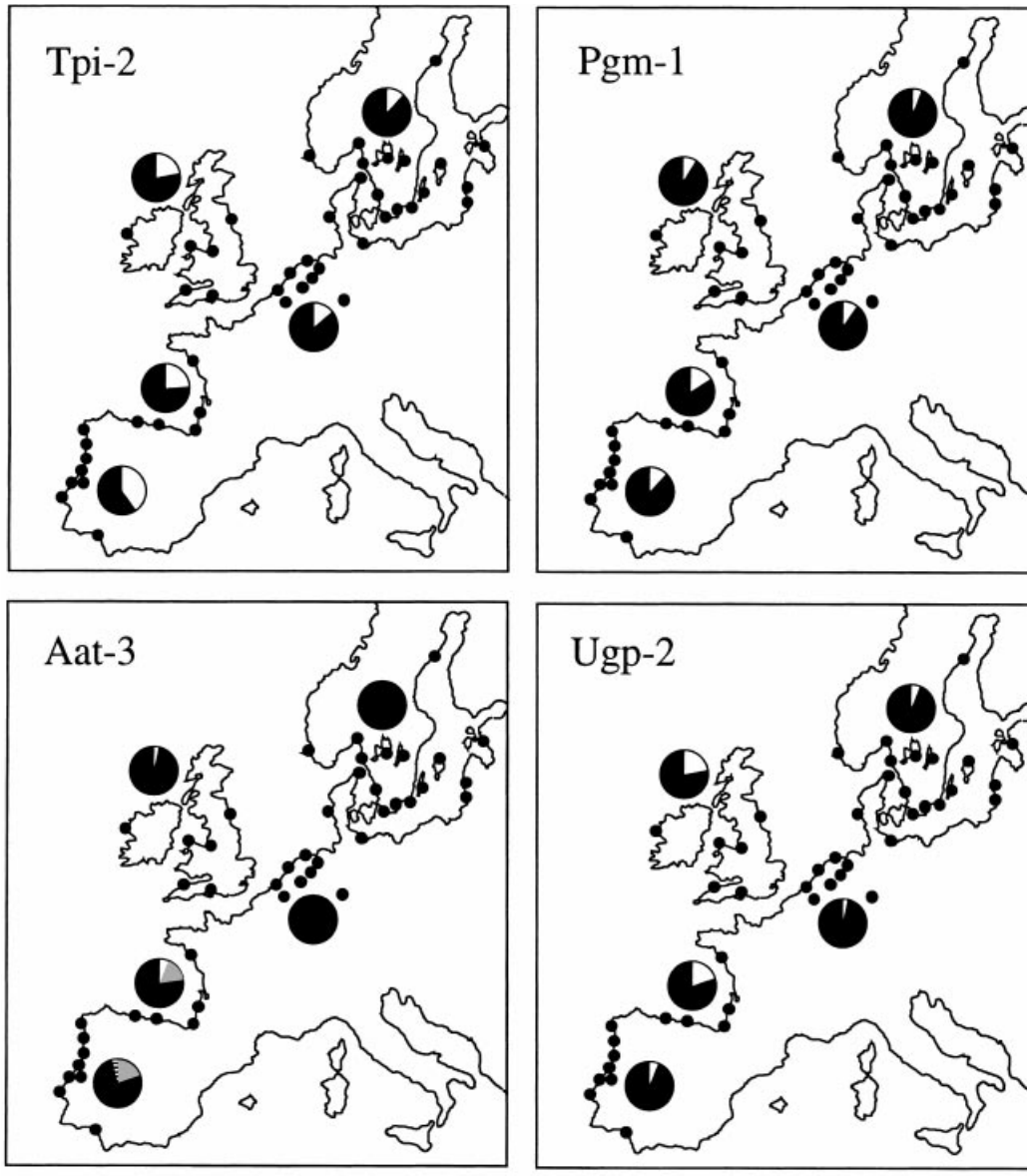

Fig. 3 Regional variation in allele frequencies at four polymorphic enzyme loci in European *Carex arenaria* L. The allele frequencies are calculated on the basis of the different multilocus genotypes (minimum number of genets) that were represented in each site. The five polymorphic loci that are not presented in the figure showed low levels of allelic variation (the commonest allele had a frequency higher than 0.99 in the total sample and within-region frequencies for the commonest allele ranged from 0.97 to 1.00). Tpi-2, Pgm-1, Ugp-2: allele 1 = black, allele 2 = white. Aat-3: allele 1 = grey, allele 2f = striped, allele 2 = black, allele 3 = white.

Table 3 Gene diversity in *Carex arenaria* L. within five geographic regions in Europe. Within-region diversity (H_{reg}) is shown for the four allozyme loci that showed the highest levels of diversity in the sampled material (and which together account for 94% and 96%, respectively, of the total gene diversity (H_{tot}) in the ramet-level and genotype-level estimates). Significance probabilities for the individual loci (*P*) refer to one-way analyses of variance. Sample sizes as in Table 2. Allele frequencies in the five regions are shown in Fig. 3

	SW Iberian Peninsula	Bay of Biscay	British Isles	North Sea Mainland	Scandinavia/ Baltic	*P*
Genotype-level H_{reg}						
Tpi-2	0.486	0.380	0.354	0.249	0.231	**
Pgm-1	0.233	0.270	0.160	0.195	0.090	NS
Ugp-2	0.086	0.305	0.354	0.036	0.068	***
Aat-3	0.372	0.330	0.035	0	0	***
Ramet-level H_{reg}						
Tpi-2	0.478	0.339	0.220	0.133	0.114	***
Pgm-1	0.231	0.296	0.181	0.121	0.027	***
Ugp-2	0.030	0.375	0.282	0.007	0.031	***
Aat-3	0.420	0.269	0.013	0	0	***

***$P < 0.001$, **$P < 0.01$, NS = non-significant.

accounted for 94% of the total gene diversity in the genotype-level calculations), there were large between-region differences in gene diversity (H_{reg}) and a general tendency for within-region diversity to decrease from SW to NE in Europe (Table 3). The differences in gene diversities between regions were statistically significant for Tpi-2, Ugp-2 and Aat-3 in both the ramet-level and genotype-level calculations (Table 3). In a two-way ANOVA including the four most diverse loci, H_{reg} differed significantly between regions and between loci. The region × locus interaction was also significant (both in ramet-level and genotype-level calculations; $P < 0.001$ in all cases). The Ugp-2 locus, in contrast to the other three loci, had a low H_{reg} value in the SW Iberian Peninsula region. The Aat-3 locus showed the strongest SW-NE decline in gene diversity (Table 3). The SW-NE decline in diversity is also reflected in the between-region differences in mean (over all loci) gene diversity (the highest diversity was found in the Bay of Biscay region and the lowest diversity in the Scandinavia/Baltic region; Table 2). Within-site variation accounted for 92.4% of the gene diversity in the total European material while the between-site and between-region components of diversity were 2.5% and 5.1%, respectively.

There were small differences between the mean (over loci) observed heterozygosity and the mean gene diversity (= 'expected heterozygosity' under Hardy–Weinberg equilibrium) within regions and in the total material (Table 2). Tests for within-region deviations from expected genotype frequencies under Hardy–Weinberg equilibrium at individual loci (genotype-level data) revealed eight cases where the smallest expected cell values were ≥ 1. Only one of these cases (Ugp-2 in the British Isles) showed a significant deviation from Hardy–Weinberg expectations.

Clonal structure and diversity within sites

The ability to discriminate between clones depends on the number of variable loci that are present within a site (Ellstrand & Roose, 1987; Widén *et al.*, 1994), or represented within a sampled ramet. The low levels of allozyme variation in *Carex arenaria*, with 18% of the study sites (cf. Table 1) and 35% of all samples being homozygous for the most common allele at all loci, meant that the information on clonal (genet) diversity within sites was limited. At many of the *C. arenaria* study sites, the number of observed multilocus genotypes is thus likely to be an under-estimate of the number of genets (cf. 'G' and 'pl', Appendix 1).

DISCUSSION

The present investigation of genetic variation in *Carex arenaria* in Europe covers the entire native range of the species and shows a successive reduction in regional levels of genetic variation (both at the genotype- and ramet-level) from the Iberian Peninsula and Bay of Biscay in the SW, northwards to the coastal areas of the Baltic Sea (Fig. 3; Tables 1, 2 and 3). The species shows levels of genetic variation that are unusually low for a rhizomatous sedge and the levels of variation in the northern sites and regions are more similar to those that are characteristically found in caespitose (usually inbreeding) sedges. However, the small between-region (5%) and between-site (3%) components of within-species gene diversity and the lack of significant deviations from Hardy–Weinberg equilibrium within regions suggest that *C. arenaria* is not predominantly self-fertilized. The low levels of genetic variation in *C. arenaria* compared with other rhizomatous sedges are likely to be a consequence of the species' history of refugial survival and postglacial spread rather than an indication of higher levels of inbreeding than in other rhizomatous sedges.

Genetic variation within regions declines northwards in Europe

Many European and North American plant and animal species overwintered the Pleistocene glaciations in refugia to the south of the ice sheets and recolonized the glaciated areas after the retreat of the ice (e.g. Soltis *et al.*, 1997). Pollen analytical data provide information on patterns of migration and reveal that, during the Holocene, tree species have recolonized central and northern Europe from one or more refugial areas to the south or east of the northern European ice sheets (Huntley & Birks, 1983; Huntley, 1990; Bennet *et al.*, 1991). *Carex arenaria* occupies areas in northern Europe (the Scandinavia/Baltic region, Fig. 1) that were under ice during the last glaciation (Andersen & Borns, 1994). Adjacent areas to the south of the ice sheets (the North Sea Mainland and British Isles regions; Fig. 1) had tundra-like habitats at the glacial maximum (c. 20 000–18 000 years BP) and may not have been available for colonization by *C. arenaria* until the early Holocene (cf. Noble, 1982).

The percentage of variable sites (sites with more than one allele at any locus) decreases from 100% in the SW Iberian Peninsula region to 61% in the Scandinavia/Baltic region (Table 1). The less common alleles (with frequencies between 0.01 and 0.25 in the total material) at polymorphic loci are widespread in Europe, but show a successive decline in frequency towards the NE (Fig. 3). The within-region gene diversity (H_{reg}) at each of the four most variable loci (which, together, account for 94% of the total gene diversity) also shows a general decline towards the NE in Europe (Table 3) as does the mean (over loci) within-region gene diversity (Table 2). The percentage of polymorphic loci (P) and the mean number of alleles per locus (A), based on the '99% criterion', decline towards the NE (Table 2).

The geographic pattern of allozyme variation in *C. arenaria* is consistent with a scenario of successive postglacial colonization of N Europe from a refugial area on the Atlantic coast and with the loss of genetic variation during migration northwards and eastwards. The wide distribution of the alleles at polymorphic loci among regions (the three loci which have the highest gene diversity in the total material, Tpi-1, Pgm-1 and Ugp-2, have all alleles present in all regions; Fig. 3) and the lack of abrupt geographical changes in allele presence/absence or allele frequencies, suggest an origin from a single refugium. At individual loci, the most common allele was the same in all regions (with regional frequencies ranging between 0.60 and 1.00). The source populations during postglacial colonization are also likely to have been dominated by these alleles.

The incidence of local *C. arenaria* sites that are fixed (with a few exceptions, for the commonest allele) at individual loci increases towards the NE in Europe. For example, at Tpi-2 (the locus with the highest level of gene diversity in the total material) only a few sites (18%) are fixed for one allele in the SW Iberian Peninsula region while most of the sites (63%) in the Scandinavia/Baltic region are fixed for the most common allele (Table 1). The patchy distribution of the less common alleles at individual loci within regions may be a consequence of

the low numbers (one to seven) of genets that could be discriminated within individual sites (Appendix 1). However, it is likely that founder effects during the colonization of new sites by a low number of genets, have also contributed to the local variation in allele presence/absence. The regional data (Fig. 3; Tables 1, 2 and 3) show a clearer pattern of clinal variation in Europe than do the site data (Fig. 2).

Rare alleles in the Scandinavia/Baltic region

A higher number of rare alleles (five) was present in the Scandinavia/Baltic region than in any other region and the Scandinavia/Baltic region consequently showed higher values for P and A — in the calculations including the rare alleles — than other regions (Table 2). Three of the five rare alleles in this region were present in two adjacent sites on Öland, where the closely related species *Carex ligerica* Gay. grows in mixed populations with *C. arenaria*. *Carex ligerica* was not present at any of the other investigated sites. Previous studies including common garden experiments (Urbaniak, 1994 and references therein) have suggested that *C. ligerica* and *C. arenaria* are morphologically distinct. Our present results suggest that there might be introgression between *C. arenaria* and *C. ligerica* (cf. Runyeon & Prentice, 1996), but electrophoretic investigation of *C. ligerica* is needed to confirm this suggestion.

Carex arenaria shows low overall levels of genetic variation compared to other rhizomatous sedges

The fact that most of the total gene diversity (H_{tot}) in *Carex arenaria* is explained by within-site variation, and the fact that there is little deviation from Hardy–Weinberg equilibrium within regions, suggest that the species has low levels of self-fertilization. However, the levels of gene diversity in *C. arenaria* (especially in the northern regions of Europe) are low for an out-crossing, rhizomatous sedge and are more similar to those that are typical for caespitose, inbreeding, sedges. Within-species diversity (mean over loci H_{tot}) was 0.070, the mean within-region diversity (mean over loci $\bar{H}_{reg}$) was 0.066 and the mean within-site diversity (mean over loci $\bar{H}_{site}$) was 0.064 in *C. arenaria* (genotype-level data; figures for the ramet-level data are considerably lower; cf. Table 2). A review of allozyme data within the Cyperaceae shows that rhizomatous sedges are generally outbreeding (having populations with low fixation indices) and have high levels of allelic polymorphism and gene diversity ($\bar{H}_{site} = 0.167$, $N = 13$ species). In contrast, caespitose sedges generally show high levels of inbreeding (having populations with high fixation indices) and low levels of alleleic polymorphism and gene diversity ($\bar{H}_{site} = 0.059$, $N = 26$ species) (B.O. Jonsson, M. Hedrén, A. Nilsson & P. Stenroth, in prep.). The highest level of diversity in *C. arenaria* was found in the Bay of Biscay region (mean over loci $H_{reg} = 0.10$). The low levels of diversity in *C. arenaria*, compared to those that are generally found in rhizomatous sedges suggests that *C. arenaria* may have lost genetic variation as a result of genetic bottlenecks during episodes of small population size — at some stage during its postglacial or earlier history (cf. Barrett & Kohn, 1991; Hewitt, 1996). The other Eurasian and American rhizomatous sedges that have been surveyed for allozyme variation, and which show much higher levels of allelic polymorphism and gene diversity than *C. arenaria*, may not have had particularly restricted distributions during the glacial periods. The majority of these species are characteristic of wetlands or tundra habitats (e.g. Ford *et al.*, 1991; McClintock & Waterway, 1993; Jonsson *et al.*, 1996) and are likely to have been relatively abundant in areas south of the ice-sheets during the glacial periods.

Clonal diversity, dispersal and establishment

Carex arenaria may colonize new areas either by seedling establishment or by the establishment of vegetative propagules. Observations of *C. arenaria* on newly created sandbanks show that the species has a substantial ability to colonize by seed or by detached rhizome fragments. Site S6 (S Sweden), was located on a 9-year-old sand bank, 150 m from the nearest source population, and contained dense stands of *C. arenaria* that belonged to at least three genets (Appendix 1).

The *C. arenaria* sites in the present study were generally found to be multiclonal, but the results suggest that there are some monoclonal

sites at the southern margin of the species' distributional range (Appendix 1). The two southernmost sites (E1, E2), which probably contain only clone each, have a Mediterranean-type climate with summer drought. The populations at these sites may have been formed by single genets and it is possible that there is no seedling recruitment in these sites at present. *Carex arenaria* seedlings are sensitive to drought (Tidmarsh, 1939) and are likely to have a lower chance of survival in these sites than further to the north in Europe.

In the British Isles, *C. arenaria* rhizomes are capable of growing up to 4 m during a year (Tidmarsh, 1939). *Carex arenaria* clones would thus have been able to spread at least 80 km (in one direction) by vegetative growth since the last glacial maximum 20 000 years ago. Longer-distance clone dispersal by sea is suggested by observations of rhizome fragments that have been washed ashore on beaches (B.O. Jonsson & H.C. Prentice, personal observations). The tolerance of rhizomes to salt water is not known but, in a growth experiment, seedlings showed low rates of survival at NaCl concentrations above 0.7% (Tidmarsh, 1939). If rhizomes are unable to survive prolonged periods with high NaCl concentrations, clone-dispersal by sea may not be of importance outside the Baltic Basin.

The buoyant *C. arenaria* seeds are dispersed on the surface of water in flooded dune slacks (Noble, 1982) but are also likely to be transported long distances by rivers and the sea. Seeds can germinate in NaCl concentration of up to 1.5% (Tidmarsh, 1939), but we know of no studies that have experimentally tested the germinability of seeds that have been exposed to North Sea salt water concentrations. Birds may contribute to the long-distance dispersal of *C. arenaria* seed in Europe. Germinable *C. arenaria* seeds have been extracted from pheasant and partridge droppings and pigeons have been observed feeding in fruiting *C. arenaria* stands (Tidmarsh, 1939). Several duck species (e.g. *Anas acuta*, *A. crecca* and *A. penelope*) overwinter at present in large numbers along the costs of W Europe and migrate in the spring towards their breeding grounds in the NE of Europe (Cramp, 1977). These ducks may transport *C. arenaria* seeds which were swallowed during feeding on the surface of the water. Noble (1982) suggests that rabbits, cattle, horses and humans are likely to be the most important animal vectors of *C. arenaria*.

Accidental or deliberate introductions by humans may have affected the geographical structure of genetic variation in *C. arenaria*. The species has been found in ships' ballast (Mackenzie, 1931) and *C. arenaria* has been planted recently as a sand-stabilizer along the German coast (P. Janiesch, personal communication 1997). In the 18th century, over-grazing led to severe erosion and the development of extensive areas of mobile sands in Skåne (southernmost Sweden). In 1721, farmers in eastern Skåne were ordered by the local governor to plant *C. arenaria* as a sand-stabilizer. Farmers that ignored the order were liable to prosecution (Lindal, 1947). The artificial propagation and spread of *C. arenaria* may explain the high proportion of sites in Skåne that are fixed at all 13 investigated allozyme loci (Appendix 1).

The recruitment of new ramets within established *C. arenaria* populations occurs mainly by clonal propagation (Noble *et al.*, 1979). Seedlings are rarely observed in natural populations and seedling survival is low and may only occur under favourable conditions in moister depressions (Tidmarsh, 1939; Noble, 1982). However, the presence of the same alleles in many different genets in *C. arenaria* suggests that, as in other rhizomatous sedges (e.g. Ford *et al.*, 1991; McClintock & Waterway, 1993; Jonsson *et al.*, 1996), sexual recruitment — and thus seedling establishment — is important for explaining the structure of genetic variation in the species. Viable seeds are produced in large quantities in *C. arenaria* populations each year (Tidmarsh, 1939). Multiclonal populations are common in clonal plant species, even in species, such as *C. arenaria*, with little or no observed seedling recruitment (Ellstrand & Roose, 1987; Widén *et al.*, 1994). Even a low rate of sexual recruitment may result in multiclonal populations and high levels of clonal diversity (cf. Eriksson, 1993; Jonsson, 1995).

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Appendix 1 *Carex arenaria*: sampling sites (cf. Figure 1), sample sizes and within-site genetic variation. R = the number of sampled ramets, *G* = the number of identified multilocus genotypes and pl = the number of polymorphic loci. Further details of geographical locations, population extent, ramet density and habitat are available from the first author on request

SW Iberian Peninsula (11 sites)

E1 6 km SE Mazagon, R = 24 *G* = 1 pl = 2; **E2** 2 km SE Mazagon, R = 2 *G* = 1 pl = 2; **P1** Torres Vedras (Sta. Cruz), R = 15 *G* = 2 pl = 2; **P2** 2 km S Lago de Eruedoira, R = 10 *G* = 3 pl = 2; **P3** Lago de Eruedoira, R = 6 *G* = 3 pl = 2; **P4** Laguna dos tres bracos, R = 16 *G* = 3 pl = 2; **P5** 3 km SSE Furadouru, R = 15 *G* = 7 pl = 4; **P6** 3 km S Furadouru, R = 9 *G* = 3 pl = 2; **P7** Esposende, R = 16 *G* = 3 pl = 3; **E3** Raxo, R = 18 *G* = 5 pl = 4; **E4** S Muros R = 7 *G* = 3 pl = 3

Bay of Biscay (13 sites)

E5 La Arena, R = 5 *G* = 3 pl = 2; **E6** Xago, R = 25 *G* = 2 pl = 2; **E7** Barro, R = 9 *G* = 1 pl = 2; **E8** 4 km NV San Vicente, R = 15 *G* = 4 pl = 2; **F1** Ondres Plage, R = 20 *G* = 6 pl = 4; **F2** Labenne Ocean, R = 10 *G* = 4 pl = 3; **F3** Plage des Casernes, R = 10 *G* = 2 pl = 1; **F4** Bicarose Plage, R = 10 *G* = 2 pl = 2; **F5** Dune du Pilat, R = 6 *G* = 5 pl = 5; **F6** La Govelle, R = 9 *G* = 2 pl = 1; **F7** Plage Valentin, R = 10 *G* = 4 pl = 2; **F8** Pen-bron, R = 18 *G* = 3 pl = 1; **F9** Merquel, R = 10 *G* = 1 pl = 0

British Isles (9 sites)

I1 3 km NW Ballyconneely, R = 10 *G* = 4 pl = 2; **I2** 4 km WNW Ballyconneely, R = 10 *G* = 4 pl = 2; **U1** Hurn Airport, R = 8 *G* = 1 pl = 1; **U2** Saunton Sands, R = 9 *G* = 2 pl = 1; **U3** Anglesey, R = 1 *G* = 1 pl = 0; **U4** Anglesey, R = 12 *G* = 7 pl = 3; **U5** West Kirby, R = 10 *G* = 5 pl = 3; **U6** Bamburgh, R = 11 *G* = 3 pl = 2; **U7** Goswick, R = 9 *G* = 2 pl = 2

North Sea Mainland (14 sites)

B1 Kalmthout, R = 18 *G* = 2 pl = 1; **B2** Kalmthout, R = 6 *G* = 2 pl = 1; **H1** Oostvoorne, R = 19 *G* = 2 pl = 1; **H2** Vogelzang, R = 3 *G* = 1 pl = 0; **H3** Castricum aan Zee, R = 17 *G* = 6 pl = 3; **H4** Apeldoorn, R = 15 *G* = 1 pl = 0; **H5** Ommen, R = 10 *G* = 2 pl = 1; **H6** Gasteren, R = 10 *G* = 1 pl = 0; **H7** Gasteren, R = 2 *G* = 1 pl = 0; **H8** Schiermonnikoog, R = 5 *G* = 2 pl = 1; **H9** Schiermonnikoog, R = 10 *G* = 2 pl = 1; **H10** Schiermonnikoog, R = 12 *G* = 3 pl = 2; **D1** Augustdorfer, R = 10 *G* = 2 pl = 1; **DK1** Nymindegap, R = 8 *G* = 1 pl = 0

Scandinavia/Baltic (30 sites)

DK2 Skagen, R = 8 *G* = 2 pl = 1; **N1** Ölberghamna (Sola), R = 3 *G* = 2 pl = 1; **N2** Solasanden (Sola), R = 5 *G* = 2 pl = 1; **N3** Åven, R = 16 *G* = 2 pl = 1; **S1** 2 km E Fiskebäckskil, R = 21 *G* = 2 pl = 1; **S2** Tönnersta ('Laxvik'), R = 10 *G* = 5 pl = 4; **S3** Järavallen, R = 6 *G* = 1 pl = 0; **S4** Habo camping, R = 6 *G* = 1 pl = 0; **S5** 2 km N Lomma, R = 4 *G* = 1 pl = 0; **S6** Falsterbo, Nabben, R = 19 *G* = 3 pl = 2; **S7** Everöd, R = 10 *G* = 1 pl = 0; **S8** Lyngsjö, R = 10 *G* = 1 pl = 0; **S9** Juleboda (S Åhus), R = 10 *G* = 1 pl = 0; **S10** 2 km S Åhus, R = 8 *G* = 1 pl = 0; **S11** Utlängan, R = 10 *G* = 1 pl = 0; **S12** Sandby Borg, R = 7 *G* = 1 pl = 0; **S13** Sandby, R = 4 *G* = 3 pl = 4; **S14** Gårdby, R = 12 *G* = 7 pl = 5; **S15** Haga Park, R = 6 *G* = 1 pl = 0; **S16** Irevik (Hangvar), R = 10 *G* = 3 pl = 1; **S17** Nässja, R = 12 *G* = 2 pl = 1; **S18** Källby badplats, R = 2 *G* = 1 pl = 0; **S19** Hattarevik (Torsö), R = 15 *G* = 1 pl = 0; **S20** Obbola, R = 9 *G* = 1 pl = 0; **D2** Timmerdorfer Strand, R = 10 *G* = 3 pl = 3; **LU** 1 km V Juodkranté, R = 10 *G* = 2 pl = 1; **L1** 1.5 km S Pape, R = 5 *G* = 1 pl = 0; **L2** Pape (Rucava), R = 15 *G* = 1 pl = 0; **L3** 1 km N Pape, R = 5 *G* = 2 pl = 1; **ET** S of Pärnu, 3 km N Ikla, R = 11 *G* = 3 pl = 3.