A preliminary investigation of genetic variation in Western European *Carex depauperata* Curtis ex With. (Cyperaceae), Starved Wood-sedge

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ABSTRACT

AFLP genetic fingerprinting studies of seven samples of *Carex depauperata* from England, Ireland, France and Spain were undertaken to investigate the level of genetic variation. There was a high degree of similarity between five of the samples (two English, two French and one Spanish). The other two samples (from Chateau Bellefontaine, France and Cork, Ireland) were relative outliers, and the Irish sample was the most distinct. Numbers of bands scored per individual were more or less uniform, and these differences thus appear to reflect real genetic differences rather than artifacts due to DNA quality.

KEYWORDS: AFLP, Conservation.

INTRODUCTION

Carex depauperata Curtis ex With. is a very rare plant in Britain. It is listed as Critically Endangered in Britain and is on Schedule 8 of the Wildlife and Countryside Act 1981. It is also regarded as endangered in north-western Europe. It has been the subject of conservation work at Cambridge Botanic Garden, in Plantlife's 'Back from the brink' project, and in English Nature's Species Recovery programme. Rich & Birkinshaw (2001) have summarised its current status and conservation in Britain.

In Britain there are two extant populations, each of which are likely to have been derived from a single genotype. In the 1960s the site near Cheddar consisted of a single plant, but through transplants and habitat management the population has now increased to more than 50 plants. The last of the three Godalming sites became 'extinct' in c. 1972 due to a landslide, but one plant was rediscovered in 1992 after increased light levels resulting from the Great Storm of 1987 stimulated buried seed to germinate. This population has now increased to four plants, again following conservation work. It was discovered in its sole site in County Cork, Ireland by T. O'Mahony in 1973; only two tussocks were present in 2000 (O'Mahony 2001).

Rich & Birkinshaw (2001) noted that, despite being widespread in Europe and Asia with many scattered, disjunct localities, it showed little morphological variation. In cultivation four different genotypes (two from England, one from France and one from Ireland) showed no significant morphological differences (Rich & Birkinshaw 2001). However, subsequently there has been some indication that the Irish plants have a significantly shorter life-span in cultivation and in the wild, indicating that there may be some genetic variation (T. O'Mahony, pers. comm. 2001). As four collections were available in cultivation (which were supplemented with three additional wild-collected samples), the opportunity was taken to undertake a preliminary investigation into the genetic variation between sites in western Europe.

The studies were carried out using amplified fragment length polymorphism (AFLP™) fingerprinting, a highly reproducible method of obtaining genetic fingerprints from small amounts of DNA consisting of fragments of different sizes (numbers of base pairs) which are then visualised as bands on a gel (Vos *et al.* 1995). This technique has proved effective with other *Carex* species (*C. muricata* L.: Fay *et al.* 2001; *C. otrubae* Podp. and *C. vulpina* L.: Cowan *et al.* 2002) and with other members of Cyperaceae (*Schoenoplectus* spp.: Fay *et al.* 2003; Fay & Cowan 2001).

MATERIALS AND METHODS

Shoots from one individual from each of seven separate localities (Table 1) were collected directly into desiccant silica gel in 2001, using the method of Chase & Hills (1991). The exact locality of the plant from southern central France (Cdep 3) was not recollected (P. Harmes, pers. comm. 2001). Vouchers are held in the National Museums and Galleries of Wales (**NMW**).

DNA was extracted from approximately 0·2 g of dried material using a modified 2×CTAB (cetyltrimethyl-ammonium bromide) procedure (Doyle & Doyle 1987), purified and quantified using a spectrophotometer.

AFLP fingerprints were performed according to the AFLP Plant Mapping Protocol of Applied Biosystems Inc. For each specimen, 0·5 µg of DNA was digested using the restriction enzymes MseI and EcoRI. The primer combinations selected for use with *C. muricata* (Fay *et al.* 2001) were used for this study. Amplification reactions were separated on a 5·0% polyacrylamide gel using an ABI 377 automated sequencer. Genescan 2.1 and Genotyper 2.0 were used to analyse bands. Data presented here are for the primer combination EcoRI-AC and MseI-CAC.

Bands were scored as either present (1) or absent (0) for all individuals and exported from Genotyper as a binary matrix which was analyzed using the UPGMA (Unweighted Pair-Group Method using Arithmetic Averages) algorithm in the software package PAUP version 4.,0 for Macintosh (Swofford 1998) and by principle coordinates analysis (PCOA) in the R Package for Multivariate Analysis version 4.0 (Casgrain & Legendre 2001) using Jaccard's coefficient (Jaccard 1908).

TABLE 1. DETAILS OF *CAREX DEPAUPERATA* MATERIAL

Accession	Locality	NMW voucher.	No. of DNA bands on gel
Cdep 1	Chantilly, Oise, France	V.2001.025.1203	92
Cdep 2	Cheddar, U.K.	V.1999.045.33	93
Cdep 3	S. C. France	–	92
Cdep 4	La Hermida, Spain	V.2001.033.006	93
Cdep 5	Godalming, U.K.	V.1999.045.29	91
Cdep 6	Cork, Ireland	V.1999.045.30	90
Cdep 7	Chateau Bellefontaine, France	V.2001.033.1202	87

RESULTS

In total, 102 bands were scored, of which 24 (23·5%) were variable. Band number was more or less uniform, ranging from 87 in sample 7 to 93 in samples 2 and 4 (Table 1).

The UPGMA dendrogram is shown in Figure 1, in which the plants tested fell into one relatively tight cluster of five closely related individuals (two from England, two from France and one from Spain). The sample from Chateau Bellefontaine (Cdep 7) fell outside this cluster, and the sample from Cork (Cdep 6) was the most distinct.

In the PCOA, the first two coordinates accounted for 79·8% and 15·4% respectively of the variation. The plot of the first two coordinates against each other is shown in Figure 2. In this analysis, the same groupings were identified, i.e. a cluster of five individuals and two outliers.

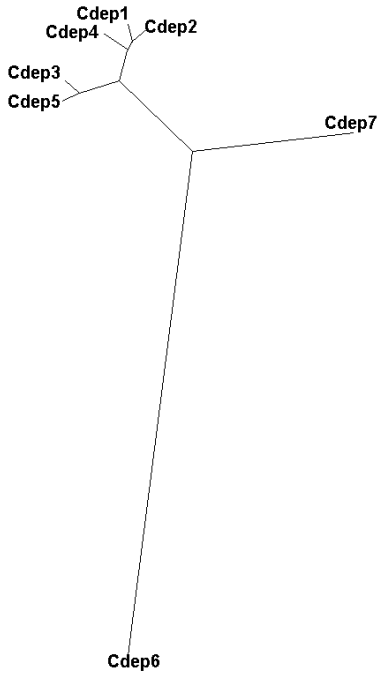

FIGURE 1. UPGMA dendrogram showing relationships of *Carex depauperata* samples (see Table 1 for sources).

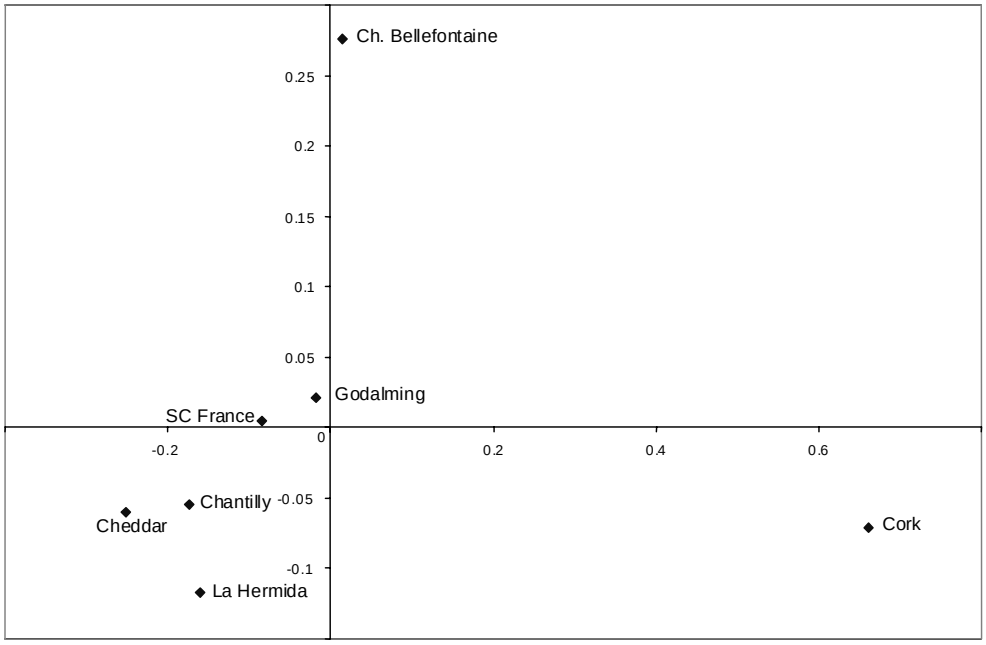

FIGURE 2. Plot of the first and second axes from the principle coordinates analysis of *Carex depauperata* samples.

DISCUSSION

The level of variation reported here appears to be relatively low, compared to other *Carex* species. For example, in a study of British populations of *C. muricata* L. subsp. *muricata* (Fay *et al*. 2001), 84 bands were scored, of which 55 (65%) were variable, although this may be partly a reflection of the larger number of plants included in that study. At this level of sampling there does not appear to be a clear geographical pattern in *C. depauperata*, and larger numbers of continental samples would be necessary to clarify the geographical origin and relationships of the English and Irish plants.

The distinctness of the Irish sample (and to a lesser extent, the Chateau Bellefontaine sample) is unexpected, given the genetic homogeneity shown by the cluster of five samples. This is in sharp contrast to *Schoenoplectus triqueter* (L.) Palla, where Irish material was genetically closely related to English material (Fay *et al*. 2003; Fay & Cowan 2001). When one sample falls in an isolated position, this can be for various reasons, including:

1. Degraded DNA (recognisable by a lower number of bands and by examination of the DNA on an agarose gel),
2. Hybridization (recognisable by a higher number of bands than expected), and
3. Misidentification of material (related species often share some bands).

Degraded DNA and hybridization can be discounted on the basis of the number of bands, which for the two outlying samples is close to that seen in the other five samples. The high level of overall genetic similarity also indicates that misidentifications are unlikely.

These results thus appear to reflect real genetic differences and indicate that the plants sampled are genetically distinct. Five of the samples are closely related, but the samples from Chateau Bellefontaine, France and Cork, Ireland were relative outliers. The Irish plants have a shorter lifespan, also indicating a genetic basis for the differences; the Chateau Bellefontaine material has not been cultivated.

We would welcome further live samples of *Carex depauperata* from different parts of its range to extend the studies.

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