

## Clonal diversity in two recently discovered English populations of *Carex vaginata* Tausch (Cyperaceae)

G. C. FRENCH, P. M. HOLLINGSWORTH\*

Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh, EH3 5LR

R. W. M. CORNER

Hawthorn Hill, 36 Wordsworth Street, Penrith, Cumbria, CA11 7QZ

F. J. ROBERTS

Eden Croft, 2 Wetheral Pasture, Carlisle, Cumbria, CA4 8HU

and

I. TAYLOR

English Nature, Juniper House, Murley Moss, Oxonholme Road, Kendal, Cumbria, LA9 7RL

### ABSTRACT

Two populations of *Carex vaginata* have recently been discovered in the northern Pennines representing the first records of this species for England. We have used Random Amplified Polymorphic DNA (RAPD) fingerprinting analyses to investigate the population structure of *Carex vaginata* in its English localities and have also compared these findings with two populations from the Southern Uplands of Scotland. Extensive clonal mats (up to 6 m in length) were detected, although in some cases, individual patches were made up of a small number of spatially structured different clones. The presence of extensive clonal mats within the English populations suggests that the plants must have been present at these sites for a considerable period of time. In addition, no genotype was found in more than one local patch within a population. This restriction of individual genotypes to single patches, and the presence of different genotypes in different patches, suggests that sexual reproduction and seed dispersal, rather than vegetative dispersal, has been the primary mechanism for the formation of new patches.

KEYWORDS: British flora, sedge, grazing, clonal growth

### INTRODUCTION

*Carex vaginata* Tausch (Cyperaceae), Sheathed sedge, is a circumboreal arctic-alpine (Hultén & Fries 1986) with a locally abundant British distribution (Preston *et al.* 2002). Within Britain, this perennial rhizomatous sedge is a characteristic constituent of flushed montane grassland of the Breadalbane and Cairngorm mountains of Scotland (Jermy *et al.* 1982), with the most southern British populations located in the Southern Uplands of Scotland. These sites are located in the Moffat Hills, Dumfriess. (v.c. 72, Ratcliffe 1959); the upper Ettrick valley, Selkirks. (v.c. 79); and the Tweedsmuir Hills, Peebles. (v.c. 78, Corner 1981). In 2002, however, two further sites were discovered which extended the UK range of the species southwards to England (Dufton Fell, Westmorland, v.c. 69; and Green Fell, Cumberland, v.c. 70) (Corner 2004). The English sites are approximately 85 km south of the most southern Scottish site (Ettrick valley).

Email: p.hollingsworth@rbge.org.uk

One of the English sites (Dufton Fell) was surveyed as part of the Flora of Cumbria project in 1991 but during these surveys, *C. vaginata* was not detected (Corner 2004). The failure to detect this species was perhaps attributable to the generally shy flowering nature of *C. vaginata* (Jermy *et al.* 1982), and the heavy sheep grazing in the northern Pennines (Robinson 2003). In 2001, however, the foot and mouth outbreak led to extensive sheep culls, and grazing was effectively eliminated over two successive summers (Robinson 2003). This decline in sheep grazing led to the "blooming" of Cross Fell (Roberts 2003), and extensive flowering of *Alopecurus borealis* at Green Fell (Robinson, 2003) and *C. bigelowii* at Dufton Fell (Corner, 2004). It was following this reduction in grazing that the two new *C. vaginata* sites in England were discovered and up to 50 flowering spikes were observed in one patch on Dufton Fell in 2002 (F.J.R. pers obs.).

To contribute towards ongoing research on the effects of sheep grazing on upland floras (English Nature, 1993; English Nature 2001), we have examined the population structure of *C. vaginata* at the two new English sites. Specifically we wished to assess the levels of clonal diversity. Although there are few other published studies on rhizomatous sedges, those that have been undertaken have shown high levels of genotypic diversity, indicating an important role of sexual reproduction in perpetuation and dispersal, despite a lack of field observations of seedling recruitment (McClintock & Waterway 1993; Jonsson *et al.* 1996).

Plants of *C. vaginata* show a clumped distribution within populations in the Pennines, and occur in a series of discrete patches. It is, however, uncertain as to whether these patches consist of single clones, or instead consist of multiple sexually derived individuals. Field observations of flowering material have not detected any viable seed, leading Corner (2004) to suggest that the plants could be derived from single clones with inbreeding taking place. Corner (2004) also noted that the Pennine plants generally looked less vigorous than their counterparts from the Southern Uplands, but stressed that this may simply be attributable to their recent release from heavy grazing, rather than being attributable to a genetically determined lower level of fitness.

In the current paper we have used Random Amplified Polymorphic DNA (RAPDs; Williams *et al.*, 1990) to assess patterns of clonal diversity in *C. vaginata*. Our goals have been to establish the extent to which the English populations consist of single or multiple clones, and how their population structure compares with the Southern Upland sites. RAPDs have been used to assess patterns of clonal diversity in several other plant species, and represent a cost-effective and efficient tool for assessing the distribution of different genotypes (Esselman *et al.* 1999; Kreher *et al.* 2000; Hollingsworth & Bailey 2000; Persson & Gustavsson 2001).

#### MATERIALS AND METHODS

##### PLANT MATERIAL

The two English localities of *Carex vaginata* were visited by F.J.R., R.W.M.C. and M. S. Porter in August 2003. Within each locality, leaf material was collected at approximately 0.5–1 m intervals, along rough transects in all known patches. Samples were placed in silica gel for long-term storage. In total, five patches were sampled at Dufton Fell and three patches at Green Fell, including a new patch discovered during this field work by Linda Robinson. The number of samples collected ranged from 3–10 per patch (Table 1). Despite dense growth of *C. vaginata*, with thousands of vegetative shoots within each patch, only one inflorescence was noted from either population. Therefore, identification of each sample was performed in the field, by R.W.M.C., using vegetative characters only.

To compare population structure in the English populations with Scottish populations, samples from two populations of *C. vaginata* were collected from the Etrick valley, southern Scotland, by R.W.M.C. in August 2003. A similar sampling strategy was performed for each population, with a total of nine patches sampled at Bught Hill and three patches at White Shank. The number of samples collected ranged from 1–4 per patch, with sample numbers reflecting patch size (Table 1). No inflorescences were noted in either population so identification was again based on vegetative characters.

TABLE 1: LOCATION OF TWO ENGLISH AND TWO SCOTTISH POPULATIONS OF *CAREX VAGINATA*, WITH THE GRID REFERENCE AND ALTITUDE OF EACH PATCH RECORDED USING A GPS WITH ACCURACY OF 5 M. THE APPROXIMATE SIZE OF EACH PATCH AND NUMBER OF SAMPLES FROM EACH PATCH IS ALSO GIVEN

Location	Vice County	Grid Reference	Altitude (m)	Approx. patch size (m)	Number of samples
ENGLAND					
Dufton Fell	Westmorland (v.c. 69)				
Patch A		NY7442029892	727	10 × 7	4
Patch B		NY7528329848	723	10 × 4	7
Patch C		NY7531529838	721	3 × 3	10
Patch D		NY7532929856	723	15 × 4	6
Patch E		NY7534729883	723	8 × 10	5
Total					32
Green Fell	Cumberland (v.c. 70)				
Patch A		NY6678836480	700	3 × 4	3
Patch B		NY6668236334	700	6 × 4	6
Patch C		NY6667335999	710	8 × 4	7
Total					16
SCOTLAND					
Bught Hill	Selkirkshire (v.c. 79)				
Patch A		NT1766910845	520	3 × 2	2
Patch B		NT1771510951	490	1 × 1	1
Patch C		NT1774010827	520	3 × 2	2
Patch D		NT1776710813	520	5 × 3	4
Patch E		NT1807110738	520	3 × 2	2
Patch F		NT1811910720	520	4 × 2	3
Patch G		NT1814310710	520	5 × 3	4
Patch H		NT1819810666	520	5 × 3	4
Patch I		NT1822710653	520	3 × 2	2
Total					24
White Shank	Selkirkshire (v.c. 79)				
Patch A		NT1705508523	530	3 × 2	2
Patch B		NT1711808523	510	3 × 2	2
Patch C		NT1724508393	530	3 × 2	2
Total					6

#### DNA EXTRACTION AND RAPD ANALYSIS

DNA was extracted from approximately 25 mg of dried leaf material, using a modified 2xCTAB (cetyltrimethyl-ammonium bromide) method (Doyle & Doyle 1987). The quality and quantity of the extracted DNA was assessed on 1.0% agarose (Bioline) gels. DNA was diluted 1:20 with autoclaved deionised water, prior to RAPD analysis, performed using the protocol of Hollingsworth *et al.* (1998), with the addition of 2% formamide (Amresco). The resultant DNA fragments were separated on 1.6% agarose gels, stained with ethidium bromide and visualised under UV light. One individual from each population was initially screened with 34 decamer primers. Of these, five primers (OPG1, OPG5, OPG6, OPC6 and OPP13 (MWG-Biotech)) yielding polymorphic and reproducible bands were selected to screen all 78 individuals. To reduce PCR amplification artifacts, for each primer, all individuals were amplified during one PCR run on the same PTC-200 Peltier Thermal Cycler (MJ Research). In addition, DNA from one individual was amplified twice within each PCR run. A subset of the samples was subjected to repeat DNA extractions and repeat PCRs to check that the fragment profiles were reproducible. Amplification across all five primers was successful in 76 out of the 78 individuals. Two individuals that showed poor amplification were excluded from further analyses.

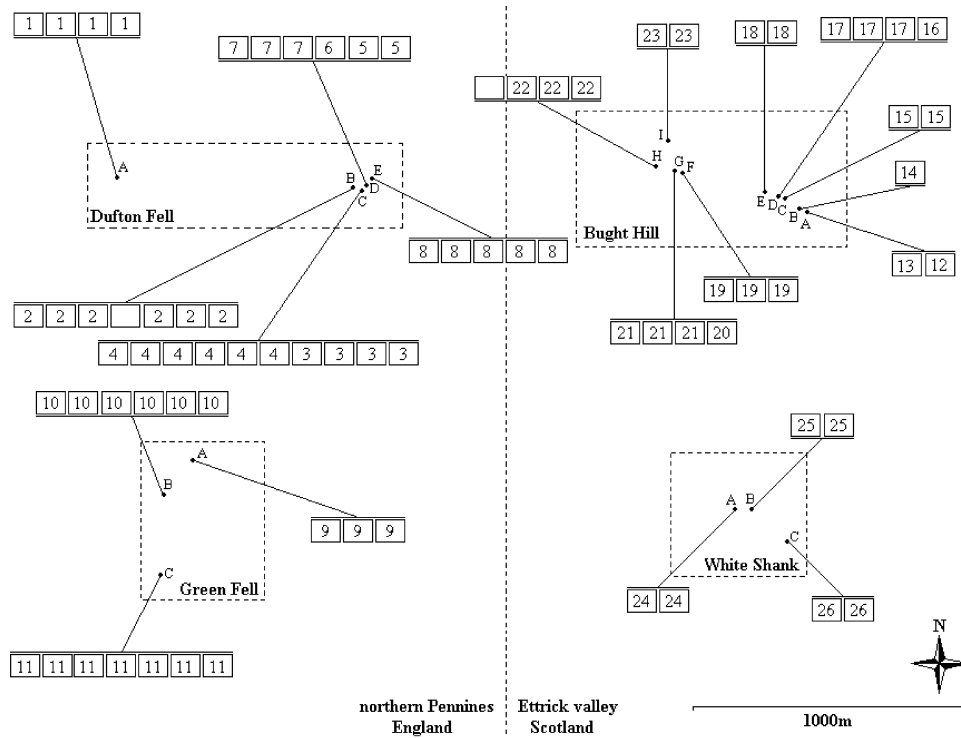


FIGURE 1. Genets of *Carex vaginata* from two northern English and two southern Scottish localities, sampled August 2003. 26 genotypes (genets) were distinguished using 52 polymorphic RAPD markers. Sites within localities represented by letters (see Table 1), samples represented by small squares (arranged along transects), genotypes represented by numbers (1–26), failed amplification represented by empty square.

Bands were scored manually as either present or absent, generating a binary matrix. From this matrix, the RAPD phenotypes for all 76 individuals were compared. Plants showing identical banding patterns were considered to belong to the same multilocus genotype and were interpreted as being members of the same clone (=genet). Each of the different multilocus genotypes were assumed to correspond to a different genet, with each plant (sample) considered as an individual ramet (potentially independent part of a genet (Cook 1983)). The spatial distribution of each putative clone (genet) was represented on a schematic diagram, with intra-population clonal diversity assessed by calculating the mean number of ramets per genet,  $R/G$ , for each population.

RESULTS

Of the 59 bands scored, 52 (88.1%) were polymorphic. In terms of their distribution among individuals, from the 76 samples, 26 multilocus genotypes (putative genets) were identified. Reproducibility tests showed that all of the banding differences investigated were reliable based on an independent re-extraction of the sample DNA and re-amplification using the RAPD primers. The number of bands amplified in each genotype ranged from 20–35, with an average of  $15.7 \pm 3.8$  band differences between genotypes (range 2–24).

The number of putative genets per population ranged from three at Green Fell and White Shank, eight at Dufton Fell, to the maximum of twelve at Bught Hill (Fig. 1, Table 2). Within each of the sites, each patch contained 1–3 putative genets, with 15 out of the 20 patches consisting of a single genet (Fig. 1). The most extensive distribution of a single genotype was at Green Fell with one

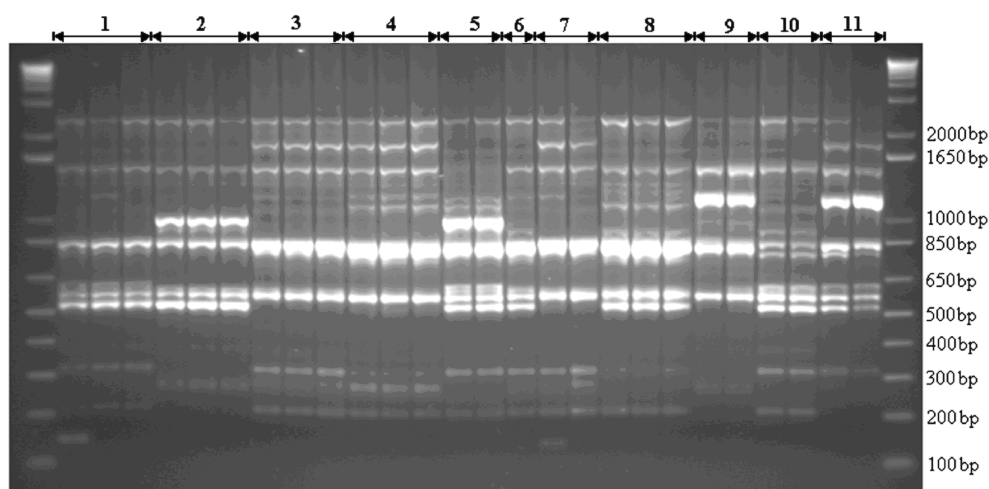


FIGURE 2. RAPD band profile of 26 individuals from Dufton Fell and Green Fell, using primer *OPC6*. Eleven genets (numerical codes as in Fig. 1) are indicated above the lanes. Genets 3 and 4 which show intensity, but not discrete banding differences were scored as identical for this RAPD primer, but showed clear differences with other primers.

TABLE 2: CLONAL DIVERSITY IN FOUR POPULATIONS OF *CAREX VAGINATA*, TWO IN THE NORTHERN PENNINES, ENGLAND AND TWO IN SOUTHERN SCOTLAND. P = NUMBER OF PATCHES SAMPLED; R = NUMBER OF RAMETS SAMPLED; G = NUMBER OF PUTATIVE GENETS (CLONES); R/G = MEAN NUMBER OF RAMETS PER GENET

	P	R	G	R/G
ENGLAND				
Dufton Fell	5	31	8	3.88 ± 1.81
Green Fell	3	16	3	5.33 ± 2.08
SCOTLAND				
Bught Hill	9	23	12	1.92 ± 0.90
White Shank	3	6	3	2.00 ± 0.00
Total	20	76	26	2.92 ± 1.79

genet being spread over at least 6 m (Site C) (Fig. 1). Where multi-clonal patches were detected, the ramets were spatially aggregated, with adjacent samples having identical genotypes. An example of a RAPD banding profile is shown in Figure 2.

No multilocus genotypes were detected that were shared between patches. All patches and populations contained different multilocus genotypes (genets) (Fig. 1).

#### DISCUSSION

*Carex vaginata* populations in England consist of a series of plants sharing multi-locus RAPD profiles. This is consistent with the presence of several clonal mats. The finding of these extensive clonal mats at the two English populations of *C. vaginata* supports the notion that the species has been present at these sites for a long period of time, rather than being a recent colonist. Cessation of grazing following the foot and mouth outbreak has resulted in the sedge becoming more conspicuous as it has been allowed to grow and flower in the absence of extensive sheep cropping.

Without a reliable estimate of clonal growth rates in *C. vaginata*, assessing the ages of these

populations is difficult. A similar study on clonal diversity on another rhizomatous sedge (*C. bigelowii* in Iceland), has, however, estimated a relatively slow growth rate with new ramets being produced on average 3.4 cm apart every 1.5 years (Jonsson 1996). If the rates of clonal spread in *C. vaginata* are similar, then a (highly speculative) extrapolation would suggest that the maximum clone size detected here (e.g. at least 6 m in Patch C at Green Fell), would indicate a persistence of the species at these sites in excess of 250 years. Of course such an estimate should be interpreted with extreme caution as there are many confounding variables, but it does seem safe to conclude that the plant's arrival is at least not very recent, and its existence at the site is likely to far pre-date the Flora of Cumbria surveys. Indeed, *C. vaginata* in the northern Pennines may represent another example of a "relict" species whose presence may be measured in thousands of years, as has been postulated for other species within the Upper Teesdale flora (Clapham 1978).

On both Dufton Fell and Green Fell, multi-clonal patches were detected. This suggests that sexual reproduction has been important in producing diversity within these English populations, and that genotypic diversity has been maintained, despite long-term intensive grazing. However, the apparent restriction of genotypes to single patches at these and the Scottish sites (no genotype was found in more than one patch) suggests that founding of new patches within populations is due to seed and not vegetative dispersal. Thus although the species, and some level of clonal diversity, has persisted under heavy grazing, it is evident that repeated removal of flowering spikes by grazing is likely to retard the dispersal ability of the species, and decrease the likelihood of colonisation of new patches. It is also worth noting that if heavy grazing reduces opportunities for sexual reproduction, then by definition this also reduces the opportunities for new genotypes to be produced. With a limited production of new genotypes, there will be a limit to the raw genetic material available for natural selection, and hence a limit to the opportunities for the species to adapt at these sites in the face of future environmental change. Thus a long term reduction in grazing in the northern Pennines may not only be beneficial for the performance of these plants in the short term, but may also maximize their chance of survival and evolution in the longer term.

#### ACKNOWLEDGMENTS

We would like to thank Jane Squirrell, Keith Watson, Heather McHaffie, Mike Porter and Linda Robinson for their help with the fieldwork and Michelle Hollingsworth and Alex Ponge for their assistance within the molecular lab. This work received funding supported from English Nature; the Royal Botanic Garden Edinburgh is supported by the Scottish Executive Environment and Rural Affairs Department.

#### REFERENCES

- CLAPHAM, A. R. (1978). *Upper Teesdale: The area and its natural history*. Collins, London.
- COOK, R. E. (1983). Clonal plant populations. *American Scientist* **71**: 244–253.
- CORNER, R. W. M. (1981). *Carex vaginata* Tausch in southern Scotland. *Watsonia* **13**: 317–318.
- CORNER, R. W. M. (2004). *Carex vaginata* Tausch (Cyperaceae): a sedge new to England. *Watsonia* **25**: 127–130.
- DOYLE, J. J. & DOYLE, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochemical Bulletin* **19**: 11–15.
- ENGLISH NATURE (1993). Review of the impact of extensive livestock farm systems on nature conservation and the environment. *English Nature Research Reports – No. 68*.
- ENGLISH NATURE (2001). The upland challenge. *English Nature Magazine* **55**: 6–7.
- ESSELMAN, E. J., JIANQIANG, D. J., CRAWFORD, D. J., WINDUS, J. L. *et al.* (1999). Clonal diversity in the rare *Calamagrostis porteri* ssp. *insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and intersimple sequence repeat (ISSR) markers. *Molecular Ecology* **8**: 443–451.
- HOLLINGSWORTH, M. L., HOLLINGSWORTH, P. M., JENKINS, G. I., BAILEY, J. P. *et al.* (1998). The use of molecular markers to study patterns of genotypic diversity in some invasive *Fallopia* spp. (Polygonaceae). *Molecular Ecology* **7**: 1681–1691.
- HOLLINGSWORTH, M. L., & BAILEY, J. P. (2000). Evidence for massive clonal growth in the invasive weed *Fallopia japonica* (Japanese Knotweed). *Botanical Journal of the Linnean Society* **133**: 463–472.

- HULTÉN, E. & FRIES, M. (1986). *Atlas of north European vascular plants north of the Tropic of Cancer. Vol. 1*. Koeltz Scientific Books, Königstein.
- JERMY, A. C., CHATER, A. O. & DAVID, R. W. (1982). *Sedges of the British Isles*. Botanical Society of the British Isles, London.
- JONSSON, O., JÓNSDÓTTIR, I. & CRONBERG, N. (1996). Clonal diversity and allozyme variation in populations of the arctic sedge *Carex bigelowii* (Cyperaceae). *Journal of Ecology* **84**: 449–459.
- KREHER, S. A., FORÉ, S. A. & COLLINS, B. S. (2000). Genetic variation within and among patches of the clonal species, *Vaccinium stamineum* L. *Molecular Ecology* **9**: 1247–1252.
- MCCLINTOCK, K. A. & WATERWAY, M. J. (1993). Patterns of allozyme variation and clonal diversity in *Carex lasiocarpa* and *C. pellita* (Cyperaceae). *American Journal of Botany* **80**: 1251–1263.
- PERSSON, H. A. & GUSTAVSSON, B. A. (2001). The extent of clonality and genetic diversity in lingonberry (*Vaccinium vitis-idaea* L.) revealed by RAPDs and leaf-shape analysis. *Molecular Ecology* **10**: 1385–1397.
- PRESTON, C. D., PEARMAN, D. A. & DINES, T. D. (2002). *New Atlas of the British and Irish Flora*. Oxford University Press, Oxford.
- RATCLIFFE, D. A. (1959). The mountain plants of the Moffat Hills. *Transactions of the Botanical Society of Edinburgh* **37**: 229–250.
- ROBERTS, F. J. (2003). After foot and mouth, Cross Fell in bloom. *The Carlisle Naturalist* **10**: 33–42.
- ROBINSON, L. (2003). Observation on *Alopecurus borealis* Trin. (Alpine Foxtail) at Green Fell in the northern Pennines, Cumbria, after foot and mouth. *BSBI News* **93**: 11–12.
- WILLIAMS, J. G. K., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A. *et al.* (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**: 6531–6535.

(Accepted April 2004)

