The status of *Carex flava* L. s.s. (Cyperaceae) in the British Isles.

Introduction.

Carex flava L. s.s (fig. 1) is a rare plant in the British Isles. It was formerly thought to be extant at only one location, Roudsea Woods, Cumbria (vc 69). Two further historic populations from Ennerdale, Cumbria (vc 70), discovered by J. Dickinson in 1836, and Hebden, W. Yorks (vc 64), discovered by T. W. Edmondson in 1906, are known from only single voucher specimens held at **LIV** and **GH** respectively. Three further putative *C. flava* x *C. lepidocarpa* Tausch. (= *C*. x *pieperana* P. Junge) hybrid populations have been described from Malham Tarn Moss (vc 64), Greywell Moors (vc 12) and Coolagh Fen (vc H17) suggesting that *C. flava* was formerly more widespread than it is today. The first of these populations, Malham Tarn Moss, was originally thought to have been found by G. Shaw in 1946 (Shaw 1946) but a recent discovery by Dr Paul Ashton in the British Museum (**BM**) indicates that a specimen was collected from this site by A. W. Bradley in 1913 (Ashton pers. com.). The

Fig. 1 *Carex flava* L. photo: Blackstock 2009

Malham Tarn Moss population was considered by many botanists to be *C. flava* s.s. (Shaw, 1946; Davies, 1953; Clymo, 1960 etc.) but doubts to its true identity were raised by Jermy and Tutin (1968) who stated that "the only typical material" of *C. flava* in Britain "is from N. Lancs (vc 69)" i.e. Roudsea Wood. Jermy *et al.* (1982) further stated that plants intermediate to *C. flava* and *C. lepidocarpa* occur at Malham Tarn Moss. It is this treatment that was adopted by Stace (1997).

A further population at Gait Barrows, Cumbria (vc 60) is known to have been introduced in 1998. Seedlings and smaller plants failed to become established but larger genets are still thriving. This may suggest that competition plays an important role in recruitment (Blackstock unpublished data).

A detailed morphometric analysis incorporating populations from Britain, Northern mainland Europe and North America of *C. flava* and *C. lepidocarpa* presented by Blackstock and Ashton (2001) challenged the view that the Roudsea Wood population was the only native population of *C. flava* in the British Isles. They found that the Malham Tarn Moss population could not be separated from the N. American, British or European populations of *C. flava* but was distinct from all populations studied of *C. lepidocarpa* and therefore concluded that the Malham Tarn Moss was *C. flava* s.s. Given the taxonomic difficulties within the *C. flava* complex it was desirable that molecular markers, such as allozymes, combined with multivariate statistical analyses of morphological data, were used to provide an additional line of evidence to support this conclusion. Allozymes have been used to successfully resolve taxonomic problems within the genus *Carex* (Bruederle and Fairbrothers 1986; McClintock and Waterway, 1993; Ford *et al*., 1991) and specifically within the *C. flava* complex (Bruederle and Jensen 1991; Hedrén 1996; Hedrén and Prentice 1996; Hedrén 2003). This study will address the identity of the putative *C. flava* x *C. lepidocarpa* hybrids from Malham Tarn Moss, Greywell Moors and Coolagh Fen through: 1) identification of morphological variation within *C. flava* and *C. lepidocarpa* and the putative hybrid populations; 2) Identification of genetic markers that differentiate *C. flava* from *C. lepidocarpa*; 3) Identify whether these markers are present in the putative hybrid populations in an additive manner thus confirming hybrid origins; 4) consider pairwise genetic identities within *C. flava, C. lepidocarpa* and the putative hybrid populations.

Method.

Specimens were collected from 27 populations from the British Isles, Northern Europe and North America. These included 13 populations of *C. flava*, 11 populations of *C. lepidocarpa* and the three putative hybrid populations of Malham Tarn Moss, Greywell Moors and Coolagh Fen. Eleven morpholagical characters and 11 enzyme systems giving 17 putative loci and 31 alleles were identified. Full details of the morphological characters and enzyme systems, along with the full methodology are given in Blackstock (2007).

Results.

A scatter plot (fig. 2) derived from a principal component analysis along PC I and PC II indicates there are two distinct clusters, although separation is minimal. These clusters could be described as a *C. flava* cluster and *C. lepidocarpa* cluster. The *C. flava* cluster includes all populations described as *C. flava* as well as the putative hybrid populations from Malham Tarn Moss and Coolagh fen, although there is some separation of the latter population along PC II. The *C. lepidocarpa* cluster included all of the *C. lepidocarpa* populations and the Greywell Moors population.

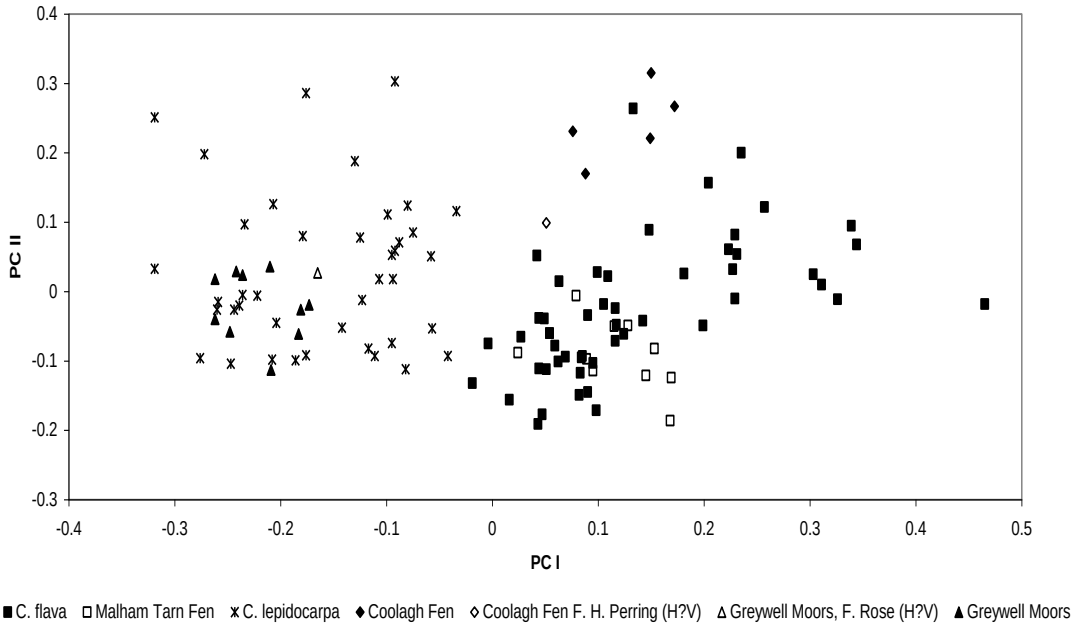

Fig. 2 A scatter plot derived from a principal component analysis along PC I and PC II of all C. flava, C. lepidocarpa and putative hybrid populations.

The allozyme analysis found 6 loci to be monomorphic thus leaving 11 polymorphic loci. A phenogram (fig. 3) derived by UPGMA clustering from a matrix of pairwise comparisons of Nei's (1978) genetic identities (Table 1) clearly shows two distinct clusters that can be ascribed to *C. flava* and *C. lepidocarpa.*

Table 1. Matrix of mean genetic identity coefficients (Nei 1978) derived from pairwise comparisons of all populations of the C*. flava* agg. sampled: observed range in values given below mean values.

	C. flava	*C. lepidocarpa*	Malham Tarn Moss	Greywell Moors	Coolagh Fen
C. flava	0.9839 (0.9045 – 1.0000)	0.6610 (0.5854 – 0.7647)	1.000 (0.9045 – 1.0000)	0.8149 (0.7632 – 0.8235)	0.7593 (0.7550 – 0.7748)
C. lepidocarpa		0.9329 (0.8406 – 0.9970)	0.6610 (0.5854 – 0.7647)	0.7960 (0.7642 – 0.8371)	0.8218 (0.7759 to 0.8825)
Malham Tarn Moss				0.8149	0.7593
Greywell Moors					0.8176

The low levels of genetic variation within *C. flava* is reflected by there being no differentiation for these populations ascribed to this taxon with the exception of three sites, Roudsea Wood, Västanå and Dalbyn. Malham Tarn Moss clearly falls within the *C. flava* cluster with the Greywell Moors population being slightly separated. The separation of the latter is, in part, due to the inclusion of the 6PGDb allele which is absent from *C. flava* but common to *C. lepidocarpa*. Four other alleles (ADHa, PGM-1b, PGM2b and

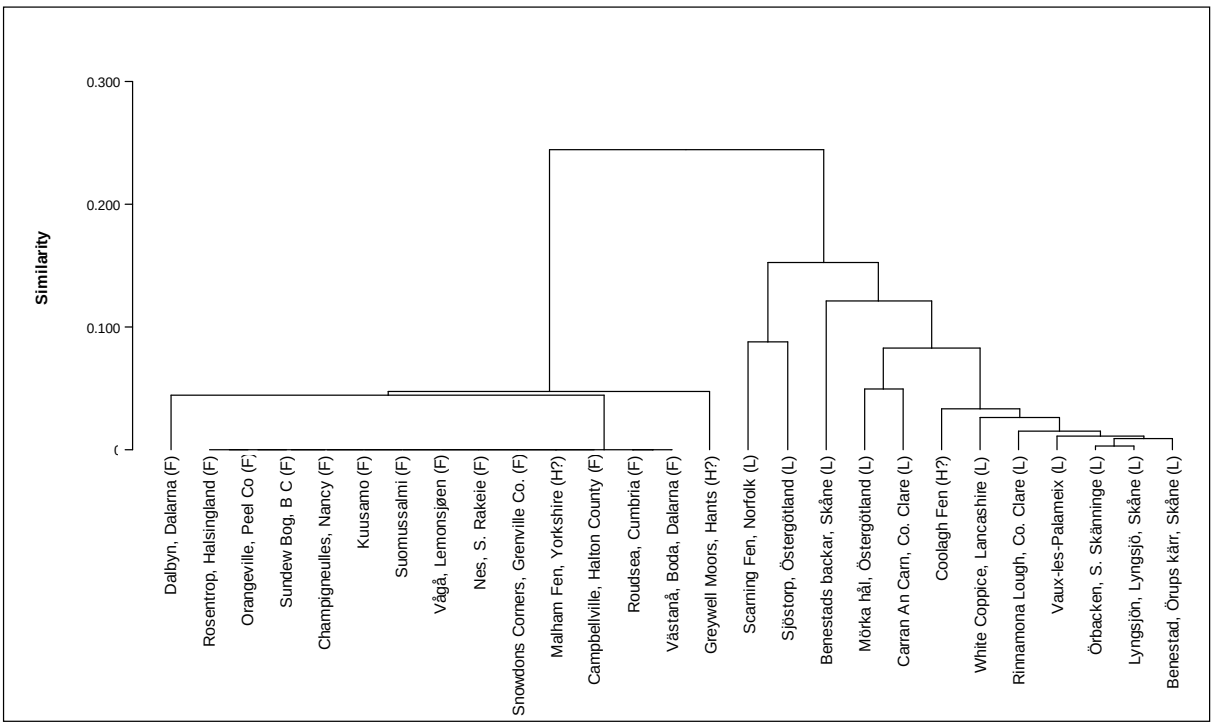

FIG 3 Phenogram of UPGMA AHC of allele frequencies between sites of *Carex flava* (F), *C. lepidocarpa* (L) and Putative hybrids (H?)

GDHa) that are fixed within *C. flava* but only found at low frequencies within *C. lepidocarpa* (<0.37) are fixed within the Greywell Moors population. This is a strong indication that *C. flava* was probably extant at Greywell Moors but is now extinct. All alleles found within the Coolagh Fen population are common within *C. lepidocarpa*. Of these, five alleles (6PGDb, ADHb, PGM-1a, PGM-2a and GDHb) found to be fixed in the Coolagh Fen population are absent from all *C. flava* populations sampled. The Malham Tarn Moss population was found to be identical to all *C. flava* populations samples, with the exceptions of Roudsea Wood, Västanå and Dalbyn mentioned above.

Conclusion.

The morphological analysis supports Blackstock and Ashton (2001) that the Malham Tarn Moss is *C. flava* s.s. The Greywell Moors population belongs to *C. lepidocarpa* and that the Coolagh Fen population belongs to *C. flava*, although there is partial separation along PC II. This partial separation raises some doubts that are further emphasised by some qualitative differences between *C. flava* s.s and the Coolagh Fen population. The clustering with *C. flava* is primarily due to the unusually large utricles found within this population. That Coolagh Fen is not *C. flava* is supported by the allozyme analysis that strongly indicates that this population is an extreme phenotype of *C. lepidocarpa*. It is suggested that this may be, in part, due to the prevailing ecological conditions at Coolagh Fen.

Morphologically the Greywell Moors population cannot be separated from *C. lepidocarpa* but it has several alleles common to *C. flava* that are either not found or very rare in *C. lepidocarpa*. Schmid (1980) indicates that introgressive hybridization between sympatric taxa of the *C. flava* agg. does occur and that introgression followed by natural selection for *C. lepidocarpa* characteristics would leave individuals with a predominantly *C. lepidocarpa* morphology. There is some evidence to support this hypothesis from a study of *Carex vulpina* L. and *Carex otrubae* Podp. where the two species are sympatric at sites in Southern Britain (Smith and Ashton, 2006).The signature of introgression remains in allozyme patterns at Greywell Moors as these alleles are considered to be selectively neutral and therefore more likely to remain in the population. It is possible that a small population of *C. flava* was once extant at Greywell Moors but has subsequently become extinct, with some *C. flava* alleles remaining within the population of *C. lepidocarpa* as a product of introgression.

The Malham Tarn Moss population cannot be separated by either morphology or allozyme analysis from *C. flava*. Indeed it could be argued that the Malham Tarn Moss population is more typical of *C. flava* than that found at Roudsea

Wood where there is some indication of introgression between *C. flava* and *C. demissa*. The Malham Tarn Moss population is very small (c. 10 genets, Blackstock, unpublished data) although the population does appear to be stable. This population needs to be carefully monitored to establish population numbers and stability and may benefit from periodic seed collection every two or three years in case the population is further threatened. Surrounding areas should also be surveyed to identify any hitherto unidentified genets.

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