Distinction between the sedges *Carex vulpina* L. and *C. otrubae* Podp. and the potential for identification of hybrids

C. SMITH*[1] AND P. A. ASHTON*[2]

NGAS Edge Hill College of Higher Education, St. Helens Road, Ormskirk, Lancs. L39 4QP

ABSTRACT

Carex vulpina and *C. otrubae* are difficult species to distinguish using floral characters. As an alternative, SEM of the nutlet surface, stomatal morphology and isozymes have been used as taxonomic tools to distinguish between the two taxa. The three methods show differentiation along species lines and are suitable to identify hybridisation. Stomatal morphology and isozymes reveal evidence of possible introgression between the two species.

KEYWORDS : False Fox-sedge, True Fox-sedge, hybridisation, isozymes, nutlet morphology, SEM, stomata.

INTRODUCTION

Carex. vulpina L. (Fox sedge) and *C. otrubae* Podp. (False Fox sedge) have been recognised as distinct species since separation by Ernest Nelmes (1939). Nelmes identified a broad geographical separation between the two species, considering *C. vulpina* to be a plant predominantly of eastern Europe while *C. otrubae* is more typical of western Europe. However, both species occur in Britain, with *C. vulpina* being a rare plant of wet meadows in southern Britain found in only eleven hectads (Preston *et al.* 2002) while *C. otrubae*, with a wider ecological tolerance, is more common, being found in a variety of damp habitats. The two species are sympatric at some sites. Hence distinction between the two plants is important to allow the ecology of *C. vulpina* to be understood and to allow informed conservation.

The two species have long been considered difficult to separate due to their morphological similarity and the character variability within the two species. The initial separation (Nelmes 1939) was based upon differences in cell morphology, culm shape, leaf colour and character of the inflorescence (bracts, glumes and utricle shape). Subsequent authors have added to the list of supposedly distinguishing morphological features, Erskine & Lambrick (2000) listed 29 such characteristics. These have typically been qualitative and have included such features as ligule shape (Nelmes 1946), bract shape (Senay 1950) and the timing of utricle fall (Erskine & Lambrick 2000). However Foley & Porter (1999) consider many of these to be of little practical use. Porley (1999), following the pioneering work of Crawford (1910) and Metcalfe (1971) on the distinctiveness of the leaf and stem anatomy of many British *Carex* species, suggested that internal leaf anatomy could be used to discriminate between the two species, although our preliminary investigations using this method have proved inconclusive.

Blurring of the delineation between the two species will result if the species hybridise. Nelmes (1939) suggested that hybrids between *C. vulpina* and *C. otrubae* had been found close to the River Medway, at Tonbridge (v.c. 16) and from Amberley Wild Brooks (v.c. 13), although Stace (1975) noted that these records are unconfirmed. However, Stace (1975) also considers that "hybrids between the two species will be revealed by careful searching". In Britain crosses have also been reported between *C. vulpina* or *C. otrubae* with members of the *Carex muricata* group and *C. paniculata* (Marshall 1897), although further studies of these specimens is required to substantiate their status (Stace 1975).

Discrimination between other morphologically cryptic Cyperaceae taxa has been undertaken using alternative characters to gross floral morphology. SEM microscopy of the nutlet surface has been successfully used by Schuyler (1971) to distinguish between morphologically similar species of *Scirpus* and *Eriophorum* while Flatberg (1972), among other approaches, utilised differences in stomatal measurements to investigate the occurrence of a new hybrid between *Carex canescens* and *C. chordorrhiza* in central Norway. Starr & Ford (2001) found that SEM of nutlet micromorphology combined with leaf anatomy and morphology (including stomatal

*[1]e-mail: smithco@edgehill.ac.uk
*[2]e-mail: ashtonp@edgehill.ac.uk

TABLE 1A. *CAREX VULPINA* SITES AND SAMPLE DETAILS

Site	Map Reference	Vice County	Isozyme Sample Nos.	Stomatal Sample Nos.
Amberley Wild Brooks (AMB)	TQ038136	v.c. 13	1	3
Love Farm East (LF)	TQ066276	v.c. 13	1	1
Besley Farm Wet Meadow	TQ016149	v.c. 13	1	0
Besley Farm (BF)	TQ018149	v.c. 13	1	2
Blackthorne (BMP)	SP636204	v.c. 23	1	1
Otmoor (Nutlets) (OT)	SP578128	v.c. 23	1	4
Asham Meads (AM)	SP590146	v.c. 23	0	2
Ashleworth Ham (AH)	SO828261	v.c. 33	3	5
Hawbridge (HA)	SO849277	v.c. 34	1	1
R. Porley (RP)	Probably Otmoor	v.c. 23	0	1
Stuttgart Bot. Garden (GE)			1	5
Czech Republic (CZR)			0	1
Total			11	26

TABLE 1B. *CAREX OTRUBAE* SITES AND SAMPLE DETAILS

Site	Map Reference	Vice County	Isozyme Sample Nos.	Stomatal Sample Nos.
Ashleworth Ham (AH)	SO828216	v.c. 34	1	5
Coombe Hill (CH)	SO873270	v.c. 34	1	2
Point of Ayr (PA)	SJ122848	v.c. 50	1	1
Donington (D)	TF210355	v.c. 53	1	2
Rixton (RI)	SJ685903	v.c. 59	1	3
Crossens (CR)	SD363214	v.c. 59	1	1
Hesketh Outmarsh	SD425255	v.c. 59	1	0
Gait Barrows (GB)	SD418772	v.c. 60	1	1
Port William (PW)	NX315488	v.c. 74	1	1
Total			8	16

characters) distinguished between members of *Carex* section *Phyllostachys* along traditional taxonomic lines. A number of authors have also used isozymes to address distinctiveness between morphologically similar species (Ford *et al*. 1991; McClintock & Waterway 1993;Ford *et al*. 1998; Reinihammar 1999; Tyler 2003).

This paper aims to investigate the usefulness of using three of the methods outlined above (SEM of the nutlet surface, stomatal morphology and isozymes) to distinguish between *C. vulpina* and *C. otrubae*. In addition we comment on the potential of each method for assessing the presence of hybrids.

METHODS

COLLECTION AND PROPAGATION

Species were identified *a priori* using a range of characters. *C. vulpina* was distinguished by a stout, sharply winged flowering culm, presence of dark auricles below the inflorescence, the presence of spare, chaffy material around the ligule and the possession of wrinkled leaf sheaths. These are recognised as the most useful diagnostic morphological characters (Rich & Jermy 1998). *C. vulpina* was collected from eight out of the eleven hectads where it is present in England, (Preston *et al*. 2002) in addition to two overseas samples (Table 1a). *C. otrubae* was collected from nine sites across a large part of the range of the species in Britain (Table 1b). At two sites (Amberley Wild Brooks and Ashleworth Ham) the two species were observed growing sympatrically.

All of the *C. otrubae* and most of the *C. vulpina* material were grown from ramets collected from the various sites. From five *C. vulpina* locations (Otmoor, Amberley Wild Brooks, Asham Meads, Germany and the Czech Republic) nutlets, rather than vegetative material, were collected and subsequently germinated.

Successful germination of nutlets was achieved by selecting undamaged, full utricles that were hard to the touch. These were then cleaned of dry debris and stored in paper envelopes at 4 °C. in a jar containing silica gel to ensure a low moisture content. These were collected in late summer/ early autumn. In the following February the utricles were spread on wet filter paper in Petri dishes and kept in the dark at 4 °C. After one month the closed Petri dishes were moved into light in an unheated glasshouse until germination occurred. Throughout these stages drying out was prevented. Within two weeks signs of germination became evident. When the root growth was established and the cotyledons reached about 5 mm in length then the plants were transferred to potting compost in small pots and then grown on outside. Within six weeks from placing the plants in the glasshouse the two species exhibited germination rates between 36–96%. Individuals grown from nutlets were kept until flowering and seed set when identification could be confirmed. Tables showing percentage germination rates for the species in the years 2002 and 2003 are shown in Tables 4a and 4b (see Appendix).

SEM METHODS

Nutlets were prepared for SEM using a method modified from Starr & Ford (2001). The *C. vulpina* material was from Ashleworth Ham (v.c. 34) and the *C. otrubae* material came from Rixton (v.c. 59). The utricles were removed and the nutlets were acetolysed in 1:9 sulphuric acid – acetic anhydride solution using a modified form of Starr & Ford's protocol. Both sets of nutlets were immersed in the solution and placed in an ultrasonic bath at a temperature of 55 ºC. *C. otrubae* needed an immersion time of 15 minutes whilst *C. vulpina* required 35 minutes. They were then rinsed in three changes of distilled water and allowed to dry overnight. Two utricles of each species were prepared for scanning. Financial constraints prevented a more extensive sample being taken.

STOMATAL MORPHOLOGY

Using fresh material from the central section of a mature leaf, microscope slides of he abaxial epidermis were prepared using a method modified from Starr & Ford (2001). This involved immersing the leaf in bleach and scraping away unwanted tissue with a scalpel, rinsing in water, dehydrating in ethanol and staining in 1% safranin prior to mounting. Ten randomly selected stomata were then measured from the prepared slides at 400 × magnification. Two measurements were recorded for each stoma, the maximum length, (the overall length of the stomatal complex running coaxially with the stomatal opening) and maximum width, measured across the outer margins of the guard cells (Fig. 1).

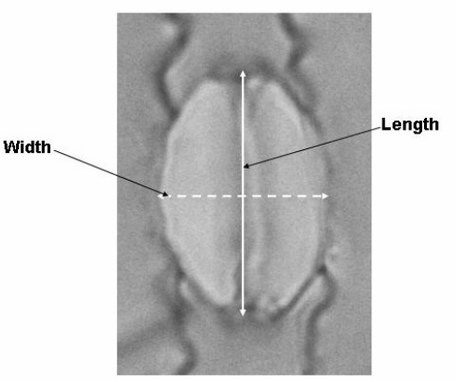

FIGURE 1. Showing the axes from which stomatal measurements were taken.

Dividing the maximum length by the maximum width gave the stomatal index (SI). In all cases the stomatal opening was closed. Stomata from 26 individuals of *C. vulpina* originating from eight English sites plus the two European samples were examined (Tables 1a and 1b). In the case of *C. otrubae* stomata from sixteen individuals from eight British sites were measured. Following checking for homogeneity of variances these data were analysed using one-way ANOVA. *Post hoc* testing was undertaken using the Tukey test.

ISOZYME METHODS

The two species gave regular consistent banding when assayed on three isozyme systems (glucose-6-phosphate isomerase (GPI) E.C. 5.3.1.9, 6-phosphogluconate dehydrogenase (6-PGD) E.C. 1.1.1.44, and menadione reductase (MNR) E.C. 1.6.99.2.).

These isozymes had previously been shown to be useful in addressing taxonomic questions in *Carex* (e.g. Schell & Waterway 1992; Jonsson & Prentice 2000) and had also been found to exhibit interspecific variation in other *Carex* species during previous work in our laboratory (Blackstock, Smith & Ashton unpub.).

Isozyme methods were modified from Abbott (1993), Ashton (1990) and Soltis (1990). 12% starch gels were used with GPI being run on a discontinuous histidine citrate

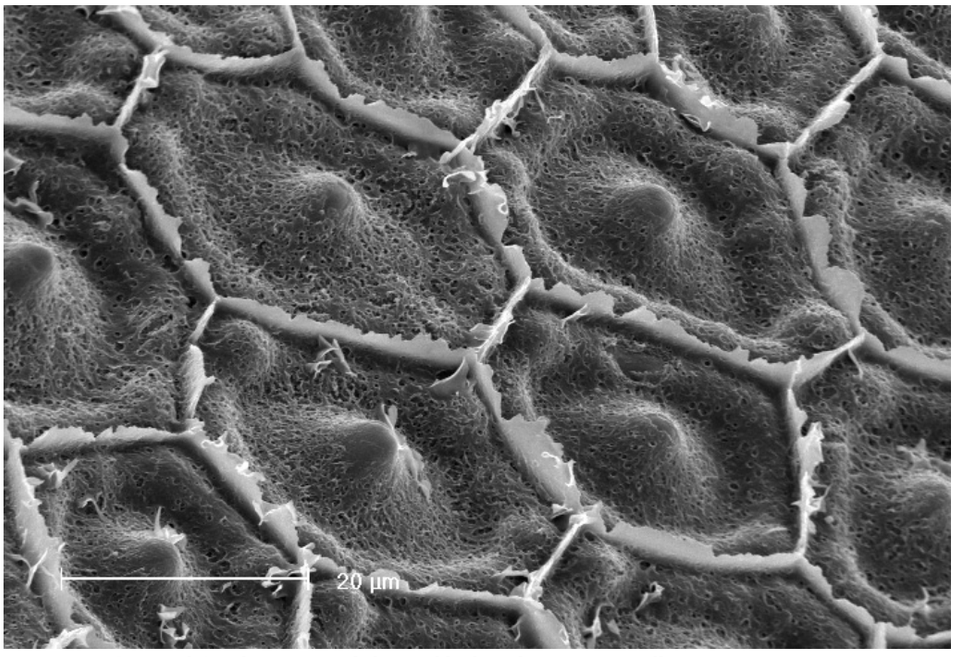

FIGURE 2a. *Carex vulpina* showing rounded central body and prominent satellites.

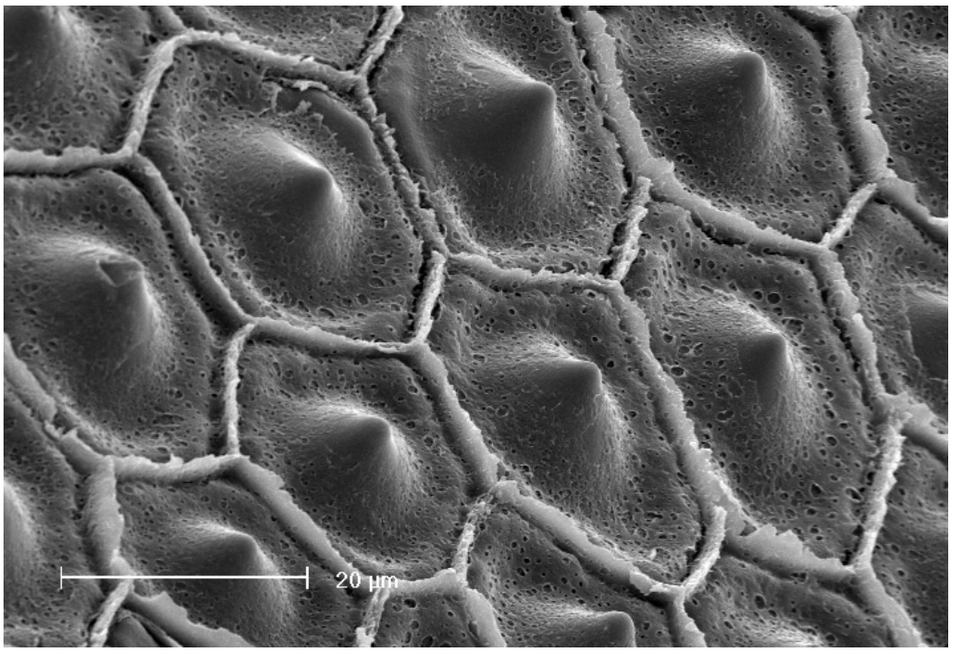

FIGURE 2b. *Carex otrubae* showing pointed central body and less obvious satellites.

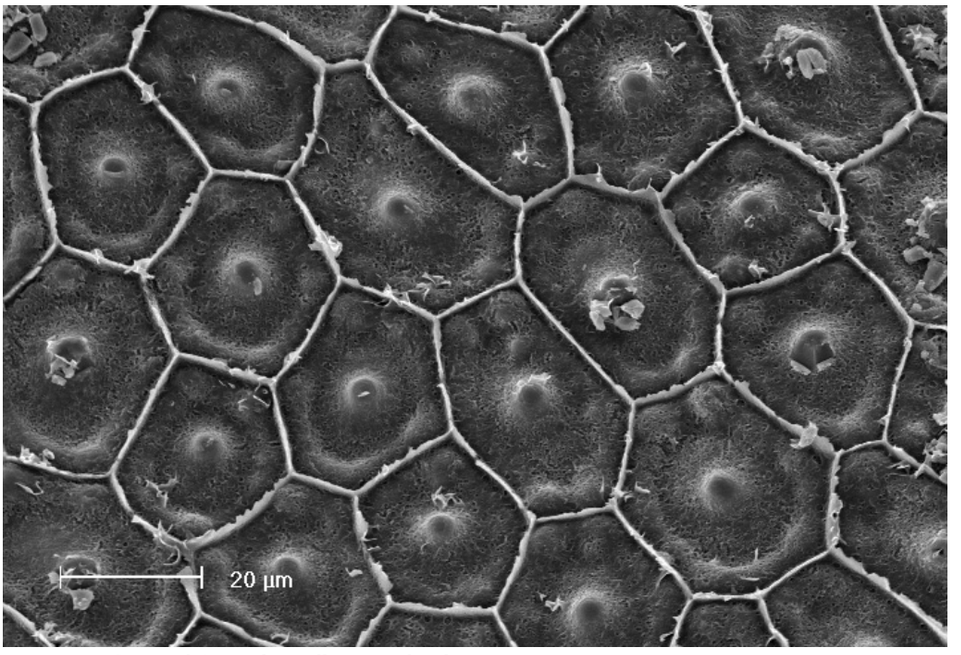

FIGURE 2c. *Carex vulpina* surface cells more orbicular.

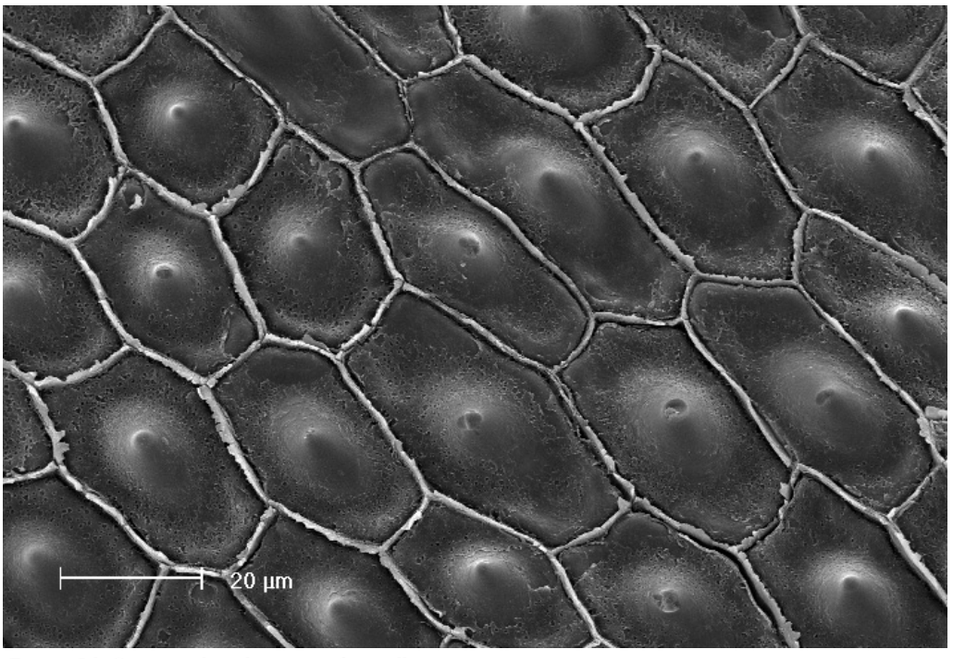

FIGURE 2d. *Carex otrubae* elongate surface cells.

buffer system and 6-PGD and MNR run on a discontinuous tris citrate buffer system (Wendel 1990). Each gel contained samples from both species. The origin of each individual used in each lane is listed in Table 2.

RESULTS

The two plates, Figs. 2a and 2b, show the differences in the nutlet surface cell morphology between the two species. The terms used to describe the features follow Schuyler (1971). Note the more rounded central body and the presence of smaller, raised satellite bodies offset towards the cell wall in *C. vulpina* (Fig. 2a). *C. otrubae* has a more sharply pointed central body and less prominent peripheral satellites (Fig. 2b). When viewed perpendicularly surface cells of *C. otrubae* (Fig. 2d) nutlets are longer along the axis that passes from the stipe to the beak of the nutlet, while in *C. vulpina* these cells are rather orbicular in outline (Fig. 2c).

In all cases the gels were loaded such that lanes 1–11 were *C. vulpina* and lanes 12–20 were *C. otrubae*. The three isozyme systems reveal distinction between the two taxa. GPI shows interspecific variation, *C. vulpina* being monomorphic for the *GPI-1a* allele and *C. otrubae* being monomorphic for the *GPI-1b* allele (Fig 3a).

A similar pattern is observed in 6-PGD, with *C. otrubae* being monomorphic for the *6-PGD-1c* allele while *C. vulpina* typically possesses the *6-PGD-1b* allele at this locus (Fig 3b). However two *C. vulpina* individuals possess a different allele. The specimen from Amberley Wild Brooks (lane 2) having the same allele as that found in *C. otrubae* (*6-PGD-1c*) while the specimen from Hawbridge (lane 8) expressed a unique allele (*6-PGD-1a*).

MNR reveals a more complex pattern than in the previous isozymes considered (Fig 3c). This has been interpreted as consisting of two loci. *C. otrubae* expresses only one of these loci (MNR-1) and is monomorphic.

The full range of variation is observed in *C. vulpina*. In this species some individuals have the same pattern as that found in *C. otrubae* (Amberley Wild Brooks and Hawbridge; tracks 2 and 8 respectively) or they express the slower form of this gene (*MNR-1b*, tracks 5 and 6, Ashleworth Ham). Alternatively the MNR-1 band is absent and individuals exhibit either a single *MNR-2a* band (lanes 9 and 11; Stuttgart and Besley Wet Meadow) or a two- banded pattern combining either *MNR-2b* and *2c* (lanes 1, 4 and 7; Blackthorne, Ashleworth Ham and Love Farm) or *MNR-2a* and *2b* (Lanes 3 and 10; Ashleworth Ham and Besley Farm).

There is considerable overlap in mean stomatal length in *C. vulpina* and *C. otrubae* and some overlap in stomatal width (Table 3).

Stomatal shape differences between the two species are clearest when both measurements are considered together (Fig. 4). *C. otrubae* stomata are wider than they are long (below the diagonal line in Fig. 4) having a stomatal index (SI) of less than 1. By comparison *C. vulpina* has stomata that are above the diagonal line in Fig. 4, being longer than they are wide (SI > 1). When the mean values of all populations, when $n = 10$, are considered using one-way Anova there is a statistically significant division ($F_{47,432} = 39{\cdot}97$, $P < 0{\cdot}05$). The only sample which does not follow this pattern is one of the *C. otrubae* specimens from Ashleworth Ham, which clusters with *C. vulpina*. SI values for each individual surveyed are given in Tables 5a and 5b (in appendix).

DISCUSSION

Discrimination between the two species can be achieved using stomatal shape, isozyme pattern and nutlet micromorphology. Within the small sample used for SEM there is consistency within a species and a difference between the two species. *C. otrubae* has a more sharply pointed central body with few, if any, peripheral satellite bodies. *C. vulpina* displays the outer satellite bodies with a blunter central body. Nutlet surface cells in *C. vulpina* tend to be orbicular in outline while *C. otrubae* cells have a pronounced longer axis in a line extending from the distal to the proximal end of the nutlet. Similarly when stomatal shape is compared *C. vulpina* has elliptical (sub-orbicular) stomata while *C. otrubae* tends to have rounder stomata (orbicular).

The differences in isozyme pattern are slightly less clear cut. The interspecific variation coupled with the absence of intraspecific variation renders GPI the most useful system for delineating between the two taxa. However the patterns observed in 6-PGD and MNR are almost as informative. Despite the apparent complexity of MNR in *C. vulpina*, only 20% of *C. vulpina* expressed the same pattern as that found in *C. otrubae*.

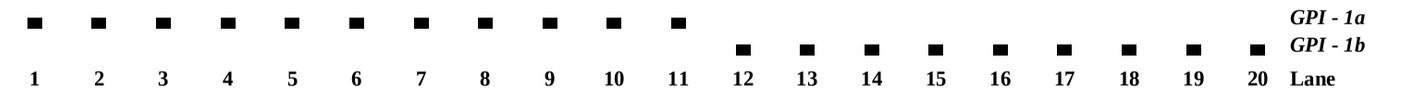

FIGURE 3a. ZYMOGRAM FOR THE ENZYME GPI.

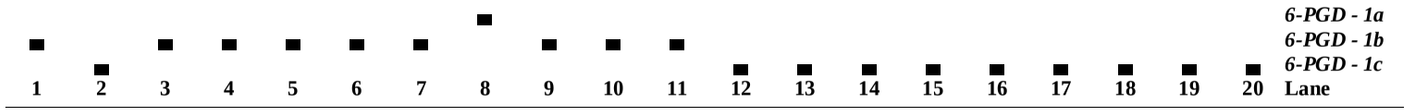

FIGURE 3b. ZYMOGRAM FOR THE ENZYME 6-PGD.

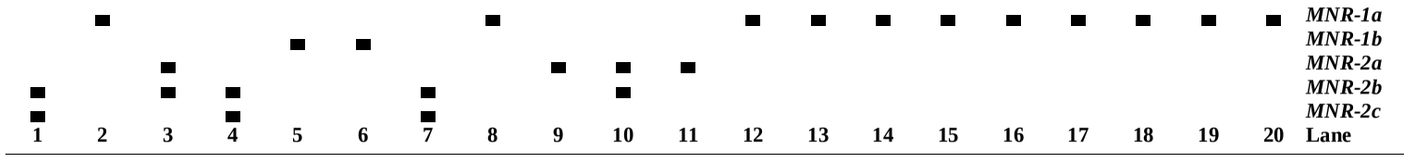

FIGURE 3c. ZYMOGRAM FOR THE ENZYME MNR.

TABLE 2. LANE IDENTIFICATION FOR ISOZYME RESULTS

Lane no.	Site	Lane no	Site	Lane no	Site	Lane no	Site
1	Blackthorne	6	Ashleworth	11	Besley Farm Wet	16	Port William
2	Amberly Wild Brooks	7	Love Farm	12	Donnington	17	Gait Barrows
3	Ashleworth	8	Hawbridge	13	Rixton	18	Crossens
4	Ashleworth	9	Germany	14	Ashleworth	19	Hesketh Marsh
5	Ashleworth	10	Besley Farm	15	Coombe Hill	20	Point of Ayr

Tracks 1–11 inclusive are *Carex vulpina*, LANES 12–20 are *Carex otrubae.*

TABLE 3. STOMA MEASUREMENT RANGES FOR *CAREX VULPINA* AND *C. OTRUBAE*

Character	Data	*C. vulpina*	*C. otrubae*
	Sample size *n*	260	160
Stomatal length (um)	Mean	29·9	29·28
	Range	(22·15–41·13)	(22·15–39·97)
Stomatal width (um)	Mean	24·18	31·88
	Range	(18·95–31·64)	(25·94–37·87)
Stomatal index	Mean	1·24	0·92
	Range	(1·07–1·5)	(0·71–1·31)

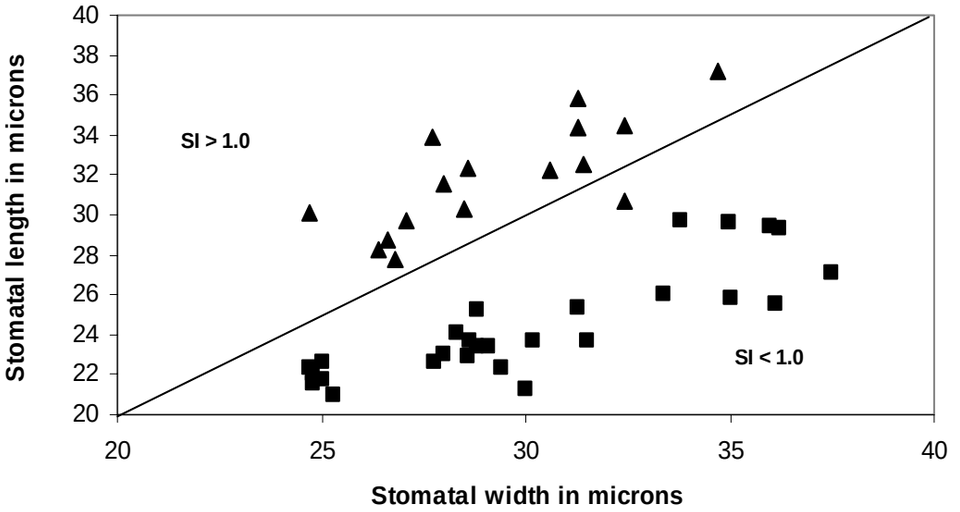

FIGURE 4. Comparison of stomatal index (SI) between *Carex otrubae* (▲) and *C. vulpina* (■).

The interspecific variation in nutlet surface features revealed by SEM coupled with an absence of intraspecific variation, if confirmed across a wider population range, suggests that this method would be a useful tool in identifying hybrids. Schuyler (1971) has used such an approach within morphologically similar *Scirpus* and *Eriophorum*, hybrids revealing intermediate morphology, although some hybrids such as those between *Scirpus atrovirens* and *S. hattorianus*, proved difficult to evaluate due to the absence of viable seeds. The pattern of variation found in *C. vulpina* and *C. otrubae* avoids the problem of

insufficient variation found by Olgun & Beyazoglu (1997) among Turkish members of *Carex* section *Mitratae* where nutlet micromorphology is uniform and thus of little use in delineating between the three species of the section. However the small sample size makes it difficult to confirm if nutlet micromorphology in these species avoids the problem of too much variation, as recorded by Salo *et al*. (1994), whose study of the *Carex flava* complex revealed a high level of intraspecific variation with no fidelity of characters within taxa.

The patterns of stomatal shape and isozyme banding are not universal within the two species. One of the *C. otrubae* individuals from Ashleworth exhibits the stomatal shape of *C. vulpina* and two of the *C. vulpina* plants display at least one isozyme pattern typical of *C. otrubae*. These are from Amberley Wild Brooks (6-PGD and MNR) and Hawbridge (MNR only). It is unlikely that this is due to initial mis-identification as the other characters examined in this study in these individuals are consistent with the original identification. The presence of isozyme bands in *C. vulpina* that are much more common in *C. otrubae* may be part of the variation present in *C. vulpina* which will be revealed by a more extensive sample. However this is less likely in the stomatal survey which is already extensive. Moreover the different stomatal shape found in the Ashleworth individual of *C. otrubae* is absent in the other four individuals surveyed from this site, indicating that this is not simply interpopulational variation in this species.

It is notable that two of the three locations yielding anomalous results are sites where the two species were observed to be clearly sympatric. At Ashleworth Ham the two species grow less than 50 m apart and at Amberley Wild Brooks they are immediately adjacent. It is also likely that the two species are sympatric at Hawbridge, the third site showing anomalous results. It is therefore possible that hybridisation between the two species has occurred followed by backcrossing and introgression. The resultant transfer of genes may then result in individuals expressing characters more commonly associated with the other species.

Flatberg (1972) utilised differences in stomatal measurements between *Carex canescens* and *C. chordorrhiza* to confirm the occurrence of a new hybrid between these two species, *C. ×lidii*, in Central Norway. However, *C. ×lidii* is a sterile F1 hybrid with no record of introgressants and it is approximately intermediate across a range of characters. This is in contrast to the individual at Ashleworth that has *C. otrubae* characteristics but with *C. vulpina*-shaped stomata.

The best indication of ongoing introgression may be the presence of alleles that are close to fixation in one species but very rare in another (Tyler 2003), such as were observed here with 6-PGD and MNR. Isozymes have been used to detect local introgression between *Carex pallens* and *C. ornithopoda* or *C. digitata* in Scandinavia (Tyler 2003). Moreover the position of *C. vulpina* in England, consisting of small populations at the edge of the species' distribution in Europe may increase the likelihood of introgression. Choler *et al.* (2004) showed that ecologically marginal populations of *Carex curvula* exhibited considerable introgression whereas there was no introgression recorded in those in typical habitats.

CONCLUSION

An extensive survey has shown stomatal shape to be a reliable quantitative method for distinguishing between *C. vulpina* and *C. otrubae.* The data have been found to be statistically robust and show a clear and unambiguous division along species lines. It is by no means a field test but reliable results can be had within half an hour. It is achieved at low cost and with equipment found in most laboratories. Where anomalies have been found it is on sites where the species grow sympatrically. From this preliminary study isozymes and SEM of nutlet surface also allow distinction between the two species. In addition the methods and pattern of variation described here provide the tools for a fuller investigation into the extent and nature of hybridisation and introgression between *C. otrubae* and *C. vulpina*.

ACKNOWLEDGMENTS

This work was undertaken with the aid of a grant from the B.S.B.I. to cover expenses for fieldwork and laboratory costs. Our gratitude is also expressed to Mary Briggs, Frances Abraham, Mike Porter, Michael Foley, Nigel Blackstock, Nicola Barrett, Camilla Lambrick, Stan Woodell, Evelyn and Paul Smith, Sarah Watson-Jones and Kees Veldkamp for help in various stages of this study. Ron Porley from English Nature was responsible for creating the interest in the problem.

REFERENCES

ABBOTT, R. J. (1993). Genetic diversity in *Primula scotica* Hook. Unpublished SNH report number SNH/030B/93/IBB.

ASHTON, P. A. (1990). Multiple Origins of *Senecio cambrensis* Rosser, and related evolutionary studies in British *Senecio*. Unpublished Ph.D. thesis. St. Andrews University.

CRAWFORD, F. C. (1910). *Anatomy of the British Carices*. Oliver and Boyd, Edinburgh.

CHOLER, P. *et al.* (2004). Genetic introgression as a potential to widen a species' niche: Insights from *Carex curvular*. *Proc. Ntl. Acad. Sci. USA* **101**:171–176.

ERSKINE, S. E. & LAMBRICK, C. L. (2000). True Fox Sedge, *Carex vulpina*, in Oxfordshire in 2000. Ashmolean Natural History Society of Oxfordshire, Rare Plants Group, Oxford.

FLATBERG, K. I. (1972). *Carex* × *lidii* Flatb. = *C. canescens* L. × *chordorrhiza* Ehrh., a New Hybrid. *Norwegian Journal of Botany* **19**: 91–106.

FOLEY, M. J. Y. & PORTER, M. S. (1999). *Carex vulpina* L. – morphological differentiation from *Carex otrubae* Podp. *BSBI News* **81**:74.

FORD, B. A., BALL, P. W. & RITLAND, K. (1991). Allozyme diversity and genetic relationships among North American members of the short-beaked taxa of *Carex* sect. *Vesicariae* (Cyperaceae). *Systematic Botany* **16**: 116–131.

MARSHALL, E. S. (1897). A new British hybrid sedge from Surrey. *The Journal of Botany, London* **35**: 491–492.

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.

METCALFE, C. R. (1971). *Anatomy of the Monocotyledons. vol. V. The Cyperaceae*. Oxford University Press, Oxford.

NELMES, E. (1939). Notes on British Carices – IV. *The Journal of Botany, London* **77**: 259–266.

NELMES, E. (1946). Plant Notes. *Bot. Soc. and Exchange Club of the British Isles.* 692.

PORLEY, R. D. (1999.). Separation of *C. vulpina* and *C. otrubae* Podp. (*Cyperaceae*) using transverse leaf sections. *Watsonia* **22**: 431–432.

PRESTON, C. D., PEARMAN, D. A. & DINES, T. D. (2002). *New Atlas of the British and Irish Flora*. Oxford University Press, Oxford.

REINIHAMMAR, L. G. (1999). Allozyme differentiation between the lowland *Carex capitata* and the alpine *Carex arctogena* (Cyperaceae) in Scandinavia. *Biological Journal of the Linnean Society* **67**: 377–389.

SCHUYLER, A. E. (1971). Scanning electron microscopy of achene epidermis in species of *Scirpus* (Cyperaceae) and related genera. *Proc. Acad. Nat. Sci. Phila.* **123**: 29–52.

SENAY, P. (1950). Le *Carex Vulpina* et ses Allies. *Bull. Mus. Hist. Nat.* **Second series,** 332–339.

SOLTIS, D. E. S., P.S. (1990). *Isozymes in Plant Biology*, vol. 1 (ed. T. R. Dudley). Chapman & Hall., London.

STACE, C. A. (1975). *Hybridization and the Flora of the British Isles.* Academic Press., London.

STARR, J. R. & FORD, B. A. (2001). The taxonomic and phylogenetic utility of vegetative anatomy and fruit epidermal silica bodies in *Carex* section *Phyllostachys* (Cyperaceae). *Can. J. Bot.* **79**: 362–379.

WENDEL, J. F. (1990). Visualisation and interpretation of plant isozymes, in SOLTIS, D. E. S., P.S. (1990). *Isozymes in Plant Biology*, vol. 1, pp. 5–45. Chapman & Hall, London.

(*Accepted October 2005*)

APPENDIX

TABLE 4A. GERMINATION RATES FOR *CAREX VULPINA* YEAR 2002

Population	Species	Start date	Completion date	% Germination
Hawbridge	*C. vulpina*	12/02/2002	05/04/2002	53%
*R. Porley	*C. vulpina*	12/02/2002	05/04/2002	70%
Ashleworth	*C. vulpina*	12/02/2002	05/04/2002	62%
		AVERAGE % GERMINATION		61.70%

* This material was supplied by the named person and probably came from Otmoor.

TABLE 4B. GERMINATION RATES FOR *CAREX VULPINA* FOR YEAR 2003

Population	Species	Start date	Completion date	% Germination
Besley Farm	*C. vulpina*	28/01/2003	24/03/2003	36%
Hawbridge	*C. vulpina*	28/01/2003	24/03/2003	90%
Asham Meads	*C. vulpina*	28/01/2003	24/03/2003	54%
*R. Porley	*C. vulpina*	28/01/2003	24/03/2003	62%
Ashleworth	*C. vulpina*	28/01/2003	24/03/2003	96%
		AVERAGE % GERMINATION		67.60%

TABLE 5A. STOMATAL INDEX OF *CAREX OTRUBAE* SAMPLE SIZE N = 10

Site	**AH Ot1**	**AH Ot2**	**AH Ot3**	**AH Ot4**	**AH Ot5**	**CH Ot1**	**CH Ot2**	**CR Ot1**
Mean	0·934	0·887	1·064	0·968	0·913	0·826	0·942	0·823
SED	0·067	0·07	0·132	0·071	0·054	0·098	0·066	0·059
Variance	0·005	0·005	0·018	0·005	0·003	0·01	0·004	0·004
Site	**D Ot1**	**D Ot2**	**GB Ot1**	**PA Ot1**	**PW Ot1**	**RI Ot1**	**RI Ot2**	**RI Ot3**
Mean	0·913	0·962	0·929	0·888	0·932	0·954	0·943	0·876
SED	0·034	0·055	0·056	0·066	0·041	0·09	0·06	0·042
Variance	0·001	0·003	0·003	0·004	0·002	0·008	0·004	0·002

TABLE 5B. STOMATAL INDEX OF *CAREX VULPINA* SAMPLE SIZE N = 10

Site	**AH V1**	**AH V2**	**AH V3**	**AH V4**	**AH V6**	**AM V1**	**AM V2**	**AMB. V1**
Mean	1·13	1·108	1·111	1·157	1·231	1·208	1·231	1·292
SED	0·072	0·08	0·134	0·131	0·061	0·054	0·076	0·108
Variance	0·005	0·006	0·018	0·017	0·004	0·003	0·006	0·012
Site	**AMB V1**	**AMB V2**	**BF V1**	**BF V2**	**BMP V1**	**CZR V1**	**GE V1**	**GE V2**
Mean	1·224	1·24	1·146	1·391	1·25	1·14	1·277	1·243
SED	0·118	0·089	0·054	0·113	0·088	0·073	0·071	0·106
Variance	0·014	0·008	0·003	0·013	0·008	0·005	0·005	0·011
Site	**GE V3**	**GE V4**	**GE V5**	**HA V1**	**LF V2**	**OT V1**	**OT V2**	**OT V3**
Mean	1·329	1·155	1·415	1·214	1·417	1·178	1·248	1·323
SED	0·089	0·074	0·154	0·077	0·07	0·047	0·098	0·097
Variance	0·008	0·006	0·024	0·006	0·005	0·002	0·01	0·009
Site	**OT V4**	**RP V2**						
Mean	1·219	1·259						
SED	0·062	0·113						
Variance	0·004	0·013						