Hybridisation between *Schoenoplectus tabernaemontani* and *S. triqueter* (Cyperaceae) in the British Isles

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ABSTRACT

Schoenoplectus samples from the Rivers Arun, Medway, Tamar and Thames were studied using AFLP genetic fingerprinting to test hypotheses concerning the origin of hybrids of *S. triqueter* with *S. lacustris* and *S. tabernaemontani*. *Schoenoplectus lacustris* and *S. tabernaemontani* were shown to be only distantly related, and therefore it is appropriate that they be treated as two distinct species rather than as two subspecies of *S. lacustris*, as recommended by some authorities. *S. lacustris* was excluded as a possible parent in all potential hybrids studied from all rivers, and evidence of additivity was found between the fingerprints of *S. tabernaemontani* and *S. triqueter* in all cases. Material collected as pure species turned out to be of hybrid origin in two cases, and identification of the hybrids and *S. tabernaemontani* appears to be difficult as a result of phenotypic plasticity. Conservation implications of the results and areas of possible future work are discussed.

KEYWORDS: AFLP, club-rushes, hybrids.

INTRODUCTION

The genus *Schoenoplectus* comprises c. 130 species and has a worldwide distribution, with the highest species diversity occurring in eastern Asia and temperate North America. The genus has often been treated as part of *Scirpus* L. s.l. (e.g. DeFilipps 1980; Walters 1984) but a combination of characters, such as the reduction of most leaves to bladeless sheaths and the pseudolateral inflorescence, distinguish it from *Scirpus* s.s. Four species of *Schoenoplectus* are present in the British Isles, namely *S. lacustris* (L.) Palla, common club-rush, *S. pungens* (Vahl) Palla, sharp club-rush, *S. tabernaemontani* (C.C. Gmel.) Palla, grey club-rush and *S. triqueter* (L.) Palla, triangular club-rush. Some workers (e.g. DeFilipps 1980; Walters 1984; Sell & Murrell 1996) have treated *S. tabernaemontani* as a subspecies of *S. lacustris*.

In the British Isles, *S. triqueter* occurs in south-western England and western Ireland. Although it was previously more widespread in southern England, it is now thought to be restricted to one site on the River Tamar (v.c. 2); the population at this site has now been reinforced with plants grown in cultivation from seeds from the Tamar. Hybrids of *S. triqueter* occur where the distributions have overlapped with *S. lacustris* and *S. tabernaemontani*. The hybrid names *S.* × *carinatus* (Sm.) Palla and *S.* × *kuekenthalianus* (Junge) D. H. Kent are used for the hybrids with *S. lacustris* and *S. tabernaemontani* respectively (Stace 1997). Due to their morphological variability, Lousley (1931) stated that 'the determination of hybrids in the *Schoenoplectus* group is by no means simple'. Both hybrids are believed to have occurred on the Tamar and possibly the Thames (Lousley 1975), although *S.* × *carinatus* is now thought to be extinct. Hybrids, thought to be *S.* × *kuekenthalianus* (Lousley 1931, 1975; Stace 1997), also occur on the Rivers Arun (v.c. 13) and Medway (v.c. 15/16).

Following an earlier study of material from the Tamar (Fay 1998), we have expanded the sampling to include material from the Arun and Medway and other material as available. The aims of the work were: (1) to establish the parentage of the hybrids from the Arun, Medway and Tamar; (2) to make a preliminary investigation into levels of genetic diversity in *S. triqueter* and (3) to provide advice to the Environment Agency relating to the management of *S. triqueter* on the Tamar and the possible reintroduction of appropriate genotypes into sites in south-eastern England.

Because amplified fragment length polymorphism (AFLP™: Vos *et al*. 1995) genetic fingerprinting had proved appropriate in the earlier study (Fay 1998), we decided to use the same technique.

TABLE 1. PLANT MATERIALS USED IN THIS STUDY

a) Samples from 1997

Field identification	Field notes	DNA No.
S. triqueter	Site 3a, River Tamar, v.c. 2	5655
S. lacustris	River Exe catchment, Clyst at tidal weir, v.c. 3	5656
S. tabernaemontani	River Exe catchment, Clyst at tidal weir, v.c. 3	5657
hybrid	Site 1, Calstock, River Tamar, v.c. 2	5658
hybrid	Site 2, Calstock Bridge, River Tamar, v.c. 2	5659
hybrid	Site 3, cottage sites, River Tamar, v.c. 2	5660
hybrid	Site 4, right bank, houseboat, River Tamar, v.c. 2	5661
hybrid	Site 5, River Tamar, v.c. 2	5662
hybrid	Site 6, island, Morwellham, River Tamar, v.c. 3	5663

b.) Samples from 2000

Field identification	Field notes/source
S. triqueter	ex hort. Nat. Mus. Wales (Limerick Bridge, River Shannon)
S. lacustris	ex hort. Kew
?*S. lacustris*	Peter Nicholson, Site 33, River Arun, v.c. 13
S. tabernaemontani	Peter Nicholson, 715 616, River Medway, v.c. 15
S. tabernaemontani	Peter Nicholson, Site 20 7129 6024, River Medway, v.c. 16
?*S. tabernaemontani*	M. C. Sheahan 128, Duke's Hollow, River Thames, v.c. 21
S. tabernaemontani	ex hort. Kew
hybrid	Peter Nicholson, Site 1, 0284 0844, River Arun, v.c. 13
hybrid	Peter Nicholson, Site 2 0284 0845, River Arun, v.c. 13
hybrid	Peter Nicholson, Site 4, River Arun, v.c. 13
hybrid	Peter Nicholson, Site 5 0305 0928, River Arun, v.c. 13
hybrid	Peter Nicholson, Site 8 0286 1006, River Arun, v.c. 13
hybrid	Peter Nicholson, Site 61 0322 1628, River Arun, v.c. 13
hybrid	Paul Smith, 0255 1175, River Arun, v.c. 13
hybrid	Paul Smith, 025 1118, River Arun, v.c. 13
hybrid	Paul Smith, 028 098, River Arun, v.c. 13
hybrid	Peter Nicholson, Site 10, 7132 6143, River Medway, v.c. 15

MATERIALS AND METHODS

PLANT MATERIALS

The plant material used in this study is listed in Table 1. All DNAs were extracted from stem material dried in silica gel using a modified 2xCTAB (cetyltrimethyl-ammonium bromide) procedure (Doyle & Doyle, 1987), then purified and quantified using a spectrophotometer.

GENETIC FINGERPRINTING

AFLPs were conducted according to the AFLP Plant Mapping Protocol of PE Applied Biosystems Inc. (AB). Two primer combinations (EcoR1-ACA/Mse1-CAG and EcoR1-AAG/Mse1-CTC) were used for all individuals. The fragments were separated and visualised as bands on acrylamide gels using an AB377 Automated Sequencer. Only bands with sizes ranging from 50–500 base pairs (bp) were included in the analysis as bands outside this size range cannot be accurately sized. Data extraction from the gels was carried out using Genescan 2·1 and Genotyper 2·0. Nested analyses were then carried out. In the first, all samples of the three species were analysed with representative hybrids, and in the second all samples of *S. triqueter* and *S. tabernaemontani* and the hybrids were included (see Results). The bands were scored as either present (1) or absent (0)

for each individual, resulting in a binary matrix which was analysed using the neighbor joining (NJ) algorithm in the software package PAUP (version 4·0d64 for Macintosh, Swofford 1998) and by principal coordinates analysis (PCO) in the R Package for Multivariate Analysis (version 4·0, Casgrain & Legendre 1999) using Jaccard's coefficient (Jaccard 1908).

RESULTS & DISCUSSION

Preliminary investigation of the AFLP traces showed that those for *S. lacustris* were quite distinct from those of *S. triqueter* and *S. tabernaemontani*; representative traces showing this are presented in Fig. 1. This high level of distinctness can cause problems in the assessment of homology of individual fragments, and for this reason we chose to carry out nested analyses. The first analysis was used to investigate whether *S. lacustris* was implicated as a parent of any of the hybrids and the second (excluding *S. lacustris*) was used for a detailed study of the hybrids and their relationships to each other and to *S. triqueter* and *S. tabernaemontani*.

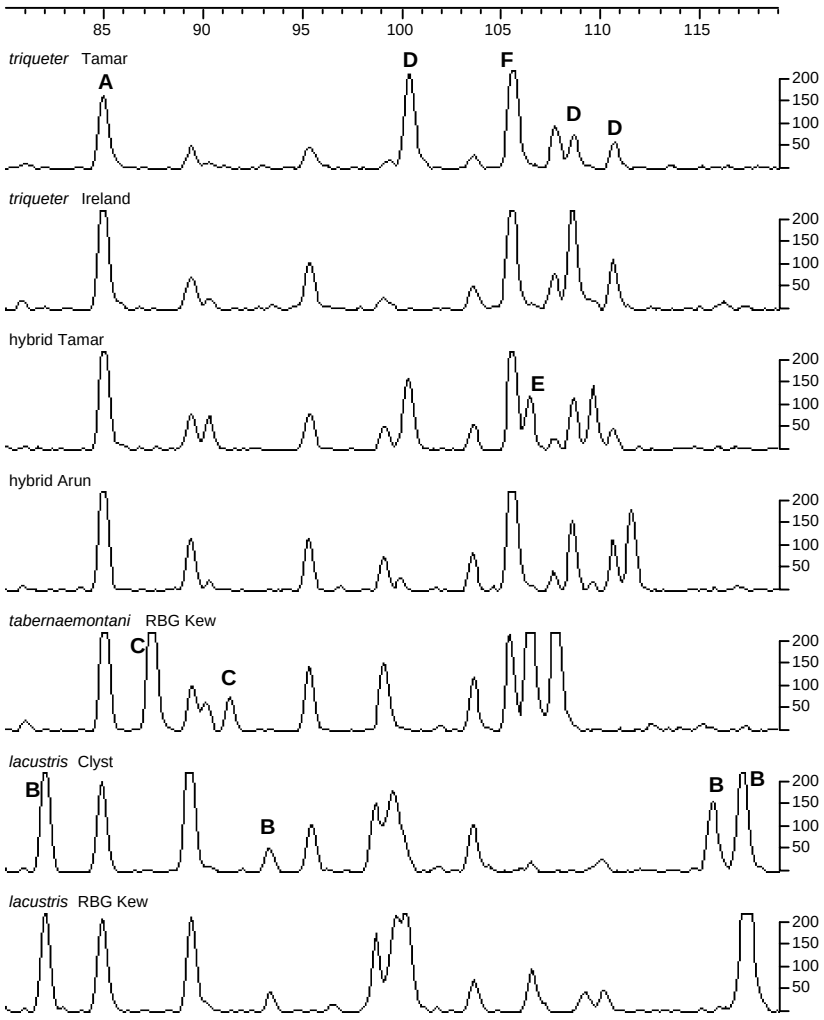

FIGURE. 1. Representative AFLP traces for three *Schoenoplectus* spp. and putative hybrids. Different classes of bands are indicated by letters: A – found in all taxa, B – only found in *S. lacustris*, C – only found in *S. tabernaemontani*, D – only found *S. triqueter* + hybrids, E – found in *S. tabernaemontani* + hybrids, F – found in *S. triqueter* + *S. tabernaemontani* + hybrids. Numbers on the horizontal axis indicate the size of the fragments in base pairs. Numbers on the vertical axis indicate the strength of fluorescence in arbitrary units.

TABLE 2. THE TYPES OF BANDS AND THEIR RELATIVE FREQUENCIES

a) Analysis 1

Type of band[1]	Number	Percentage
L	46	23
L + TA + TR + H	41	21
TA	27	14
TA + TR + H	23	12
TA + H	17	9
TR + H	12	6
L + TA + H	11	6
L + TA	6	3
H	6	3
TR	4	2
L + TR + H	3	1
Total	196	100

b) Analysis 2

Type of band[1]	Number	Percentage
TA + TR + H	64	45·1
TA + H	27	19
TA	26	18·3
TR + H	12	8·5
H	10	7·0
TR	2	1·4
TA + TR	1	0·7
Total	142	100

[1] L = *S. lacustris*, TA = *S. tabernaemontani*, TR = *S. triqueter*, H = hybrids

In the first analysis, 196 bands were scored, of which 25 were found in all individuals of the three species and in the hybrids. The bands were of eleven types, and these types and their relative frequencies are shown in Table 2a. The dendrogram is shown in Fig. 2 and the plot of the first two coordinates from PCO (accounting for 49 and 19% of the variation, respectively) is shown in Fig. 3. *Schoenoplectus lacustris* was clearly the most distinct taxon in this analysis; this is indicated by the high number of bands unique to this species (Fig. 1, Table 2a) and by its isolated position in Figs. 2 and 3. These data confirm the treatment of *S. lacustris* and *S. tabernaemontani* as distinct species, as recommended by Stace (1997), among others.

There was no evidence of *S. lacustris* being involved in the parentage of any of the hybrids examined in this study as no bands were shared exclusively by *S. lacustris* and the hybrids. (This does not prove that *S.* × *carinatus* does not occur in England, only that we did not sample it if it does). Samples collected as ?*S. lacustris* on the Arun (Site 33) and as ?*S. tabernaemontani* (MCS128) on the Thames (v.c. 21) were clearly of hybrid origin. Examination of herbarium material for the latter sample confirmed the absence of any of the main morphological features generally considered to be diagnostic of the hybrids. However, the sample appeared to be mostly sterile (as reported by Lousley 1931, 1975) and it is possible the hybrids show more variability in their vegetative parts than is indicated in the keys, making the characters used to distinguish them unreliable. Based on the sterility of the specimen from the Thames and the genetic results we would conclude that the specimen is a hybrid. This is in line with the observations made by Peter Nicholson of widespread morphological plasticity (even within individual clumps) and difficulty in deciding whether material was of pure species or hybrid origin when he was collecting material

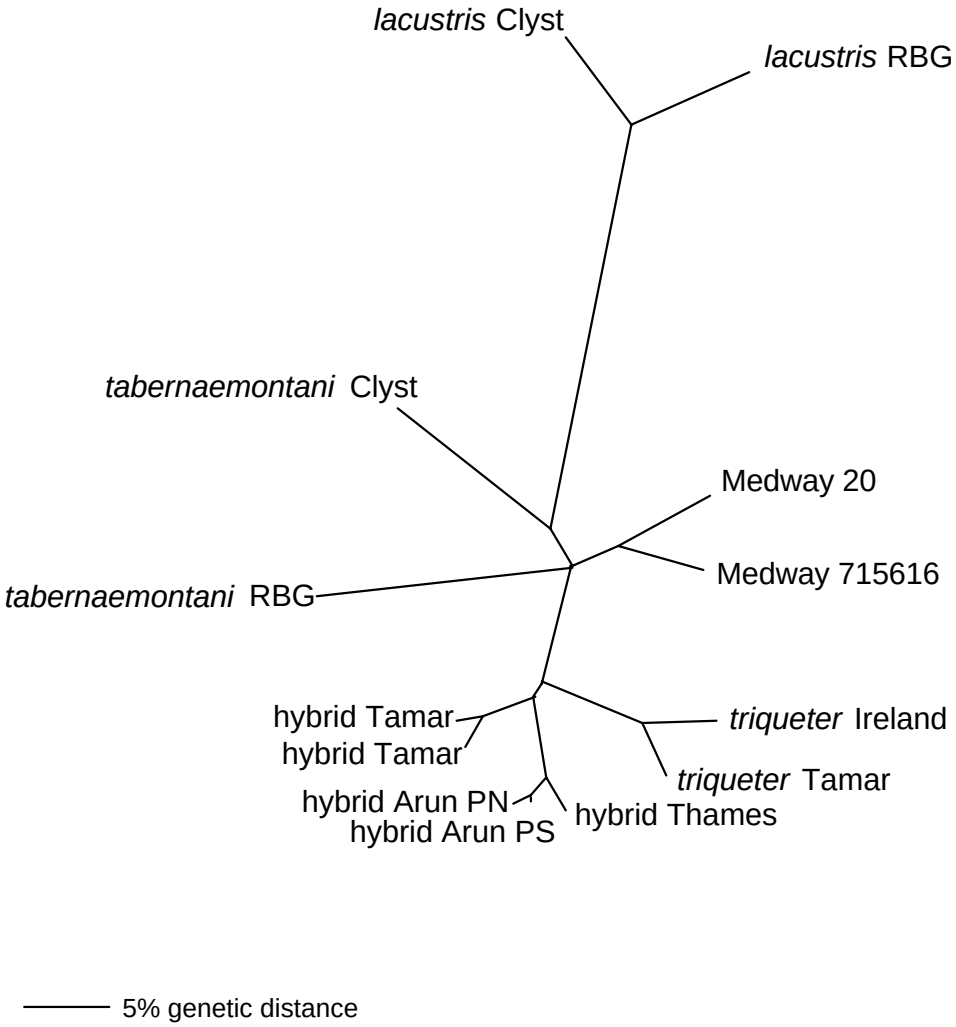

FIGURE 2. Neighbour joining analysis of *Schoenoplectus* spp. and putative hybrids. Note that Medway 20 and 715616 appear to be *S. tabernaemontani*, not hybrids.

for this project (P. Nicholson, pers. comm.). Lousley (1975) stated that the record of *S.* × *kuekenthalianus* from the Thames by Perring & Sell (1968, p. 145) required confirmation. The data presented in this paper provide that confirmation.

In the second analysis, 142 bands were scored, of which 42 were present in all individuals of *S. tabernaemontani*, *S. triqueter* and hybrids. The bands were of seven types, and these types and their relative frequencies are shown in Table 2b. There was strong evidence of additivity in the hybrids between the bands found in the two putative parents, *S. tabernaemontani* and *S. triqueter*. The samples of *S. triqueter* were genetically very close to each other, despite the obvious size difference even in cultivated material, whereas *S. tabernaemontani* was more variable. In addition, *S. triqueter* had far fewer unique bands than *S. tabernaemontani* (two vs. 26), and both bands unique to *S. triqueter* were found only in the sample from the Shannon. The sample collected as a putative hybrid on the Medway (Site 10) does not appear to be of hybrid origin as it clustered with the samples of *S. tabernaemontani*. The NJ dendrogram is shown in Fig. 4 and the plot of the first two coordinates from PCO (accounting for 38 and 17% of the variation, respectively) is shown in Fig. 5.

TABLE 3. COMPARISON OF FIELD IDENTIFICATION AND RESULTS OF GENETIC STUDIES. THE THREE CASES WHERE THE FINGERPRINTING RESULT DID NOT AGREE WITH THE FIELD IDENTIFICATION ARE SHOWN IN BOLDFACE

Sample information	Field identification	Fingerprinting result
ex hort. Kew	*lacustris*	*lacustris*
ex hort. Kew	*tabernaemontani*	*tabernaemontani*
ex hort. Nat. Mus. Wales	*triqueter*	*triqueter*
Tamar	*triqueter*	*triqueter*
Site 1, Calstock, Tamar	hybrid	hybrid[1]
Site 2, Calstock Bridge, Tamar	hybrid	hybrid
Site 3, cottage sites, Tamar	hybrid	hybrid
Site 4, right bank, Tamar	hybrid	hybrid
Site 5, Tamar	hybrid	hybrid
Site 6, Morwellham, Tamar	hybrid	hybrid
Exe catchment, Clyst	*lacustris*	*lacustris*
Exe catchment, Clyst	*tabernaemontani*	*tabernaemontani*
Arun site 1 0284 0844	hybrid	hybrid
Arun site 2 0284 0845	hybrid	hybrid
Arun site 4	hybrid	hybrid
Arun site 5 0305 0928	hybrid	hybrid
Arun site 8 0286 1006	hybrid	hybrid
Arun site 33	**?*lacustris***	**hybrid**
Arun site 61 0322 1628	hybrid	hybrid
Arun 0255 1175	? hybrid	hybrid
Arun 025 1118	? hybrid	hybrid
Arun 028 098	? hybrid	hybrid
Medway 715 616	*tabernaemontani*	*tabernaemontani*
Medway site 10 7132 6143	**hybrid**	***tabernaemontani***
Medway site 20 7129 6024	*tabernaemontani*	*tabernaemontani*
Thames (Chiswick) MCS128	**?*tabernaemontani***	**hybrid**

[1] 'hybrid' in this column refers to *S.* × *kuekenthalianus* (*S. tabernaemontani* × *S. triqueter*)

One interesting aspect of the data presented in Table 2b is the seeming imbalance between the proportions of bands shared between the hybrids and each of the putative parents. Out of the 79 bands found in *S. triqueter*, only three (<4%) were not shared with any hybrids. All three of these bands were only found in the Irish material and not in English *S. triqueter*, and thus 100% of bands found in English *S. triqueter* are present in at least some of the hybrids. In contrast, out of the 118 bands found in *S. tabernaemontani*, 27 (23%) were not shared with any of the hybrids. This same imbalance was already apparent in the sampling studied in the earlier report on material from the Tamar (Fay 1998), in which the hybrids shared 97% of bands with *S. triqueter* and 83% with *S. tabernaemontani*. In other situations where hybrids have been examined using AFLPs, the proportion of bands shared with each parent is variable (Table 4). In a recent study of hybrids in the *Sorbus latifolia* (Lam.) Pers. complex, thought to result from crosses between *S. aria* (L.) Crantz and *S. torminalis* (L.) Crantz, 53 out of 57 bands (93%) found in *S. aria* and 60 out of 66 (91%) found in *S. torminalis* were found in the hybrids examined (Fay *et al*. 2002). In *Dactylorhiza* allotetraploids, thought to result from hybridisation between *D. incarnata* (L.) Soó *s. l.* and *D. fuchsii* (Druce) Soó, a similar pattern to that found in *Schoenoplectus* was found, with 75 out of 79 bands (95%) found in *D. incarnata s.l.* and 73 out of 93 (78%) found in *D. fuchsii* being found in the hybrids (Hedrén *et al*. 2001). In the *Sorbus* example, both parental species appear to be quite genetically uniform with relatively few variable bands. The same applies to *S. triqueter*, where the Irish and English samples are very similar, and to *D. incarnata s.l.*, where there are

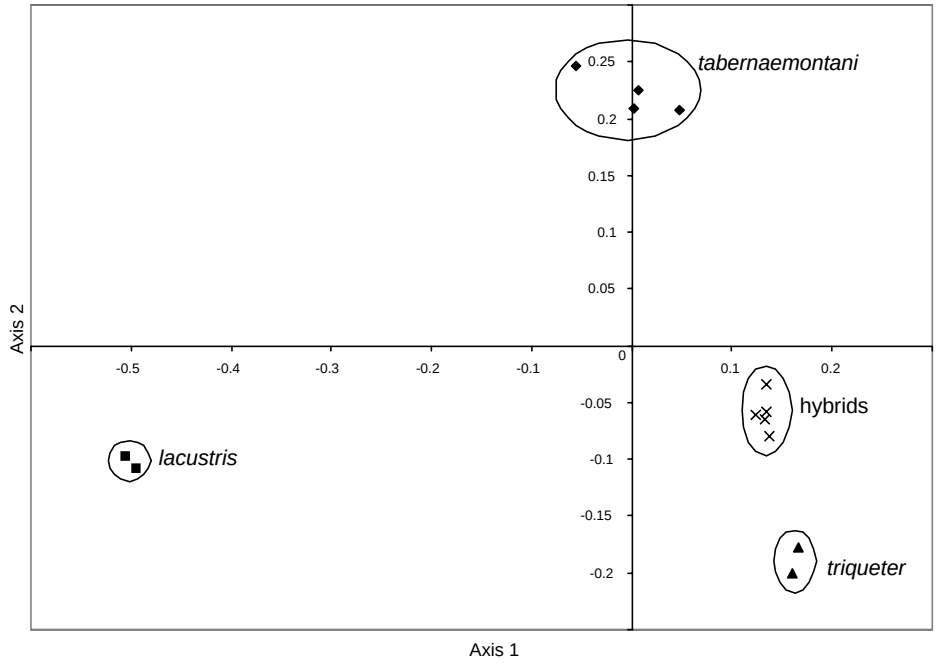

FIGURE 3. Plot of the first (x axis) and second (y axis) co-ordinates from the PCO of *Schoenoplectus* spp. and putative hybrids. The first and second co-ordinates account for 44·3 and 21·2% of the variation, respectively.

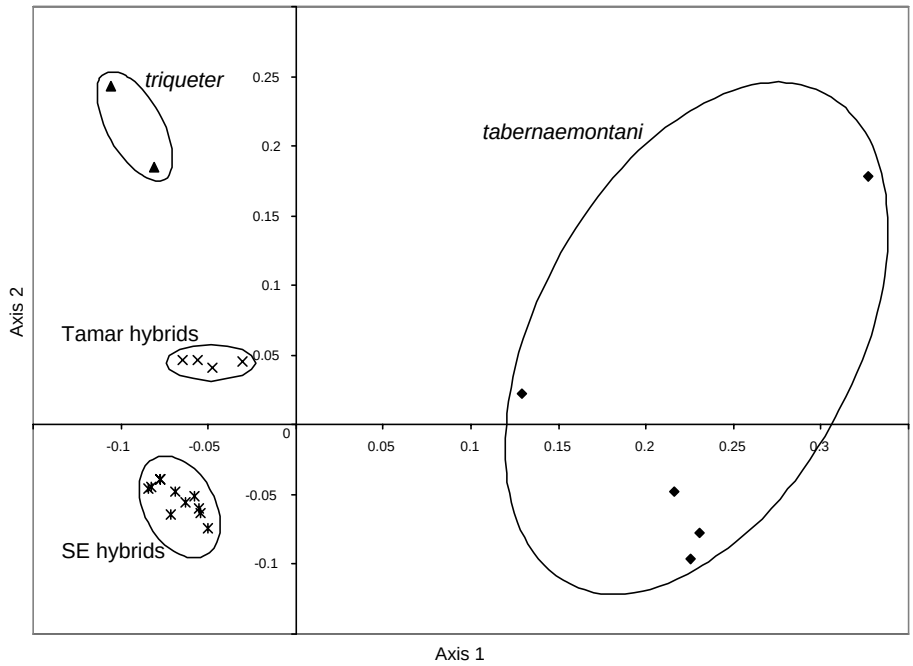

FIGURE 5. Plot of the first (x axis) and second (y axis) coordinates from the PCO of *Schoenoplectus* samples with full sampling of putative hybrids. The first and second coordinates account for 34·7 and 18·4% of the variation, respectively. SE = South-east England.

TABLE 4. COMPARISON OF PERCENTAGES OF AFLP BANDS INHERITED FROM PARENTAL SPECIES IN HYBRIDS OF *DACTYLORHIZA*, *SCHOENOPLECTUS* AND *SORBUS*

Hybrid/s	Bands from parent 1	Bands from parent 2	Reference
Dactylorhiza allotetraploids	95% (*D. incarnata*)	78% (*D. fuchsii*)	Hedrén *et al*. 2001
Schoenoplectus × *kuekenthalianus*	96% (*S. triqueter*)	77% (*S. tabernaemontani*)	this paper
Sorbus latifolia agg.	93% (*S. aria*)	91% (*S. torminalis*)	Fay *et al*., 2002

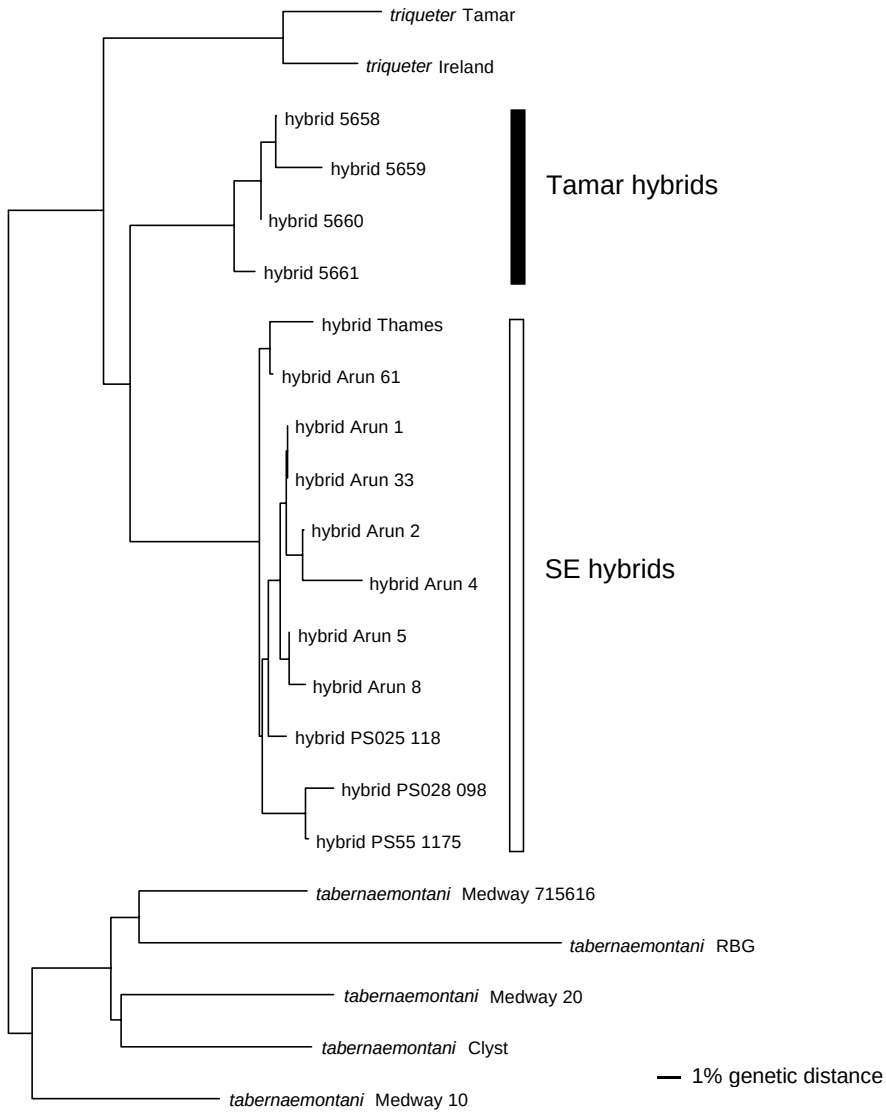

FIGURE 4. Neighbour joining tree with full sampling of hybrids showing relationships to *S. triqueter* and *S. tabernaemontani*. SE = South-east England.

relatively few variable bands even between morphologically distinguishable entities including *D. incarnata* subspp. *cruenta* (O. F. Muell.) P. D. Sell and *ochroleuca* (Boll) P. F. Hunt & Summerh. In contrast, the parent sharing a lower percentage of bands with the hybrids in both *Dactylorhiza* and *Schoenoplectus* (*D. fuchsii* and *S. tabernaemontani*) shows considerably more variation. This type of imbalance would be expected if one species was heterozygous at many loci and the other was highly homozygous, and this is the likely cause of the apparent tendency towards *S. triqueter* in this case, but the low number of samples of *S. tabernaemontani* studied so far means that our measurement of the levels of genetic variation in *S. tabernaemontani* is still provisional. In particular, further samples from the Medway should be included, given the high level of morphological and genetic diversity found in the samples of *S. tabernaemontani* tested so far and the absence of Medway hybrids in the current sampling. Samples from other areas would also be useful.

The close relationship between English and Irish material of *S. triqueter* indicates that this is probably a genuine case of a species with narrow genetic diversity, at least in the British Isles. In terms of reintroduction studies, material from the Tamar source is closer to the genotype that gave rise to the hybrids on the Arun and the Tamar, and it is thus probably appropriate to use Tamar material for such studies.

FUTURE WORK

Samples of hybrids from the Medway and further samples of *S. tabernaemontani* from a range of sites should be fingerprinted for inclusion in the analysis in order to investigate the level of variation within *S. tabernaemontani*. If possible, this should be supplemented with a study using microsatellites so that levels of heterozygosity can be assessed.

Further individuals of *S. triqueter* should be added to the analysis from plants derived from Tamar seed, the Shannon and from continental populations. This would allow investigation of the apparently low level of variation and high level of homozygosity in this species.

Sequencing studies of loci from the plastid genome should be carried out to determine the direction of the hybridisation. Finally, cytogenetic studies should be undertaken to confirm the hybrid nature of the samples shown to be hybrids with AFLPs but which have the morphology of *S. tabernaemontani*.

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