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Genetic differentiation and hybridisation among subspecies of Deergrass (*Trichophorum cespitosum* (L.) Hartman) in Northumberland

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ABSTRACT

A preliminary survey of genetic variation in Northumberland populations of *Trichophorum cespitosum* (L.) Hartman (Cyperaceae) was carried out using isozyme electrophoresis. Isozyme markers were found which differentiated between the two subspecies (subsp. *cespitosum* and subsp. *germanicum).* These markers were also used to show that sterile, morphologically intermediate plants growing in Northumberland are hybrids between subsp. *cespitosum* and subsp. *germanicum.* Based on allelic variation in the parental taxa and the hybrid for the AAT enzyme system, we suggest that the hybrid has arisen on more than one occasion (i.e. it is the product of multiple gamete fusions).

KEYWORDS: isozymes, Cyperaceae, multiple origins, molecular systematics.

INTRODUCTION

In Europe, two different forms of Deergrass (*Trichophorum cespitosum* (L.) Hartman, Cyperaceae) are recognised, namely subsp. *cespitosum* and subsp. *germanicum* (Palla) Hegi. They are primarily distinguishable by sheath morphology and stem anatomy (De Filipps 1980). The two subspecies also have somewhat different ranges; subsp. *germanicum* has an atlantic-subatlantic distribution, whereas subsp. *cespitosum* is more circumpolar (Hultén 1962; Meusel *et al.* 1965). In Britain, while subsp. *germanicum* is common and widespread, subsp. *cespitosum* has been considered either a rare plant of uncertain status (Clapham *et al.* 1987), or lacking substantiated records (Sell & Murrell 1996; Stace 1991). Recent studies by G. A. Swan, however, have provided strong morphological and anatomical evidence for the occurrence of subsp. *cespitosum* in Northumberland (Stace 1997; Swan 1999). Furthermore, Swan (1999) reported the presence of sterile, morphologically intermediate plants between subsp. *germanicum* and subsp. *cespitosum* and suggested that these are of hybrid origin (*T. cespitosum* nothosubsp. *foersteri* G. A. Swan (*T. cespitosum* subsp. *cespitosum* × *T. cespitosum* subsp. *germanicum*)).

Both of the subspecies are rhizomatous hermaphrodite perennials (Clapham *et al.* 1987), although some monoecious plants have also been reported (Hegi 1909). Examination of *Trichophorum cespitosum* in Northumberland revealed that some plants of subsp. *germanicum* are also proliferous, with small plantlets formed in the spikelets. The same phenomenon occurs in some populations of the intermediate plants, but it has not been observed in subsp. *cespitosum* in Northumberland (Swan 1999).

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CHROMOSOME NUMBERS

Trichophorum cespitosum subsp. *cespitosum* has a most commonly reported chromosome number of 2n = 104 (Jörgenson *et al.* 1958; Löve & Löve 1956; Scheerer 1940). We are not aware of any chromosome counts made on *T. cespitosum* subsp. *germanicum*. Cytological studies of *Trichophorum* populations in Northumberland are currently being carried out at the University of Newcastle-upon-Tyne by A. J. Richards. Although at an early stage, and hampered by the technical difficulties of accurately counting numerous small chromosomes, the initial results suggest that individuals of both subsp. *germanicum* and subsp. *cespitosum*, as well as nothosubsp. *foersteri*, may occur at different ploidy levels (A. J. Richards, pers. comm., 1997).

MOLECULAR MARKERS

While the morphological and anatomical studies of Swan (1999) provide strong support for the presence of two subspecies and hybrid plants of *Trichophorum cespitosum* in Northumberland, it is desirable, given the morphologically similarity of the different taxa, to have an additional line of evidence based on molecular data to confirm this.

Molecular markers are now routinely used to study hybridisation among plants and to investigate the delimitation of morphological similar taxa (Hollingsworth *et al.* 1995b, 1996; Liston *et al.* 1990; Newbury & Ford-Lloyd 1993; Rieseberg & Ellstrand 1993). They can provide powerful insights into the occurrence and frequency of hybridisation, and can be used to examine key evolutionary processes such as introgression and speciation (Abbott 1992; Arnold *et al.* 1991; Ashton & Abbott 1992; Raybould *et al.* 1991a & b; Roose & Gottlieb 1976; Wang & Szmidt 1990). Although a wide array of DNA-based molecular markers are now available and frequently used, isozyme electrophoresis remains a popular choice for biosystematic studies, due to the simplicity with which large numbers of individuals can be screened for genetic polymorphisms. Isozymes represent discrete, non-environmentally malleable markers that show straightforward co-dominant mendelian inheritance. If fixed allelic differences can be identified in the parental species, putative hybrids can be examined for the expected additive inheritance of these markers. The technique has been successfully used to identify the parentage of a number of different hybrid taxa, as well as providing information on the number of times such hybrids may have arisen (e.g. Gallez & Gottlieb 1982; Hollingsworth *et al.* 1995b, 1996; Raybould *et al.* 1991a).

In this study we have used isozymes to show that in Northumberland, *Trichophorum cespitosum* subsp. *cespitosum* is genetically distinct from subsp. *germanicum*, and that the sterile intermediate plants are hybrids between these taxa, and have arisen on more than one occasion.

MATERIALS AND METHODS

Plant material was collected from the localities listed in Table 1. These samples represent a range of populations of subsp. *germanicum* and proliferous and non-proliferous nothosubsp. *foersteri* in the Northumberland area. Additionally, two populations of subsp. *cespitosum* were sampled. Three Scottish populations of subsp. *germanicum* were included in the analysis to provide more geographically distant material for comparison. All samples were analysed for genetic variation using standard starch gel electrophoresis. Eight enzyme systems were investigated: AAT, G6PDH, PGI, PGD, PGM, SKD, MDH and IDH. G6PDH, PGM, PGI, PGD, AAT and SKD were resolved using a lithium borate buffer system (pH 8·1); MDH and IDH were resolved using a morpholine citrate buffer system (pH 8·0). All electrophoretic conditions were as described by Hollingsworth *et al.* (1995a, b) except MDH which was visualised using 50 ml 0·1M Tris-HCl pH 8·5, 750 mg malic acid sodium salt, 10 mg NAD, 10 mg MTT and 3 mg PMS.

Interpretation of enzyme banding patterns in terms of loci and alleles was based on expectations of conserved isozyme number, quaternary structure and sub-cellular compartmentalisation (Gottlieb 1981, 1982; Weeden & Wendel 1989). In the absence of progeny analyses and considering the high chromosome numbers of all of the taxa, as well as the potential for intra-taxon euploid variability (see Introduction), locus and allele designations remain putative and we avoid interpretations of the data that are ploidy-level dependent. Numerical codes to indicate multi-locus genotypes have been given to all samples (two samples sharing a multi-locus genotype have the same banding pattern for all enzyme systems).

TABLE 1. SAMPLE LOCALITIES OF *TRICHOPHORUM CESPITOSUM* (L.) HARTMAN

	Population	Code	V.c.	Grid ref.	Sample size
a. Subsp. *germanicum*	Ottercops Moss	a	67	NY/946.893	3
	Nr Gowany Knowe	b	67	NY/735.789	1
	Langleeford	c	68	NT/945.233	1
	Padon Hill (1)	d	67	NY/817.924	1
	Padon Hill (2)	e	67	NY/816.928	5
	Battle Hill	f	67	NY/950.915	1
	Creag nan Gall, Balmoral	Sco 1	92	NO/271.916	5
	Buailteach, Balmoral	Sco 2	92	NO/277.932	5
	Gleann Beag	Sco 3	89	NO/137.756	5
b. Nothosubsp. *foersteri* (non-proliferous)	Ottercops Moss	n	67	NY/948.896	1
	Greenleighton Moss	g	67	NZ/016.923	1
	Gowany Knowe	h	67	NY/731.789	2
	Felecia Moss	j	67	NY/722.775	3
	Blackheugh End	k	67	NY/827.915	1
c. Nothosubsp. *foersteri* (proliferous)	Ottercops Moss	n	67	NY/948.896	2
	Felecia Moss	j	67	NY/722.775	3
d. Subsp. *cespitosum*	Margin of Gowany Knowe	m	67	NY/727.788	4
	Blackheugh End	k	67	NY/827.915	6

TABLE 2. ISOZYME VARIATION IN EIGHT ENZYME SYSTEMS IN *TRICHOPHORUM CESPITOSUM* (L.) HARTMAN

Uppercase letters correspond to zymograms in Fig. 1. Population codes refer to Table 1.

	Code	AAT	G6PD	MDH	IDH	PGD	PGI	PGM	SKD	MLG	Sample size
a. Subsp. *germanicum*	a	A	A	A	A	A	A	A	A	1	3
	b	A	A	A	A	A	A	A	A	1	1
	c	A	A	A	A	A	A	A	A	1	1
	d	A	A	A	A	A	A	A	A	1	1
	e	A	A	A	A	A	A	A	A	1	5
	f	A	A	A	A	A	A	A	A	1	1
	Sco1	A	A	A	A	A	A	A	A	1	5
	Sco2	A	A	A	A	A	A	A	A	1	5
	Sco3	A	A	A	A	A	A	A	A	1	5
b. Nothosubsp. *foersteri* (non-proliferous)	n	A	A	B	B	B	B	A	A	2	1
	g	A	A	B	B	B	B	A	A	2	1
	h	A	A	B	B	B	B	A	A	2	2
	j	B	A	B	B	B	B	A	A	3	3
	k	A	A	B	B	B	B	A	A	2	1
c. Nothosubsp. *foersteri* (proliferous)	n	A	A	B	B	B	B	A	A	2	2
	j	B	A	B	B	B	B	A	A	3	3
d. Subsp. *cespitosum*	m	C	A	C	C	C	C	A	A	4	2
	m	A	A	C	C	C	A	A	A	5	2
	k	A	A	C	C	C	B	A	A	6	3
	k	B	A	C	C	C	C	A	A	7	3

MLG = multi-locus genotype.

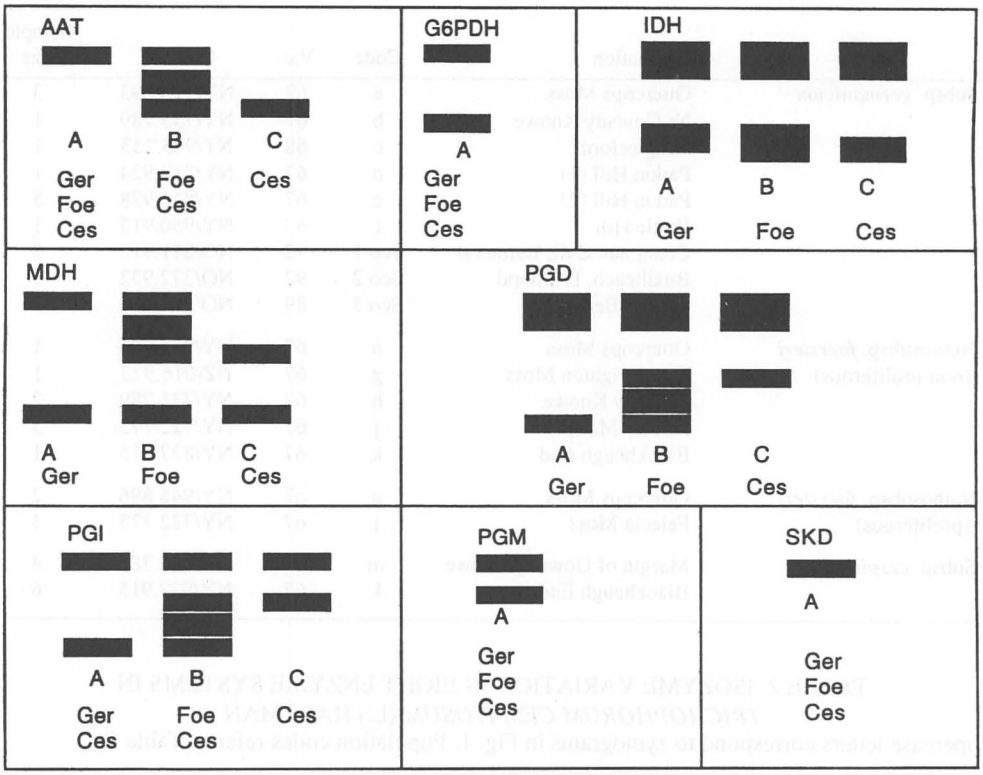

FIGURE 1. Zymogram representing banding patterns in *Trichophorum cespitosum* (L.) Hartm. in Northumberland (anode towards the top of the figure). Letters below the banding patterns represent a single enzyme genotype, the distribution of these among samples are given in Table 2. Ger = subsp. *germanicum*, Foe = nothosubsp. *foersteri* and Ces= subsp. *cespitosum*.

RESULTS

ENZYME PHENOTYPES

From the eight enzyme systems examined, a minimum of eleven anodally migrating loci was clearly resolved (Fig. 1 and Table 2). These are described in turn below.

AAT - a single putative locus was resolved, although there was a faint trace of a second faster migrating locus on the gel. At the locus that was clearly resolved, all samples of subsp. *germanicum* were homozygous for a fast allele, nothosubsp. *foersteri* was either homozygous for this fast allele, or heterozygous for this and a slower allele. Subsp. *cespitosum* showed all possible combinations of the two alleles.

G6PDH - two putative loci were resolved with both being homozygous and uniform in all three taxa.

IDH - two putative loci were resolved with the most anodally migrating locus being uniform across all samples. The other locus was variable, although resolution was poor. All individuals of subsp. *germanicum* have a fast moving zone of activity, all individuals of subsp. *cespitosum* have a slow moving zone of activity, and all individuals of nothosubsp. *foersteri* have a smear which corresponds to both zones of activity.

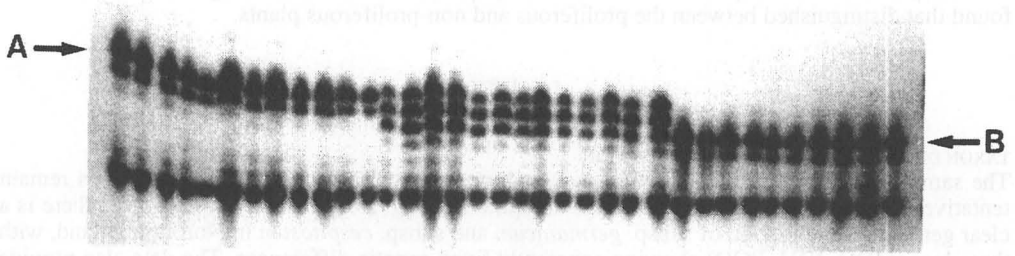

FIGURE 2. MDH evidence for hybridisation between *Trichophorum cespitosum* subsp. *cespitosum* and subsp. *germanicum* in Northumberland (anode towards the top of the figure). Two loci are shown with all taxa being homozygous and uniform for the least anodally migrating locus. At the most anodally migrating locus, all individuals of subsp. *germanicum* (from left to right, lanes 1–12) are homozygous and uniform for a fast moving allele (marked A), all individuals of subsp. *cespitosum* (lanes 26–35) are homozygous and uniform for a slow moving allele (marked B), and all individuals of nothosubsp. *foersteri* (lanes 13–25) show a heterozygous genotype (including a heterodimer) for these two alleles. Bands present just behind these two different alleles are considered to be artifactual and do not represent additional alleles or loci.

MDH - two putative loci were resolved with all taxa being homozygous and uniform for the least anodally migrating locus. At the most anodally migrating locus, all samples of subsp. *cespitosum* were homozygous and uniform for a slow moving allele, all individuals of subsp. *germanicum* were homozygous and uniform for a fast moving allele, and all samples of nothosubsp. *foersteri* showed a heterozygous genotype for these two alleles (Fig. 2).

PGD - two putative loci were detected, the most anodally migrating was invariant across all samples. At the second locus, all samples of subsp. *cespitosum* were homozygous for a fast moving allele, all samples of subsp. *germanicum* were homozygous for a slow moving allele and all samples of nothosubsp. *foersteri* were heterozygous for these two alleles.

PGI - two putative loci were detected with the most anodally migrating locus being poorly resolved but apparently uniform. At the least anodally migrating locus, all samples of subsp. *germanicum* were homozygous and uniform for a slow moving allele, all samples of nothosubsp. *foersteri* were heterozygous for this and a fast moving allele. Subsp. *cespitosum* showed all possible combinations of the two alleles.

PGM - all taxa were monomorphic for two bands. As PGM is a monomeric enzyme it is not clear whether this represents all samples being homozygous and uniform for two separate loci, or fixed heterozygosity at a single locus.

SKD - One putative locus was detected at which all taxa were homozygous and uniform.

VARIATION BETWEEN TAXA

Subsp. *cespitosum* and subsp. *germanicum* in Northumberland are clearly distinguishable by fixed genetic differences for three enzyme systems MDH, PGD and IDH. For all of these enzyme systems, nothosubsp. *foersteri* shows apparent direct additive inheritance of these taxon-specific markers.

VARIATION WITHIN TAXA

All individuals of subsp. *germanicum* showed the same multi-locus enzyme genotype, and at the loci where allelic designations could be made, all samples were homozygous.

Subsp. *cespitosum* showed diallelic variation for two enzyme systems (AAT and PGI). A total of four different genotypes was identified, two from each of the two populations sampled (Fig. 1, Table 2). For these variable loci, both homozygous and heterozygous genotypes were recovered, although the small sample sizes and our cytological ignorance preclude tests for deviations from random mating based on the Hardy-Weinberg principle.

A total of two different genotypes of nothosubsp. *foersteri* was detected based on variation at one enzyme system (AAT). No intra-population variation was detected, with four of the populations having one of the genotypes, and the other genotype being confined to the fifth population (Fig. 1, Table 2). It should be borne in mind, however, that for two populations, only one individual plant

was sampled and the sample sizes for the other populations are small. No genetic markers were found that distinguished between the proliferous and non-proliferous plants.

DISCUSSION

TAXON DIFFERENTIATION AND HYBRIDISATION

The sample sizes used in this study are low and we stress that the following conclusions remain tentative. Nevertheless, the initial findings are quite striking. Isozyme evidence suggests there is a clear genetic differentiation of subsp. *germanicum* and subsp. *cespitosum* in Northumberland, with three loci (MDH, IDH, PGD) showing apparently fixed genetic differences. The data also provide strong support for nothosubsp. *foersteri* being of hybrid origin from the two subspecies (Figs 1 & 2). The absolute concordance of data from these loci between populations (including comparisons with Scottish populations) suggests that these samples are representative for British plants, although of course further verification including wider geographic sampling is desirable.

Given the fixed genetic differences between the subspecies (based on this sample) and the sterility of the hybrid, this raises the question as to whether the subspecies should be raised to specific rank. We cannot commit ourselves to this based on such a small sample, but point out that if more intensive studies support the conclusions from this work, namely a. that there are clear fixed differences between the two sub-species, and b. that the hybrids are sterile, then specific rank for subsp. *germanicum* and subsp. *cespitosum* should be considered. However, it is worth stressing that the question of the sterility of nothosubsp. *foersteri* remains open for discussion. Although no mature fruits have ever been seen on plants of nothosubsp. *foersteri*, Swan (1999) noted that there is often high stainability of its pollen; he also reported the presence of *Trichophorum* plants growing in Northumberland that appear to be intermediate between nothosubsp. *foersteri* and subsp. *germanicum*. These may represent backcrosses and there is an obvious need to study these plants to determine whether their isozyme profiles support this suggestion.

Any possibility of trace fertility in nothosubsp. *foersteri* potentially complicates interpretations of the number of origins of the hybrid. AAT variation between plants of nothosubsp. *foersteri* could indicate that the hybrid has arisen on at least two occasions, i.e. it is the product of more than one successful gamete fusion (Fig. 1). We feel that this, rather than hybrid fertility, is the most likely explanation for the genetic variation in the samples of the hybrid examined in this study, as (a.) no disruption of the taxon specific markers (MDH, IDH, PGD) was detected amongst the hybrids, and (b.) the variation for AAT is exactly as would be predicted based on crosses between the fixed fast band of subsp. *germanicum* and both the fast and the slow alleles from subsp. *cespitosum*. In this respect it is noteworthy that the homozygous slow condition is not detected in nothosubsp. *foersteri* due to the invariant fast allelic constitution of subsp. *germanicum*.

Variation of PGI in subsp. *cespitosum* indicates that the nothosubsp. *foersteri* plants in this study result from a cross involving a subsp. *cespitosum* plant or plants with the fast PGI allele, as no individuals of nothosubsp. *foersteri* homozygous for the slow allele were observed.

Our inability to distinguish between the proliferous and the non-proliferous forms of nothosubsp. *foersteri* using isozymes may simply be a result of not sampling enough loci, and these different forms may represent additional origins of the hybrid. The difference in spikelet morphology between the two forms is quite striking, with the bristles in the non-proliferous plants being replaced by a membranous perianth in the proliferous plants. However, environmentally induced variation in spikelet morphology and function is far from unknown, and the transition from non-prolifery to prolifery is well documented (e.g. as in *Deschampsia cespitosa* (L.) P. Beauv.; Briggs & Walters 1984). The proliferous plants sampled here showed identical multi-locus genotypes with their sympatric non-proliferous counterparts (Table 2). In the absence of information from more molecular markers, and given our lack of understanding of what the mechanism would be if prolifery was under genetic control, it is difficult to comment further.

VARIATION WITHIN TAXA

The uniformly homozygote genotype detected within subsp. *germanicum* precludes us from distinguishing between clonal growth and sexual reproduction (be it self-pollination, mixed mating or outcrossing) as the major reproductive mode. The presence of hybrids with subsp. *cespitosum*, however, indicate that reproduction is probably not purely asexual or purely autogamous.

Large-scale sexual reproduction by nothosubsp. *foersteri* is considered unlikely based on the results of this isozyme survey and the fact that mature fruits have never been found (as discussed above). A more probable explanation is that nothosubsp. *foersteri* persists via a combination of clonal propagation and recurrent origins from the parental subspecies. Further sampling is required, however, to substantiate this.

Diallelic variation and the presence of both homozygote and heterozygotes for AAT and PGI in subsp. *cespitosum* provides good evidence for sexual reproduction with at least some outcrossing in this taxon. That more variation was detected in subsp. *cespitosum,* which is local and rare in Britain, than in the common and widespread subsp. *germanicum* is surprising and we as yet have no explanation for this apparent paradox. A greater sampling of both individuals and loci, set in the context of a firm cytological framework, may offer further insights into the reproductive biology and population biology of these taxa.

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