

Identification of British species of *Callitriche* by means of isozymes

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ABSTRACT

Isozyme variation was investigated among the eight species of *Callitriche* L. (Callitrichaceae) native to Britain. By this means, nearly all the species could be distinguished, particularly by the enzyme systems GPI and PGD. Although *C. brutia* Petagna and *C. hamulata* Kütz ex W. D. J. Koch could be separated from the other species they were not separable from each other. A case study involving a survey of four Norfolk rivers revealed only three species: *C. stagnalis* Scop., *C. obtusangula* Le Gall and *C. platycarpa* Kütz., of which the latter two were by far the most frequent.

KEYWORDS: allozymes, Callitrichaceae, distribution, taxonomy.

INTRODUCTION

Most British species of *Callitriche* L. are capable of living either submerged in water or on land in damp mud. This amphibious behaviour is associated with considerable phenotypic plasticity, particularly of vegetative features. Reproductive parts, especially ripe fruits and pollen, are required before material can be determined with any great confidence, and even then *C. brutia* Petagna and *C. hamulata* Kütz ex W. D. J. Koch cannot always be distinguished in the field (Schotsman 1972). Furthermore, when growing completely submerged, species such as *C. stagnalis* Scop., *C. obtusangula* Le Gall. and *C. platycarpa* Kütz., do not flower and, as a result, cannot be identified. In very many cases, therefore, it has proved impossible to make firm identifications. In Britain numerous papers and communications at botanical meetings have highlighted the confusions and disagreements among botanists (Pearsall 1935; Jones 1955; Meikle & Sandwith 1956; David 1958; Savidge 1960, 1967; Preston & Croft 1997).

One consequence of this has been uncertainty over which species are to be found in the British Isles. Even over the last 50 years, for example, *C. cophocarpa* Sendtn. or *C. palustris* L. have been credited to our flora by some authors but not by others (Clapham *et al.* 1952, 1962; Schotsman 1972; Haslam *et al.* 1982; Lansdown 1998; Kent 1992; Preston & Croft 1997; Stace 1991, 1997). These uncertainties were partly based on taxonomic problems regarding the distinction of *C. cophocarpa* from *C. platycarpa* (Savidge 1958; Schotsman 1967) and *C. lenisulca* Clavaud (Schotsman & Andreas 1974; Schotsman & Haldimann 1981). Another root of the problem has been the lack of authenticated voucher material, so it is of some interest, therefore, to note that *C. palustris* was discovered recently in Ireland (Lansdown & Bruinsma 1999) and then also in Scotland (by R. V. Lansdown) during the course of fieldwork for the present study. *Callitriche cophocarpa*, in contrast, is less likely to occur in Britain because of its primarily eastern European distribution (Savidge 1958; Schotsman & Haldimann 1981). Eight species of *Callitriche* are currently recognised as members of the British flora (Table 1).

A second consequence of not being able to identify species properly has been the difficulty in preparing reliable distribution maps (Preston & Croft 1997). Not only have there been frequent misidentifications, but also many fieldworkers have been unwilling to venture identifications at all, with the result that some species are probably under-recorded (Preston & Croft 1997; Lansdown 1998).

Although of no use in the field or herbarium, inter-specific variation in chromosome number (Table 1) has helped considerably with problems of identification, although one sometimes suspects an element of circular reasoning in some of the reports. A good example of a study that

TABLE 1. BRITISH SPECIES OF *CALLITRICHE* AND THEIR PUBLISHED CHROMOSOME COUNTS. ALL COUNTS BASED ON BRITISH MATERIAL EXCEPT FOR *C. PALUSTRIS*

Species	2n
<i>Callitriche hermaphroditica</i> L.	6
<i>Callitriche truncata</i> Guss.	6
<i>Callitriche stagnalis</i> Scop.	10
<i>Callitriche obtusangula</i> Le Gall	10
<i>Callitriche platycarpa</i> Kütz.	20
<i>Callitriche palustris</i> L.	20
<i>Callitriche brutia</i> Petagna	28
<i>Callitriche hamulata</i> Kütz.	38

has used chromosome number to help document the distribution of *Callitriche* species is that by Lewis-Jones & Kay (1977) who surveyed localities in Glamorgan. Nevertheless, with the exceptions of *C. brutia* and *C. hamulata*, no British species has a unique chromosome number and so this evidence cannot be used alone to determine identity.

The primary aim of the present study is to describe another method, using isozymes, by which vegetative material of *Callitriche* can be identified (Demars & Gornall 2001). Although equally inapplicable in the field or herbarium, the method does at least allow for the screening of large numbers of plants so that the true extent of morphological variation within each species can be assessed; this may then allow better descriptions to be drawn up and allow potentially diagnostic morphological characters to be described more precisely.

A secondary aim of the present study is to use the findings to help survey the distribution of *Callitriche* species in four Norfolk rivers (R. Wensum, Nar, Wissey and Bure). This survey forms part of a biodiversity inventory and biomonitoring scheme led by the Environment Agency (forthcoming R&D report P2-127).

MATERIALS & METHODS

PLANT MATERIAL

Reference material in fruiting and/or flowering condition was collected throughout Britain, mostly during summer 2000. Chromosome counts were made on certain plants in order to provide supporting evidence for identifications made on diagnostic morphological characteristics. Table 2 provides a summary of the reference populations sampled. Voucher specimens of most collections are preserved in **NMW** or **LTR** (Table 2). In many cases only a single plant was gathered per population, and our results must therefore be interpreted cautiously. Nevertheless, we have sampled a wide range of populations and furthermore, since most species are predominantly self-fertilising (Philbrick & Anderson 1992), we assume there is unlikely to be much intra-population variability. In Norfolk, collections were made from four river basins: those of the Rivers Wensum, Wissey, Nar and Bure, in 1999 and 2000. Details of these collections are given in Table 3.

CHROMOSOME COUNTS

Actively growing root-tips were pre-treated with 0.002M 8-hydroxyquinoline for ca20h in the field and fixed later using 3:1 absolute ethanol: glacial acetic acid. Root-tips were hydrolysed at room temperature in 5M HCl for 10 minutes before squashing, flaming and staining in aceto-orcein.

ISOZYME ELECTROPHORESIS

After collection, samples were kept cool (4–10°C) and processed usually within 5 days. Standard horizontal electrophoresis, on 12% starch gels, of isozymes from extracts of young, actively growing shoot apices was carried out as described by Wendel & Weeden (1989) using the extraction buffer of Hollingsworth *et al.* (1995). Two gel/electrode buffer systems were used: tris-borate-EDTA (TBE) (Wendel & Weeden 1989) and morpholine citrate (MC8) (Hollingsworth *et al.* 1995). Eight enzyme systems were studied: glucose-6-phosphate isomerase (GPI), malate

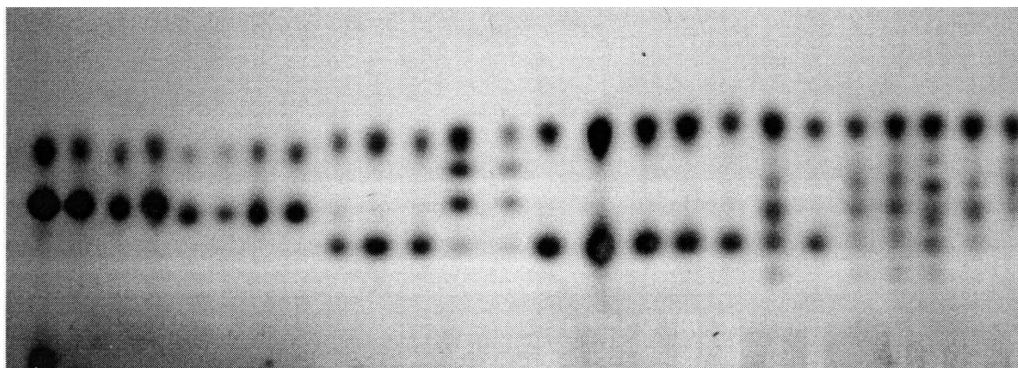


FIGURE 1. Glucose-6-phosphate isomerase (GPI) phenotypes in *Callitriche* species. (Figures in parentheses refer to the population reference numbers in Table 2.) Lanes 1–4, *C. hermaphroditica* (1, 2, 3, 4). Lanes 5–8, *C. truncata* (13, 12, 10, 11). Lanes 9–11, *C. stagnalis* (15, 16, 17). Lanes 12–13, *C. platycarpa* (33, 35). Lanes 14–18, *C. obtusangula* (31, 25, 22, 23, 26). Lane 19, *C. brutia* (41). Lane 20, *C. obtusangula* (27). Lanes 21–25, *C. hamulata* (48, 49, 50, 53, 54).

dehydrogenase (MDH), phosphogluconate dehydrogenase (PGD), phosphoglucumutase (PGM), aspartate aminotransferase (AAT), fructose-bisphosphate aldolase (FBA), isocitrate dehydrogenase (IDH) and malic enzyme (ME). AAT, GPI, FBA, IDH, ME and PGM were assayed using the TBE system, the others (MDH, PGD) using the MC8 system. Staining protocols followed Hollingsworth *et al.* (1995) for all enzymes except MDH, ME and FBA for which the recipes in Wendel & Weeden (1989) were used.

Preliminary interpretation of enzyme banding patterns in terms of alleles and loci was based on their conserved subunit structure and subcellular compartmentalization (Gottlieb 1981, 1982; Weeden & Wendel 1989) and a knowledge of the ploidy level of the species.

RESULTS

CHROMOSOME NUMBERS

Results of the chromosome number determinations are shown in Table 2. The counts obtained for the species examined, viz. *C. stagnalis* ($2n=10$), *C. obtusangula* ($2n=10$), *C. platycarpa* ($2n=20$), *C. brutia* ($2n=28$) and *C. hamulata* ($2n=38$), agreed with previous reports (Table 1).

ISOZYME PHENOTYPES

Variation within and between the species was such that species-specific isozyme phenotypes were identified only in GPI (Fig. 1), MDH and PGD. These are summarised in Fig. 2. As expected, diploid species (*C. hermaphroditica*, *C. truncata*, *C. stagnalis* and *C. obtusangula*) have the simplest phenotypes, consistent with the presence of two loci coding for each of the enzyme systems studied. Phenotypes indicative of heterozygotes were rare, as might be expected from the predominantly self-fertilizing nature of the species (Philbrick & Anderson 1992). The polyploid species (*C. platycarpa*, *C. palustris*, *C. brutia* and *C. hamulata*) usually have more complicated phenotypes, and showed evidence of heterozygosity in all samples studied.

Inspection of Fig. 2 shows that the variation allows identification of all species except *C. brutia* and *C. hamulata*, which cannot yet be distinguished from each other. Intraspecific variation in GPI or PGD occurred in *C. hermaphroditica*, *C. stagnalis*, *C. obtusangula*, *C. brutia* and *C. hamulata* (Fig. 2, Table 2). In the case of *C. hermaphroditica*, both large-fruited and small-fruited variants were sampled, but their respective isozyme phenotypes showed uncorrelated variation. In one of the cases where reference populations were sampled in some detail, variation between individuals was discovered, viz. *C. obtusangula* in the Grand Union Canal, Leicester (Table 2).

TABLE 2. DETAILS OF POPULATION LOCALITIES, SAMPLE SIZES (Ni), CHROMOSOME NUMBERS (2n) AND INFRA-SPECIFIC ISOZYME VARIATION IN GPI OR PGD (SEE FIG. 2 FOR PHENOTYPES)

Ref Species/population no.	Voucher ^a	Ni	2n	Infra-specific variation
				GPI PGD
<i>C. hermaphroditica</i>				
1 Worcs., v.c. 37, Chadwich Manor, SO975761	RL 750 & BD, 19 Jul 2000	1S ^b		B
2 Salop, v.c. 40, Colemere Mere, SJ435330	RL 751 & BD, 19 Jul 2000 (NMW)	1		A
3 Monts., v.c. 47, Montgomery Canal, SJ255197	RL 752 & BD, 19 Jul 2000 (NMW)	1		A
4 Monts., v.c. 47, Montgomery Canal, SJ237083	RL 753 & BD, 19 Jul 2000 (NMW)	1S		A
5 Lincs., v.c. 53, Swanholme lakes, SK945685	RL 766 & BD, 8 Aug 2000 (NMW)	1L ^b		B
6 W. Perth, v.c. 87, Lake of Menteith, NN583011	RL 771, BD & NW, 9 Aug 2000 (NMW)	1L		A
7 W. Perth, v.c. 87, Doune ponds, NN724023	RL 767 & BD, 9 Aug 2000	1L		B
8 E. Perth, v.c. 89, Loch Clunie, NO115437	RL 773 & BD, 10 Aug 2000 (NMW)	1L		A
9 E. Perth, v.c. 89, Fingask Loch, NO175428	RL 774 & BD, 10 Aug 2000	1L		B
<i>C. truncata</i>				
10 S. Devon, v.c. 3, River Dart, Totnes, SX802611	RL 740 & BD, 18 Jul 2000 (NMW)	1		
11 S. Devon, v.c. 3, River Axe, SY290982	RL 742 & BD, 18 Jul 2000 (NMW)	1		
12 S. Somerset, v.c. 5, Bridgewater & Taunton canal, Creech St Michael, ST275256	RL 736 & BD, 17 Jul 2000	1		
13 Leics., v.c. 55, Groby Pool, SK522083	BD (RL763), 21 Sep 1999 (NMW)	1		
<i>C. stagnalis</i>				
14 W. Cornwall, v.c. 1, SE of Grochall, SW699142	NS, 9 Mar 1996	3	10	D
15 S. Devon, v.c. 3, East Bovey Head, SX686824	RL 738 & BD, 17 Jul 2000 (NMW)	1		D
16 S. Devon, v.c. 3, River Dart, Totnes, SX803611	RL 741 & BD, 18 Jul 2000 (NMW)	1		D
17 S. Hants., v.c. 11, Ober Water, SU250039	RL 749 & BD, 18 Jul 2000 (NMW)	1		D
18 W. Norfolk, v.c. 28, River Tat, TF836312	BD, 27 Jul 1999 (LTR)	5		C
19 Cards., v.c. 46, Brynllynan, SN184482	AOC, 1997	4		D
20 Cards., v.c. 46, Pen-y-Graig, SN223519	AOC, 1997	2		D
21 Dunbarton, v.c. 99, Wards pond, NS443876	RL 769, BD & NW, 9 Aug 2000	2		C
<i>C. obtusangula</i>				
22 N. Somerset, v.c. 6, Gordano Valley, ST442733	RL 735 & BD, 17 Jul 2000 (NMW)	1		D
23 S. Hants., v.c. 11, Ober Water, SU258038	RL 746 & BD, 18 Jul 2000 (NMW)	1		D
24 E. Norfolk, v.c. 27, River Bure, Ingworth, TG193291	BD (RL 783), 15 Aug 2000	2		D
25 W. Norfolk, v.c. 28, Wendling Beck, TF966152	BD, 9 Jul 2000 (LTR)	1		D
26 W. Gloucs., v.c. 34, Over Pools, SO818194	RL 758 & BD, 20 Jul 2000 (NMW)	1		D
27 Cards., v.c. 46, Glan Rheidol, SN662793	RL & BD, 20 Jul 2000 (LTR)	1	10	D
28 Leics., v.c. 55, Grand Union Canal, Leicester, SK5702	RG, 1996	80	10	12D 68E
29 Leics., v.c. 55, Aylestone Meadows, SK5600	RG, 1996	37	10	E
30 Leics., v.c. 55, Saddington Brook, SP6691	RG, 1996	40	10	E
31 France, Deux-Sèvre, River Auxances, D59 bridge	BD (D59), 21 Jun 2000	1		D
<i>C. palustris</i>				
32 Dunbarton, v.c. 99, Wards pond, NS443876	RL 768, BD & NW, 9 Aug 2000 (NMW)	2		

TABLE 2. CONTINUED

Ref Species/population No.	Voucher ^a	Ni	2n	Infra-specific variation	
				GPI	PGD
<i>C. platycarpa</i>					
33 Dorset, v.c. 9, Stoborough, SY929867	RL 743 & BD, 18 Jul 2000 (NMW)	1			
34 E. Norfolk, v.c. 27, Saxthorpe, TG107306	BD (RL 784), 15 Aug 2000 (LTR)	1			
35 W. Gloucs., v.c. 34, Over Pools, SO818194	RL 757 & BD, 20 Jul 2000 (NMW)	1			
36 Leics., v.c. 55, Kilby Brook, Kilby, SP6294	RG, 6 Mar 1996	24	20		
37 Leics., v.c. 55, Kilby Brook, SP617954	BD, 25 Jul 1999 (LTR)	1			
38 Leics., v.c. 55, Knighton, Knighton Hall, SK599014	RG, 15 Sep 2000	25	20		
39 Leics., v.c. 55, River Sence, Little Stretton, SK6600	RG, 6 Mar 1996	4	20		
40 Leics., v.c. 55, Stonton Wyville, SP7394	RG, Mar 1996	30	20		
<i>C. brutia</i>					
41 S. Somerset, v.c. 5, Wimbleball Lake, SS977317	RL 737 & BD, 17 Jul 2000 (NMW)	1		G	G
42 Carms., v.c. 44, Afon Teifi, SN256422	AOC, 1997	4		I	G
43 Cards, v.c. 46, Brynllynan, SN184482	AOC, 1997	2		I	G
44 Cards., v.c. 46, Pen-y-Graig, SN223519	AOC, 1997	1		I	G
45 Monts., v.c. 47, Machynlleth, SH742013	BD (RL785), 23 Aug 2000 (NMW)	2		I	G
46 Merioneth, v.c. 48, Dolgellau, SH71	APC, 1996	2	28	H	H
47 Caerns., v.c. 49, N. of Aberdaron, Methlem Pond, SH174301	APC, 9 Mar 1996	6	28 ^c	I	G
<i>C. hamulata</i>					
48 S. Devon, v.c. 3, East Dart River, SX647790	RL 739 & BD, 17 Jul 2000 (NMW)	1	38	G	H
49 S. Hants., v.c. 11, Oberwater floodplain, SU265029	RL 748 & BD, 18 Jul 2000 (NMW)	1		G	H
50 S. Hants., v.c. 11, Oberwater floodplain, SU260034	RL 745 & BD, 18 Jul 2000 (NMW)	1	38	G	H
51 Worcs., v.c. 37, Swanhurst Park, SP08	JP, 1996	2	38	G	H
52 Warks., v.c. 38, Rookery Brook, SP1866	JP, 1996	2	38	G	G
53 Monts., v.c. 7, Montgomery Canal, Newton Powys, SO172970	RL 754 & BD, 19 Jul 2000 (NMW)	1	ca38	G	H
54 Monts., v.c. 47, River Wye, Llangurig, SN908797	RL 755 & BD, 19 Jul 2000 (NMW)	3	38	H	H
55 Dunbarton, v.c. 99, Wards pond, NS443876	RL 770, BD & NW, 9 Aug 2000 (NMW)	1		G	H

^a AOC, Arthur Chater; APC, Ann Conolly; BD, Benoît Demars; JP, James Partridge; NW, Nigel Willby; NS, Nick Stewart; RG, Richard Gornall; RL, Richard Lansdown. Collection numbers, where given, form part of codes assigned by RL

^b L – broad-winged fruits; S – narrow-winged fruits.

^c Count by Wentworth *et al.* (1991).

NORFOLK RIVER SURVEY

Callitriche is a very common genus in the rivers of Norfolk. Three species were identified using the isozyme phenotypes described above, supplemented by diagnostic pollen features in a few cases (Table 3). Without exception, the diagnostic characters supported the determination based on isozyme phenotype. *Callitriche obtusangula* (Fig. 3A) and *C. platycarpa* (Fig. 3B) were the most frequent species and were often found growing together, sometimes inter-mixed. *Callitriche stagnalis* appears to be restricted to the upper River Tat, a tributary of the River Wensum (Fig. 3A).

TABLE 3. OCCURRENCE OF *CALLITRICHE* SPECIES IN THE RIVERS WENSUM, WISSEY, NAR AND BURE IN NORFOLK

Locality (all v.c. 28 except where noted)	Grid Ref.	Collection	No. individuals assayed	
			<i>C. obtusangula</i>	<i>C. platycarpa</i>
<i>River Wensum</i>				
Whissonsett	TF913238	10–11 Apr 1999		5
Pear Tree Corner	TF898237	10–11 Apr 1999		6
Southmill Farm	TF882283	10–11 Apr 1999	2	2
Doughton	TF882290	10–11 Apr 1999	1	4
Sculthorpe Mill	TF894303	10–11 Apr 1999	1	1
Sculthorpe Fen	TF902297	10–11 Apr 1999		5
Fakenham – upstream STW	TF921293	10–11 Apr 1999	2	3
Fakenham – downstream STW	TF936292	10–11 Apr 1999	2	5
Pensthorpe Water Fowl Park	TF943288	10–11 Apr 1999	2	3
Pensthorpe Gravel Pit Bridge	TF953287	10–11 Apr 1999		4
Lyng, v.c. 27	TG073181	17 Aug 2000		1
Taverham, v.c. 27	TG157137	17 Aug 2000		1
Taverham, v.c. 27	TG159136	17 Aug 2000	1	1
<i>River Wensum tributaries</i>				
South Raynham Brook	TF879241	10–11 Apr 1999		5
Helhoughton Brook	TF868267	10–11 Apr 1999		5
Fakenham (ditch)	TF936293	10–11 Apr 1999		5
Langor drain	TF961291	10–11 Apr 1999	5	
River Ainse, v.c. 27	TG094213	28 Jun 2001	pollen	
River Tat, Coxford	TF846294	18 Aug 2000		3*
River Tat, Broomsthorpe	TF852284	10–11 Apr 1999	4	2
River Tat, Tatterford Common	TF867280	10–11 Apr 1999	1	2
tributary of River Tat	TF845289	10–11 Apr 1999		5
East Rudham Brook	TF845287	10–11 Apr 1999	1	4
<i>River Wissey</i>				
Hilborough	TF833008	1 Aug 2000		1
Gooderstone Common stream	TF753006	2 Aug 2000		2
Beachamwell stream	TF717005	2 Aug 2000	1	2 + pollen
Bodney	TL828988	1 Aug 2000	1	1
Ickburgh	TL808944	1 Aug 2000	2	1
Didlington	TL769968	1 Aug 2000	1	
Stoke Ferry	TL710995	3 Aug 2000	1	
Stoke Ferry Fen	TL676975	31 Jul 2000		1
<i>River Nar</i>				
Mileham	TF905186	24 Jul 2000	pollen	
Litcham	TF883172	24 Jul 2000	pollen	
Lexham Hall (pond)	TF869175	24 Jul 2000	pollen	
West Lexham	TF840170	24 Jul 2000		1*
West Acre	TF833008	25 Jul 2000	1 + pollen	
Newton	TF828154	25 Jul 2000	1	1
Newton	TF827154	25 Jul 2000		1
Narborough	TF745132	27 Jul 2000	1 + pollen	pollen*
Marham	TF723120	25 Jul 2000	pollen	
<i>River Bure</i> (all v.c. 27)				
Saxthorpe	TG123296	15–18/08/00	3*	
Buxton	TG242232	16 Aug 2000	1	
Costishall	TG266201	16 Aug 2000	1	

Plants were identified by isozyme assay (no. individuals tested) and/or pollen characters. These records are additional to the reference collections from Norfolk listed in Table 2.

* Voucher specimen in **LTR**.

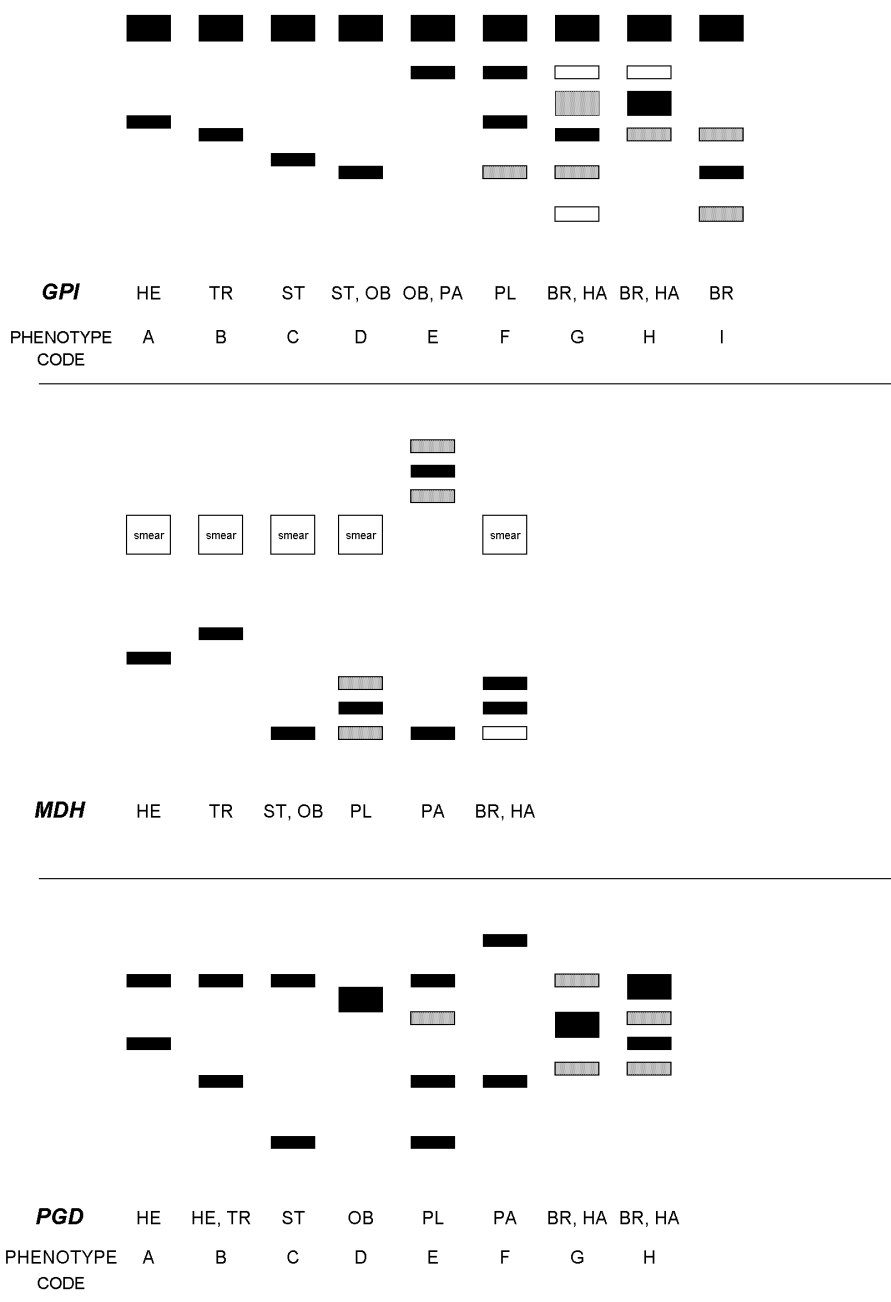


FIGURE 2. Schematic single-enzyme phenotypes recovered from *Callitriche hermaphroditica* (HE), *C. truncata* (TR), *C. stagnalis* (ST), *C. obtusangula* (OB), *C. platycarpa* (PL), *C. palustris* (PA), *C. brutia* (BR) and *C. hamulata* (HA). Differential shading is approximately proportional to allozyme band intensity. Anode towards top of figure.

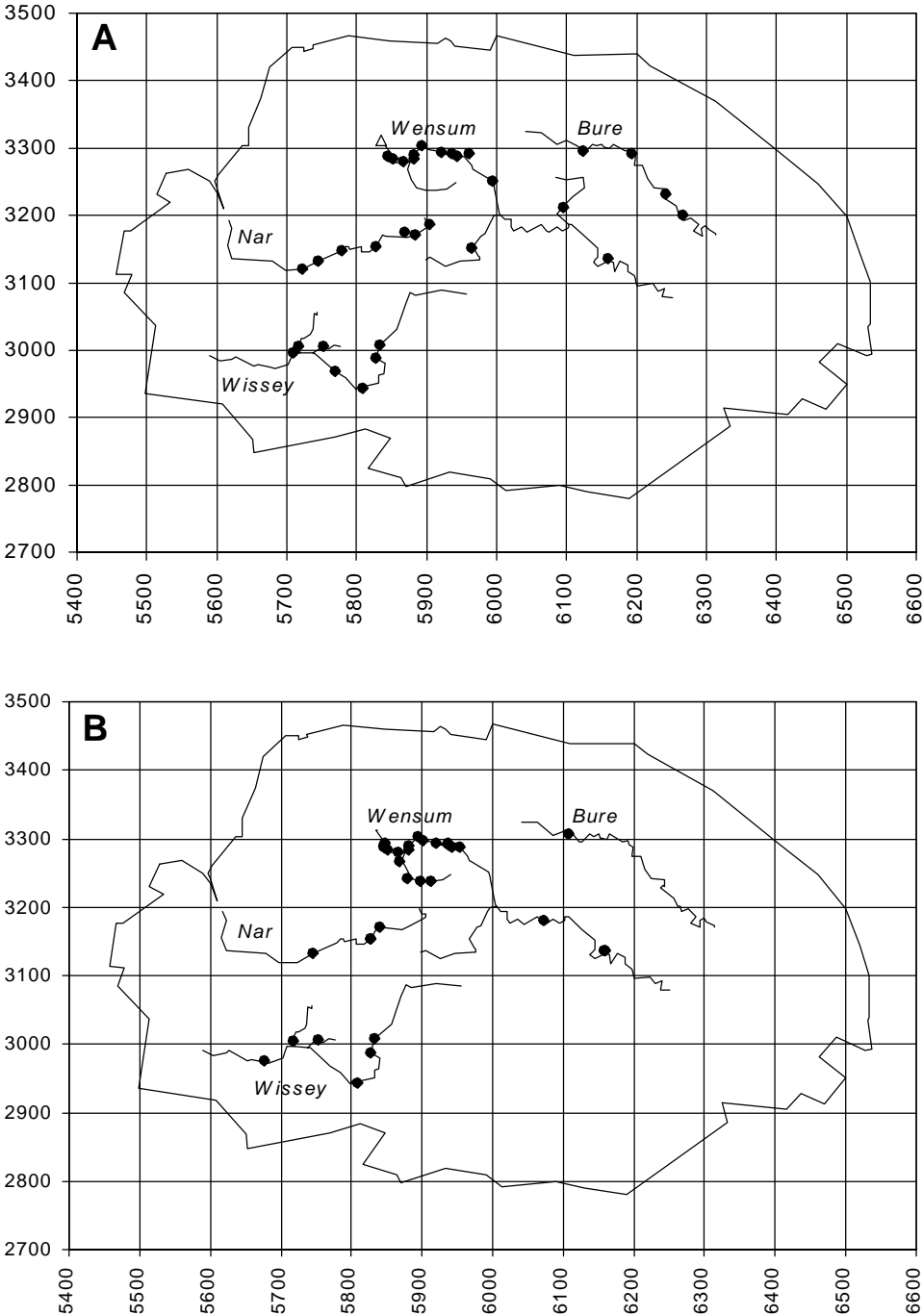


FIGURE 3. Distribution of *Callitriche* species in Norfolk rivers recorded in 1999–2000 and displayed on the 10-km British national grid. A) \triangle = *C. stagnalis*; \bullet = *C. obtusangula*; B) *C. platycarpa*.

DISCUSSION

IDENTIFICATION

The only previously published study of isozymes in the genus was by Gil Pinilla (1992) who compared 28 populations of *C. obtusangula* and *C. stagnalis* from Dorset and Wales in ten enzyme systems (AAT, ADH, G6PDH, EST, IDH, MDH, ME, PGI, SKD and SOD). She found little variation and concluded that isozymes were unlikely to provide a basis for species identification. We could not distinguish *C. stagnalis* from *C. obtusangula* using AAT, IDH, MDH or ME either. We did, however, find abundant variation, some of it apparently species-specific or nearly so, in GPI, MDH and PGD sufficient to allow separation of all taxa except *C. hamulata* and *C. brutia*. It is of course possible that further collections may well reveal additional alleles and thereby extend the range of variation such that our observed differences will become less clear. Nevertheless, we believe that the provenance of our material is sufficiently wide that this is unlikely to happen to any great extent. Thus, in earlier, preliminary studies, material assayed from a wider range of localities throughout Britain revealed only limited additional variation, and insufficient to disrupt the correlation with species identity (RJG, unpublished data).

It is perhaps no surprise that our isozyme assay could not distinguish *C. brutia* from *C. hamulata*. The morphological features of the two taxa are extremely close, so much so that they can be virtually impossible to separate at some stages of their development (Schotsman 1967, p.93) or when growing submerged (Schotsman 1967, p.88; 1972). Diagnostic characters relating to leaf shape are prone to phenotypic plasticity, and those involving hairs or fruit morphology (Schotsman 1967, p.88) are very subtle at best. Even the most commonly used character, peduncle length, may not be constant (Schotsman 1954 pl. 13a; Schotsman 1967, p. 88). The two species are clearly very closely related genetically, and this is further borne out by the fact that they share identical *rbcL* sequences in their chloroplast genome (Philbrick & Les 2000). We suggest that one explanation for this close relationship is that *C. hamulata* ($2n = 38$) could be an allopolyploid, with *C. brutia* ($2n = 28$) as one of its parents and a species with $2n = 10$ as the other. Until additional evidence can be supplied the only reliable ways of separating the two species are by cytological means or electron microscopy of the pollen (Cooper *et al.* 2000). The difference in chromosome number is mirrored also by a difference in the amount of DNA per chromosome (Pijnacker & Schotsman 1988).

Variation in fruit size among British populations of *C. hermaphroditica* was not accompanied by any correlated variation in isozyme phenotypes. Martinsson (1991a) examined Nordic populations of the two variants and concluded from multi-variate analyses that the pattern of variation in fruit morphology is complex and involves other characters as well as size. The extent and nature of the genetic difference between the large-fruited and the small-fruited variants remains to be established, although Savidge (1958, p. 55) observed that under similar experimental cultural conditions they retained their respective phenotypes.

POLYPLOIDY

Although apparently rare, hybridisation is not unknown in *Callitriche* (e.g. Martinsson 1991b). It seems to occur chiefly among species capable of wind-pollination, hence with an increased chance of outcrossing (Schotsman 1982). In this regard, Savidge (1958, 1960) suggested that *C. platycarpa* ($2n=20$) may have had an allotetraploid origin from *C. stagnalis* and *C. cophocarpa* (both $2n=10$). The evidence for this hypothesis rests on the morphological intermediacy of *C. platycarpa* and on the cytological behaviour of the hybrid *C. platycarpa* \times *cophocarpa*. The two putative parents are also sympatric in eastern Europe (Schotsman 1967). Savidge (1958, p.114), however, did not rule out a possible autotetraploid ancestry, owing to the partial sterility often seen in *C. platycarpa*. This alternative view was taken up by Schotsman (1967), who concluded that *C. platycarpa* was more likely to be an autotetraploid of *C. cophocarpa*. Evidence from chromosome number and DNA amount per chromosome is consistent with either hypothesis (Pijnacker & Schotsman 1988). Our findings would support Savidge's contention of an allopolyploid origin, assuming that the constant heterozygosity seen in *C. platycarpa* at three loci (GPI-2, MDH-2 and PGD-2) turns out to be fixed and is due neither to clonal growth nor to a sampling artefact caused by tetrasomic inheritance. Consideration of shared alleles indicates also that *C. stagnalis* is a likely candidate for one of the parents.

The other three polyploid species, *C. palustris*, *C. brutia* and *C. hamulata*, also showed complex isozyme phenotypes, consistent with heterozygosity. The implications of this remain to be demonstrated, although we have earlier speculated on a possible hybrid origin for *C. hamulata*.

DISTRIBUTION IN NORFOLK

The findings from our survey of the four Norfolk rivers indicate that both *C. platycarpa* and *C. obtusangula* are common, supporting the statements made by Petch & Swann (1968). In contrast, *C. stagnalis* is scarcely to be found in the rivers surveyed. The distribution maps provided by Beckett & Bull (1999) in their account of the flora of Norfolk, based on a survey 1985–1999, show very few records of either *C. platycarpa* or *C. obtusangula*, presumably owing to difficulties in determining non-flowering material or to a different sampling strategy. Their map of *C. stagnalis*, in contrast, shows a widespread distribution but its apparent avoidance of rivers is not clear. Our experience, therefore, is that isozyme data can provide a reliable basis for the study of the distribution and ecology of *Callitriche* species.

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