Biology, genetic variation and conservation of *Luronium natans* (L.) Raf. in Britain and Ireland

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ABSTRACT

Luronium natans (Floating Water-plantain) is a European endemic aquatic plant which is now rare and threatened across most of its extant range. Confusion with similar species has led to misunderstanding of its distribution and status in Britain and Ireland, but oligotrophic upland lakes now seem to hold the main populations. Isozyme studies show comparatively high levels of genetic variation among native populations in Wales, contrasting with the lack of variation reported in some closely-related aquatic species, but there is close similarity among samples of plants from canal populations on the Welsh borders. It has spread into this canal habitat relatively recently (post-1850), and the isozyme patterns found there indicate that the canal populations have originated from a native lake population connected to the canals by feeder streams. The greatest range of genetic variation now appears to remain in a few native "core" sites, and the survival of these populations is thus of particular importance. Conservation priorities are discussed in relation to the biology, distribution and metapopulation structure of the species in Britain and Ireland.

KEYWORDS: Floating Water-plantain, aquatic plants, isozyme variation, Wales, threatened species, oligotrophic lakes.

INTRODUCTION

Luronium natans (L.) Raf. (Alismataceae) is a stoloniferous aquatic perennial which grows in a range of habitats, and is phenotypically very plastic. The strikingly different forms that it can assume in different habitats nearly all resemble commoner aquatic species, with the result that it has often been overlooked or confused with these commoner plants (Ferguson, Briggs & Willby 1998). In shallow water *L. natans* produces stalked leaves with ovate or elliptical blades, up to about 4 × 1·4 cm, sometimes larger, which superficially resemble the floating leaves of some common *Potamogeton* species. When growing on exposed mud it produces similar but smaller ovate leaves with stiffer, shorter stalks, and then closely resembles *Baldellia ranunculoides*, even when it is in flower. In deeper water (0·7–2 m, perhaps up to 4 m in particularly clear lakes) it grows as "isoetoid" rosettes of linear-triangular leaves, about 0·5 cm wide at the base and 5–7 cm long, without any expanded lamina. These rosettes are practically identical to juvenile *Alisma plantago-aquatica*, and very similar when seen at a distance to those of *Isoetes* spp. and *Littorella uniflora*, both of which are often abundant in habitats that are suitable for *Luronium natans*, and may not be recorded in shore-based sampling. Finally, in flowing water it can produce long (50–60 cm), strap-like, flexible leaves about 0·5–0·7 cm wide, in dense masses, resembling the leaves of the species of *Sparganium* that grow in similar habitats. The forms (illustrated by Jones & Rich (1998)) appear to be phenotypically freely interchangeable, both in cultivation and in nature, with leaves of different forms being produced on one and the same rosette under appropriate conditions. Their common feature, found in otherwise similar aquatic species only in *Baldellia ranunculoides*, is the combination of slender stolons, 5 cm or more in length, with laminar leaves produced in rosettes. The stoloniferous forms of *Baldellia ranunculoides* that most closely resemble *L. natans* can be distinguished in the field by the characteristic smell of coriander in their crushed foliage.

In Britain and Ireland *Luronium natans* is known from three main habitats. Its chief natural habitat is now in oligotrophic, moderately acidic lakes and pools, in upland areas but generally not at high altitudes (up to about 400 m). In these sites it can occasionally be detected by the presence in shallow water of flowering plants with floating leaves, but more often remains inconspicuous and unsuspected as submerged isoetoid rosettes, vegetative and growing at depths of 1–2·5 m. It appears to have been lost from similar, formerly oligotrophic lakes in the lowlands, for example the Shropshire meres and the larger pools and lakes in Anglesey (Ynys Môn) as a consequence of eutrophication, mainly during the early part of the century. A second characteristic habitat, which still survives in a few sites, is in shallow oligotrophic or mesotrophic ponds or pools on grazed heathland or commonland in low-lying western maritime areas. Scattered references to its former occurrence in such sites (e.g. Davies 1813) suggest that *L. natans* may have had a fairly wide distribution in ponds of this type in the past, but has drastically declined as they have been lost or altered by drainage, eutrophication, changes in agricultural practice or overgrowth. *L. natans* is now known to grow at only two such sites in western Britain, on Ramsey Island (Ynys Dewi) and Dowrog Common, both in south-western Wales. Heathland ponds of this type are or were often man-made, with their suitability as a habitat for *L. natans* maintained or enhanced by human or livestock activity, and at least some of the populations of *L. natans* in such sites can be seen as satellite populations utilising relatively transient and essentially artificial sites. Similar small and probably transient populations found in isolated farm ponds in Pembrokeshire, in scattered sites across the English midlands, and (perhaps introduced) in Norfolk (M. Wade pers. comm., Driscoll 1992) reflect this more opportunistic aspect of the ecology of *L. natans*, as does its third presently characteristic habitat, which is in abandoned or little-used canals. It has spread into this habitat fairly recently (since about 1850), colonising canals on the Welsh border and in north-central England, sometimes becoming locally abundant (Willby & Eaton 1993) but subsequently declining in at least some sites (Briggs 1988) as a consequence of eutrophication, overgrowth, increased boat traffic and other possible factors. It seems possible that the canal habitat effectively reproduces a formerly widespread but now very rare natural habitat of *L. natans*, in clear, oligotrophic or moderately mesotrophic, slow-flowing rivers. In Britain *L. natans* has been recorded from seven such river sites, but it is now known from only two, both in Wales. As with lowland pool and lake sites, suitable river habitats would almost certainly have been more common in the past, before widespread channel-straightening, eutrophication and sediment deposition took place, and river populations of *L. natans* have most probably declined as a result (Davies 1813; Lockton and Whild 1997). Whilst the present canal populations of *L. natans* clearly grow in an artificial habitat, their relationship to other populations of the species has been uncertain. The interconnection between and superficial similarity of canals to sluggish river channels suggests that canal populations might have had river populations as their source, although it is also possible that river populations are themselves satellites of "core" populations in upland Wales. A number of the current and former sites for *L. natans* in rivers occur downstream of lakes containing large, established populations.

Luronium natans is a European endemic species, with a distribution of the Suboceanic Temperate type (Preston & Hill 1997). Its range extends from north-western Spain and Ireland in the west to Lithuania, Bulgaria and Moldavia in the east. On much of the European continent it is now very rare and declining, with a scattered and disjunct distribution, and many surviving populations are reported to be under threat from drainage, eutrophication or acidification of their freshwater habitat (Roelofs 1983; Hanspach & Krausch 1987; Fritz 1989; Ferguson 1991; Rodriguez-Oubina & Ortiz 1991; Willby & Eaton 1993). As a result the species has been listed on the Berne Convention Appendix 1 (which requires signatory states to prohibit taking, and to take measures to conserve listed species), and the EC Habitats Directive Annexes IIb & IVb (which require designation of protected areas and special protection measures for them). Consequently, *L. natans* is now listed on Schedule 8 of the United Kingdom Wildlife and Countryside Act (revised 1992), which makes it an offence to pick, uproot, sell or destroy the species, and is included among the "Short List" species of the UK Biodiversity Action Plan, with a commitment to maintain and, if possible, enhance its present range.

In the British Isles its distribution has been rather poorly understood in the past, in part because of confusion with other commoner species, but it now seems clear that *L. natans* has a stronghold in Wales, where it is fairly widespread in the uplands (Ellis 1983; Preston 1994). With the decline of heathland ponds, native or long-established lowland populations in adjacent English counties have dwindled greatly and may recently have become extinct in this habitat (S. Whild, pers.comm.). Past reports of the occurrence of *L. natans* in the mountainous Lake District of north-western England (Cumbria) were repeatedly copied and cited in County Floras and other publications (Turner & Dillwyn 1805; Smith 1828; Hooker 1831; Baker 1885; Hodgson 1898; Wilson 1938; Perring & Walters 1976; Halliday 1978; Willby & Eaton 1993), but investigation of these records, all dating from the nineteenth century, showed that they were questionable and in one case the result of a copyist's error (Kay & John 1995; Halliday 1997), and as such they were omitted from the maps published by Preston (1994) and Preston & Croft (1997). Subsequent re-investigation of likely habitats in Cumbria (G. Halliday, pers. comm.) has however shown that *L. natans* is locally abundant in at least two of the larger lakes, Derwentwater (Halliday 1997) and Bassenthwaite Lake, and it has also been found in a pool near Ullswater (G. Halliday, pers. comm.), so in this light it now seems probable that Greville's early record of the species from Derwentwater (Turner & Dillwyn 1805), which was first called into question by Hodgson (1898) was in fact correct.

Furthermore, there is now evidence that *L. natans* has been even more widely overlooked. Nineteenth-century records of its occurrence in south-western Scotland and western Ireland (e.g. Hooker 1831) had, in the absence of more recent records, come to be regarded as probable errors. However, Rich, Kay & Kirschner (1995) have recently found a new locality for the species in Ireland, and they have shown that several older records from other sites in Ireland are certainly or probably correct. It now seems likely that *L. natans*, although scarce, may be fairly widely distributed in western Ireland, at least from Killarney (where it was last seen in 1886) through Clare (1882) to Galway (1994). An equally interesting series of new records of *L. natans* has recently been reported from central and western Scotland; here, lake surveys have shown that it occurs at several sites in Argyll, although its status there is uncertain (N. Willby, pers. comm.). These records from Ireland and Scotland, and its rediscovery in the English Lake District, show that *L. natans* is considerably more widespread in western Britain and Ireland than had been thought, and suggest that it may be present but undetected in other sites.

The aims of the present study, which commenced in 1993, were to characterise the reproductive biology of *L. natans*, to assess its patterns of genetic variation using isozyme analysis, to interpret these findings in terms of its population and metapopulation structure, and to consider their implications for its conservation.

MATERIALS AND METHODS

Observations on reproductive biology were made both in the field and in cultivation. Plant samples for isozyme analysis were collected (under licence) by hand or grab sampling at intervals of at least 2 m in pond and stream populations and 5–20 m in lake populations. Grapnels do not attach well to *L. natans*, and simple diving equipment – a face-mask, snorkel and wet-suit – is the most effective means of sampling colonies below 1 m depth. One or more clones from most populations were grown on for further study, without difficulty, rooted in fine gravel in a 15 cm depth of rainwater in open-topped polythene containers 12–18 cm in diameter on a part-shaded open-air hardstanding. Isozyme analysis was carried out by horizontal starch gel electrophoresis, using about 0·5–1 cm^2 of fresh leaf material from each sample. The electrophoresis procedure was similar to that described by Shields, Orton & Stuber (1983) and Lack & Kay (1986). Staining was carried out using recipes following Shaw & Prasad (1970).

RESULTS

REPRODUCTIVE BIOLOGY

Luronium natans flowers during July and August, and appears to be adapted for both self-pollination and insect visitors. Plants growing in less than about 60 cm of water, or on exposed mud, produce bowl-shaped flowers which open to about 15 mm in diameter in sunny and calm weather, rising to or above the surface on long pedicels if the plant is submerged. In cultivation, the

TABLE 1. *LURONIUM NATANS* – SITES, SAMPLE SIZES, NUMBERS OF ISOZYME GENOTYPES AND NUMBERS OF VARIABLE LOCI FOUND

Population code, site name and grid reference	No. of samples collected	No. of isozyme genotypes found	No. of variable loci
WALES			
1. Llyn Cwellyn SH/565.548	1	1	0
2. Llyn-y-Dywarchen SH/560.534	4	2	1
3. Llyn Tegid SH/88.35	9	2	1
4. Llangollen Feeder Canal SJ/210.426	4	2	3
5. Montgomery Canal, Four Crosses SJ/258.191	4	2	1
6. Montgomery Canal, Nag's Head SO/194.990	2	2	1
7 . Montgomery Canal, Fron SO/167.965	2	2	3
8. Llyn Hir SN/789.677	4	4	2
9. Llyn Teifi (all) SN/78.67	14	4	3
9A. Llyn Teifi A (South Bay) SN/785.675	6	4	2
9B. Llyn Teifi B (South Shore) SN/784.675	8	3	2
10. Llyn Egnant SN/792.675	9	3	2
11. Afon Teifi, Cors Caron SN/69.64	4	2	1
12. Afon Teifi above 'Flash' SN/675.620	3	3	2
13. Afon Teifi below 'Flash' SN/677.640	4	3	3
14. Llyn Eiddwen SN/606.670	6	1	0
15. Llyn Fanod SN/603.644	6	1	0
16. Ramsey Island, West Pond SM/702.235	10	1	0
IRELAND			
17. Invermore Lough (Connemara) L/899.390	1	1	0

flowers last for only a day, but regularly attract small flies. Their three delicate white petals have a conspicuous yellow base, and are unscented. Their nectar provision, if any, was not quantified, but they resemble the nectar-providing flowers of *Ranunculus* subgenus *Batrachium* (water crowfoots) which grow in similar habitats. In windy conditions, or when produced on plants growing at depths greater than about 60 cm, when the flowers fail to reach the surface, the petals do not open and self-pollination takes place cleistogamously within the closed flower. G. Halliday (pers. comm.) has observed the production of several long-stalked cleistogamous flowers from each of many ascending stoloniferous stems in plants growing in about 2 m of water in Derwentwater. There are normally six stamens per flower, and up to 13 carpels (an average number of 12·5 was found in samples from Ramsey Island (Ynys Dewi)), each containing a single ovule, and forming an achene when fertilized. The pollen grains are morphologically distinctive, bluntly octagonal in cross-section; the pollen fertility of a sample from Ramsey, assessed as acetocarmine stainability, was 83·97%, with about 12000 pollen grains per flower. The pollen:ovule ratio was 973, suggesting adaptation for at least partial outbreeding. No formal test of compatibility relationships was made, but the production of seeds by submerged and apparently cleistogamous flowers indicates self-compatibility. In suitable conditions, for example on the exposed mud of Llyn Teifi (Chater 1990) or in sheltered parts of Llyn Glaslyn (Catherine Duigan, pers. comm.), and in the Montgomery and Rochdale Canals (N. Willby, pers. comm.) flowering plants can produce conspicuous displays.

It seems probable that allogamy is possible only in plants that are growing in fairly shallow water where flowering shoots can extend to the surface, or on exposed mud, and that seed production is most abundant under these conditions. The small (1–2 mm) cylindrical achenes have no special adaptations for dispersal, and in water sink when released from the receptacle, although seedlings float (Ridley 1930) and it seems likely that the seeds can be dispersed by waterfowl. Seedling establishment is sometimes conspicuous (as, for instance, along the strand-line on Llyn Teifi, Cardiganshire), but the characteristically vigorous stoloniferous spread shown by *L. natans* suggests that reproduction within individual lakes, rivers or canal systems is likely to be predominantly clonal, by vegetative spread of stolons and detached rosettes. Rosettes and stolons might also occasionally become attached to the legs or necks of waterfowl, enabling vegetative

TABLE 2. *LURONIUM NATANS* – GENE FREQUENCIES

Locus and allele	Population 1	2	3	4	5	6	7	8	9	9A	9B	10	11	12	13	14	15	16	17
PGI 1 A	0·333	0·167	0·333	0·240	0·333	0·167	0·167	0·167	0·167	0·167	0·167	0·333	0·333	0·167	0·167	0·167	0·167	0·167	0·167
B	0·333	0·333						0·333	0·333	0·333	0·333	0·333	0·333	0·167	0·111	0·333	0·333		
C			0·333	0·333	0·333	0·333	0·333							0·167	0·333			0·333	0·333
D	0·333	0·333						0·333	0·333	0·333	0·333	0·333	0·333	0·167	0·111	0·333	0·333		
E			0·333	0·333	0·333	0·333	0·333							0·167	0·111	0·333		0·333	0·333
n		0·167		0·093		0·167	0·167	0·167	0·167	0·167	0·167			0·167	0·167	0·167	0·167	0·167	0·167
Number of genotypes	*1*	*1*	*1*	*2*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*2*	*3*	*1*	*1*	*1*	*1*
PGM 1 A			0·500	0·500	0·500	0·500	0·250							0·500	0·250				
B	1·000	1·000	0·500	0·500	0·500	0·500	0·750	1·000	1·000	1·000	1·000	1·000	1·000	0·500	0·750	1·000	1·000		0·500
C																		0·500	
D																		0·500	0·500
Number of genotypes	*1*	*1*	*1*	*1*	*1*	*1*	*2*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*2*	*1*	*1*	*1*
MDH A													0·250						
B	0·500	0·250	0·667	0·278	0·750	0·750	0·667	0·250	0·250	0·250	0·333	0·333		0·500	0·500	0·333	0·500	0·500	1·000
C												0·167	0·250	0·500					
D	0·500	0·500	0·333	0·222	0·250	0·250	0·333	0·500	0·500	0·500	0·333	0·500	0·500		0·500	0·333	0·500	0·500	
E		0·250		0·500				0·250	0·250	0·250	0·333					0·333			
Number of genotypes	*1*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	*3*	*2*	*3*	*3*	*2*	*1*	*1*	*3*	*1*	*1*	*1*
SDH A	0·333	0·333	0·500	0·500	0·333	0·333	0·167	0·333	0·452	0·625	0·611	0·333	0·333	0·333	0·333	0·500	0·333	0·333	1·000
B										0·292	0·111			0·333		0·500			
C	0·333	0·333	0·500	0·352	0·444		0·417	0·440	0·190		0·333	0·333	0·333		0·500		0·333	0·333	
D												0·111							
E	0·333	0·333		0·148	0·222	0·333	0·417	0·227	0·357	0·083	0·027	0·222	0·333	0·333	0·167		0·333	0·333	
n						0·333													
Number of genotypes	*1*	*1*	*1*	*2*	*2*	*1*	*1*	*2*	*4*	*4*	*1*	*3*	*1*	*3*	*2*	*1*	*1*	*1*	*1*

dispersal to occur between separate (although probably comparatively close) bodies of water. The results of our genetic analysis provide supporting evidence for the predominant rôle of vegetative spread within lakes, canals and river systems, and also for the occurrence of seed-mediated dispersal between water bodies. Within lake and river systems our genetic analysis also provides evidence of the occurrence of reproduction by seed and consequent recombination in some areas, but the frequency of successful sexual reproduction and of allogamy cannot be determined because of the small number of variable loci and the probable occurrence of fixed heterozygosity, as described in the next section.

GENETIC VARIATION

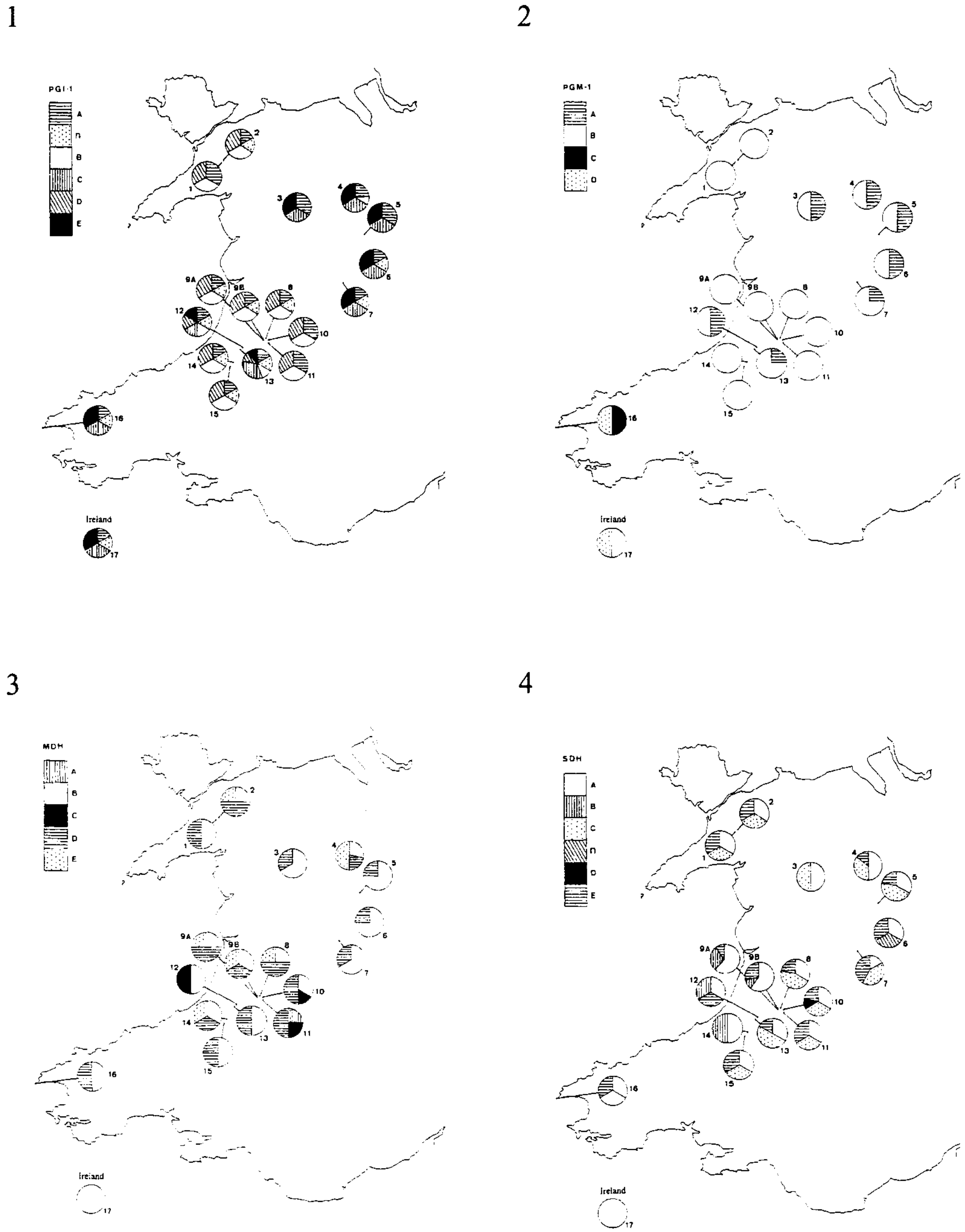
A chromosome number of 2n = 42, probably hexaploid from a basic number of x = 7, has been reported for Swedish material of *Luronium natans* by Björkvist (1961), and a count of 2n = 38, which we cannot trace, was reported for British material by Clapham, Tutin & Warburg (1962), suggesting that British material is also hexaploid. No counts on British or Irish material are documented in the Leicester Cytological Catalogue (R. Gornall, pers. comm.).

We obtained samples for isozyme studies of genetic variation from 16 populations or sub-populations in Wales, one of which was subdivided. A single sample was obtained from the newly-discovered population at Invermore Lough, Co. Galway (Connemara) in western Ireland. Details of the populations sampled are given in Table 1 and Figs. 1–4. Eleven loci in nine enzyme systems were assayed, of which only four were found to be variable, phosphoglucose isomerase 1 (PGI 1), phosphoglucomutase 1 (PGM 1), malate dehydrogenase (MDH) and shikimate dehydrogenase (SDH). Banding patterns were in agreement with the hexaploid chromosome counts reported for *L. natans*. Where all bands stained to the same intensity, it was assumed that there were equal numbers of copies of each allele. Where there were differences in staining intensities among the bands it was possible to estimate the number of copies of each allele that were present in an individual plant. Gene frequencies, together with the number of genotypes recorded at each site, are shown in Table 2. Two populations (Llyn Cwellyn and Invermore Lough) were represented by a single sample. Three of the 15 populations from which several samples were obtained (Llyn Eiddwen and Llyn Fanod, each with six widely-spaced samples, and Ramsey West Pond, with ten samples from a dense pond population) were monomorphic at all variable isozyme loci. Two or more genotypes, with a maximum of four, were found in each of the twelve remaining populations. While each genotype could represent a true-breeding inbred line, the strong vegetative spread and multiplication shown by the species suggest that apparent monomorphy, or paucity of genotypes within a population, is more likely to be the result of predominantly clonal reproduction. In a hexaploid, most isozymes will have three loci, so, in a survey of this type, it is not possible to distinguish true heterozygotes (individuals heterozygous at a single locus) from fixed heterozygotes (individuals which are homozygous for different alleles at corresponding loci in different genomes). Thus, although the observed frequency of heterozygosity was high, it was not possible either to confirm the occurrence of outcrossing and recombination, or to obtain an estimate of their frequency. The low ratios of numbers of genotypes found to numbers of variable loci (Table 2) and our examination of relative band intensities, which are normally proportional to the number of copies of each allele that are expressed (e.g. Lack & Kay 1986) suggested that the frequencies of sexual reproduction, and especially of recruitment of new recombinant genotypes within a population, were low, and possibly zero in some cases.

The distribution and frequencies of alleles at each scored locus are mapped in Figs. 1–4. The PG1 locus showed two major genotypes, one (found in Llyn Tegid, the Llangollen and Montgomery Canals, on Ramsey and in Ireland) with linked C and E alleles, and the other (from central and northern Wales) with linked B and D alleles. The Irish sample from Invermore Lough was the only one which showed monomorphy and homozygosity at two of the loci, but many of the central and northern Welsh populations were monomorphic for probable fixed heterozygosity at two or more loci.

RELATIONSHIPS AMONG POPULATIONS

An unrooted phylogenetic tree of relationships among populations, based on the isozyme data and prepared using the CONTML procedure of Felsenstein's PHYLIP package (Felsenstein 1993), is shown in Fig. 5, and Nei's (1972) measures of genetic distance between populations, also based on the four variable loci, are shown in Table 3. The distances between populations along the branches



FIGURES 1–4. The distribution and frequency of alleles at four variable loci in 17 populations or sub-populations of *Luronium natans* in Wales and Ireland (inset). Sector size in each composite circular symbol is proportional to the frequency of the corresponding allele of each enzyme in the population sample from that site. PGI 1 is shown in Fig. 1 (top left), PGM 1 in Fig. 2 (top right), MDH in Fig. 3 (lower left) and SDH in Fig. 4 (lower right).

TABLE 3. *LURONIUM NATANS* – NEI'S (1972) MEASURE OF GENETIC DISTANCE AMONG POPULATIONS

Population	2	3	4	5	6	7	8	9A	9B	10	11	12	13	14	15	16	17
1 Llyn Cwellyn	0·043	0·337	0·458	0·317	0·417	0·196	0·050	0·130	0·106	0·019	0·093	0·459	0·119	0·185	0·013	0·743	0·569
2 Llyn-y-Dywarchen		0·460	0·388	0·458	0·532	0·259	0·006	0·086	0·062	0·045	0·079	0·532	0·151	0·141	0·030	0·851	0·724
3 Llyn Tegid			0·143	0·026	0·170	0·135	0·422	0·514	0·505	0·378	0·569	0·367	0·106	0·562	0·363	0·451	0·301
4 Llangollen Canal				0·169	0·269	0·241	0·376	0·432	0·376	0·475	0·556	0·404	0·215	0·466	0·466	0·611	0·467
5 Montgomery Canal, Four Crosses					0·112	0·074	0·444	0·587	0·520	0·388	0·588	0·297	0·115	0·618	0·343	0·432	0·332
6 Montgomery Canal, Nag's Head						0·137	0·573	0·556	0·471	0·514	0·735	0·244	0·231	0·595	0·404	0·514	0·310
7 Montgomery Canal, Fron							0·265	0·437	0·363	0·258	0·393	0·328	0·065	0·476	0·183	0·436	0·418
8 Llyn Hir								0·097	0·084	0·045	0·085	0·575	0·134	0·147	0·037	0·857	0·730
9A Llyn Teifi A									0·027	0·119	0·169	0·464	0·260	0·026	0·117	1·023	0·532
9B Llyn Teifi B										0·113	0·167	0·413	0·255	0·059	0·093	0·981	0·470
10 Llyn Egnant											0·052	0·454	0·142	0·178	0·033	0·850	0·658
11 Cors Caron												0·546	0·247	0·254	0·109	1·043	0·985
12 Afon Teifi A													0·393	0·422	0·446	0·889	0·490
13 Afon Teifi B														0·322	0·106	0·473	0·436
14 Llyn Eiddwen															0·172	1·120	0·556
15 Llyn Fanod																0·730	0·556
16 Ramsey																	0·441
(17 Connemara)																	

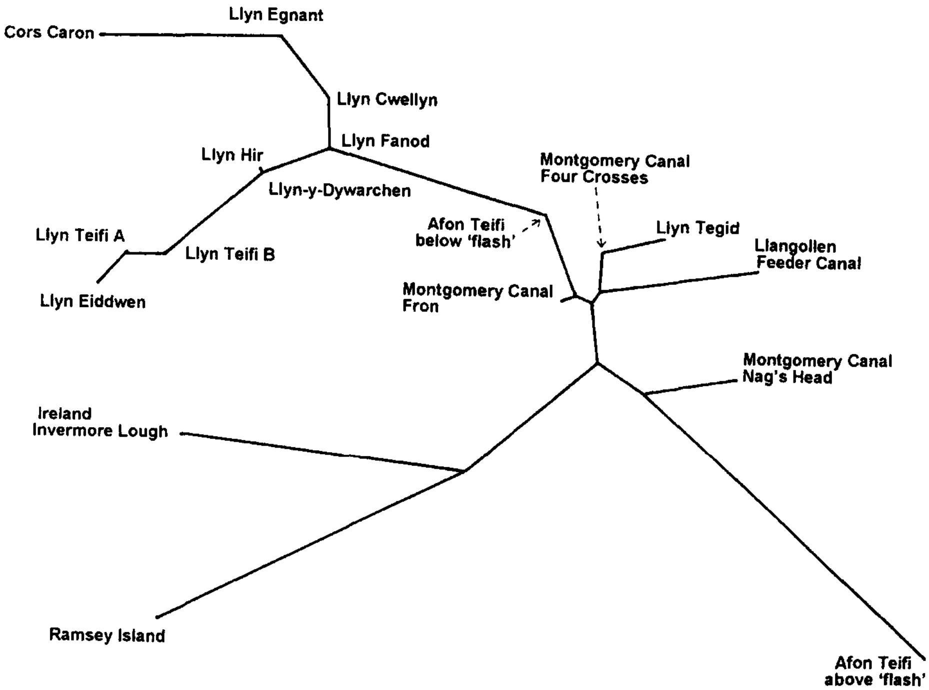


FIGURE 5. An unrooted phylogenetic tree, prepared using the CONTML procedure from PHYLIP (Felsenstein 1993), showing inter-relationships between 17 populations or sub-populations of *Luronium natans*. Distances between populations, measured along the arms of the diagram, are proportional to calculated genetic divergence.

of the tree are proportional to calculated genetic divergence. While it must be remembered that this tree is based on only four variable loci, there is clear evidence of differentiation between geographic areas. The geographically isolated Ramsey and Connemara populations, which although well separated from one another are joined on a single branch of the tree, are also fairly widely separated from the other populations, suggesting that they might be representatives (or relics) of one or more different western metapopulations. Ramsey Island, at the westernmost extremity of southern Wales, is actually closer to the Irish mainland (78 km) than to the nearest sampled populations of *L. natans* elsewhere in Wales (Llyn Fanod and Llyn Eiddwen, nearly 100 km to the north-west). Among the main group of Welsh populations, the populations from the Montgomery Canal and from Llyn Tegid (Bala Lake) and the Llangollen Feeder Canal form a relatively tight cluster in a central position on the tree, with the central and northern Welsh populations forming a much looser association. There is a surprisingly great genetic distance between the two subpopulations separated by the "Flash" on the Afon Teifi, perhaps as a result of downstream vegetative propagation of *L. natans* in the river current, from different older and effectively ancestral colonies upstream. The centre of genetic diversity of the species shown by the data within Wales lies in the upland pools and lakes around Llyn Teifi, which drain into the Afon Teifi.

Considering the pattern of genetic variability in more detail, there is some evidence of a cline of reducing genetic variability both northwards and westwards from the centre of distribution of the species in central Wales. The outermost populations have lower numbers of variable loci and lower numbers of genotypes than more central populations, although only a single individual could be found in the most northern population (Llyn Cwellyn) so there was no possibility of detecting variation in this population. However, each of the outlying populations in central and south-western

Wales (14–16) was represented by reasonable numbers of samples, and each population consisted of a single genotype, different in each case. At the PGI 1 and MDH loci, maximum variability was found in central Wales.

Because of the probable predominance of clonal reproduction in this species, it was not possible to carry out statistical correlation tests to quantify genetic erosion, but as it is likely that clones can survive for very long periods of time, potentially deleterious effects of genetic erosion would be masked for correspondingly long periods.

DISCUSSION

Our genetic analysis shows that substantial genetic variation exists among Welsh populations of *Luronium natans* as a whole, but within-population variation was usually limited. The lowest within-population variation was found in the very isolated Ramsey population (ten samples) and in the comparatively isolated but extensive Llyn Fanod and Llyn Eiddwen populations (six samples in each case) which each consisted of only a single genotype, different at each site. The greatest genetic variation and within-population diversity were found in Llyn Teifi and its surrounding lakes, which may form a particularly effective metapopulation group (that is, a cluster of sites for a species, all more or less self-contained, but linked genetically over time). Nevertheless, even here the number of genotypes that we found was small, suggesting that clonal reproduction predominates. Several genotypes are also present in the "canal" populations from the much younger (less than 200 years) habitat of the Montgomery and Llangollen Canals. However, these populations, especially the Four Crosses sample, are genetically close to the probably large and ancient Llyn Tegid lake population. As Llyn Tegid (Bala Lake) is connected to the Montgomery Canal via the Afon Dyfrdwy (River Dee) and the Llangollen Feeder Canal, it seems extremely likely that the canal populations, and their genetic variation, have been derived from the Llyn Tegid population, in agreement with the suggestions of Lousley (1970) and Willby & Eaton (1993) that the canal populations of the species in the Welsh Borders and the lowland industrial areas of central and north-western England had originated in upland Wales. Canal traffic and water flow, and the capacity of the species for rapid vegetative extension and multiplication, would have facilitated their subsequent, documented spread along the full extent of the Montgomery and Shropshire Union canal (Briggs 1988), ultimately to more than 125 km from Llyn Tegid, and perhaps also even further afield into the canals of central and north-western England.

The isozyme variability of *Luronium natans* contrasts with the extremely low or nil levels of isozyme polymorphism reported for other taxa in the same family (*Alisma* spp., Triest 1991, Triest & Roelandt 1991, and *Baldellia ranunculoides*, Triest & Vuille 1991) and emphasizes the importance of case studies of individual species and populations in conservation genetics and conservation practice, rather than generalizations and assumptions based on apparently similar situations. In *L. natans* each population or distinct metapopulation is likely to have its own genetic characteristics, and should thus be regarded as a separate unit in any plans for conservation.

The Welsh populations of *Luronium natans* (Figs 6 and 7) are probably one of its chief remaining strongholds within its world range, exhibiting the full spectrum of ecological diversity and, as we have shown, comparatively high levels of genetic variation. Their conservation and survival thus have particular importance. The declining or threatened status of *L. natans* across its world range, and the distribution, ecological status and importance of its native populations in Britain and Ireland, have not been fully appreciated in the past. In Britain, its colonization of abandoned canal systems led to the view that it was increasing (e.g. Rose 1983) and therefore had low conservation priority (Perring & Farrell 1983) although, in reality, expansion into canals probably did little more than to compensate for earlier losses from lowland pools and perhaps rivers. The observations of Briggs (1988), Willby & Eaton (1993) and Trueman *et al.* (1995) indicate that these canal populations are probably rather unstable and potentially transient. In addition to the deleterious efffects of competition, eutrophication and succession, Murphy & Eaton's observations (1983) suggest that *L. natans* might in any case be eliminated from most of its existing canal sites if the canals were fully reopened for navigation by pleasure-boats.

This presents something of a dilemma for the Countryside Council for Wales, English Nature and British Waterways, which are the agencies responsible for the conservation of *Luronium natans*,

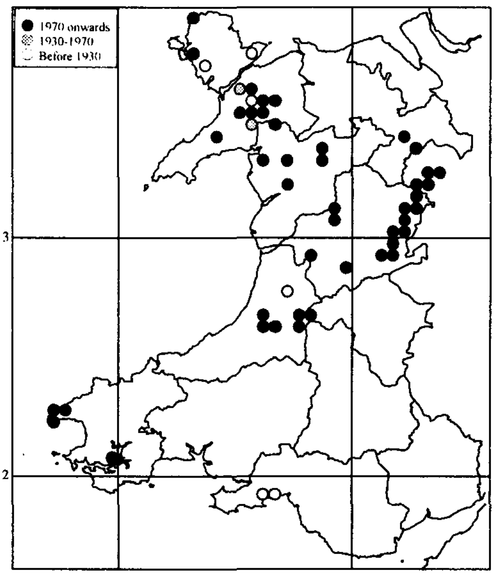


FIGURE 6. The distribution of *Luronium natans* in 5 km squares of the National Grid in Wales and the Welsh Borders. See Table 1 for details of individual populations; several 5 km squares contain more than one population. Map prepared using DMAP.

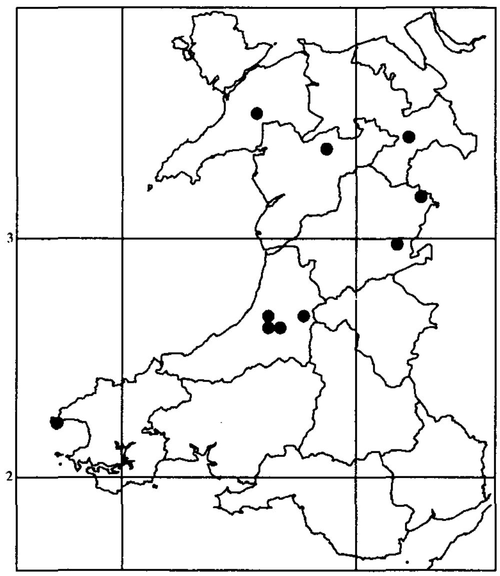


FIGURE 7. The distribution of sampled populations of *Luronium natans* in 5 km squares of the National Grid in Wales. See Tables 1 and 3 for more details; some 5 km squares contained more than one sampled population. Map prepared using DMAP.

but which also face strong pressure from recreational interests favouring re-opening or increased use of the canals for pleasure-boats. On the one hand, it can be argued that its spread into the canal habitat has reversed range decline for *L. natans* in lowland Britain, and that this habitat may now hold the largest population of the species anywhere in the world. On the other hand, this is a recent, highly artificial, and perhaps intrinsically transient distribution. British Waterways have attempted to conserve *L. natans* in a series of "off-line" canal reserves, and propose restoration of the Guilsfield Arm of the Montgomery Canal for the same purpose, but site management and population maintenance have presented considerable difficulties (Briggs 1996) and the long-term persistence of *L. natans* in such sites will probably depend largely on an appropriate interventionist management regime.

The results of this study provide a new insight into this problem. They show that, despite the considerable abundance of *Luronium natans* in the Welsh canal system in which it occurs, there is comparatively little genetic difference between plants from different parts of the canal system. Indeed, they could probably all have been raised from a small proportion of the plants in Llyn Tegid and, given appropriate management, this could take place in a relatively short space of time. The whole series of Welsh canal populations could thus be seen as a subpopulation of the plants of one upland lake (Llyn Tegid). If correct, this interpretation indicates that the canal populations are of correspondingly less importance in terms of genetic conservation. The origins of the large population of *L. natans* centred on the canals of Manchester in north-western England, which we did not sample, are less clear, but we suspect that the same basic principles of low genetic diversity will apply, even if this population did not derive from the same source. In contrast, the long-established oligotrophic lake and river populations and metapopulations, and the surviving pond population on Ramsey, have been shown to have distinct genetic identities, markedly different from those in similar habitats elsewhere, and in a few cases even quite sharply different from closely adjacent sites.

Within the main series of native populations in Wales, the highest priority should be given to the conservation of the native populations or metapopulations that show the greatest genetic diversity and of those that are long-established but disjunct. Examples of populations that grow in unusual

TABLE 4. *LURONIUM NATANS* – CONSERVATION STATUS, DATES OF RECORDS AND HABITATS OF ALL KNOWN SITES IN WALES

Vice-county and site name	Grid reference	Status	First record	Latest record	Habitat	Status
ANGLESEY (52)						
Mynachdy Reservoir	SH/31.92		1983	1987	Modified heath pond	Probably extinct
Llyn Dinam	SH/31.77	SSSI	1813	1984	Lowland formerly oligotrophic lake	Probably extinct
Llyn Coron	SH/37.70	SSSI	1813	1895	Lowland formerly oligotrophic lake	Probably extinct
Llyn Bodgylched	SH/58.77	SSSI	1834	1834	Lowland formerly oligotrophic lake	Probably extinct
CAERNARFON (49)						
Llyn Glasfryn	SH/40.42	SSSI	1987	1987	Lowland formerly oligotrophic lake	Probably extinct
Llyn Nantlle	SH/51.53		1834	1992	Upland oligotrophic lake	Extant
Llyn Cwellyn	SH/56.54	SSSI	1895	1994	Upland oligotrophic lake	Extant
Afon Rhythallt	SH/54.63		1895	1967	Upland oligotrophic slow-moving river	Unknown
Llyn-y-Dywarchen	SH/56.53		1895	1994	Upland oligotrophic lake	Extant
Llyn-y-Gadair	SH/56.52		1964	1992	Upland oligotrophic lake	Extant
Llyn Padarn	SH/57.61	SSSI	1848	1997	Upland formerly oligotrophic lake	Extant
Afon y Bala	SH/585.601		1773	1985	Upland oligotrophic slow-moving river	Probably extinct
Llyn Peris	SH/59.59		1805	1905	Upland oligotrophic lake	Probably extinct
Llyn Idwal	SH/64.59	SSSI/ NNR	1971	1971	Upland oligotrophic lake	Unknown
Llyn Cwmffynon	SH/64.56	SSSI	1992	1992	Upland oligotrophic lake	Extant
Llyn Llydaw	SH/62.54	SSSI/ NNR	1971	1971	Upland oligotrophic lake	Unknown
Afon Glaslyn	SH/59.47	?	1950	1950	Lowland oligotrophic slow-moving river	Unknown
MERIONETH (48)						
Llyn Cwmorthin	SH/67.46		1961	1997	Upland oligotrophic lake	Extant
Llyn Eiddew Bach	SH/64.34	SSSI	1955	1955	Upland oligotrophic lake	Probably extinct
Llyn Cwmbychan	SH/64.31	SSSI	1921	1997	Upland oligotrophic lake	Extant
Afon Eden	SH/70.30		1960	1997	Upland oligotrophic slow-moving river	Extant
Llyn Cynwch	SH/73.20		1888	1996	Upland oligotrophic lake	Extant
Llyn Tegid	SH/89.31	SSSI	1805	1996	Upland oligotrophic lake	Extant
DENBIGH (50) & FLINT (51)						
Llangollen Canal	SJ/296.397 –SJ/20.43		1862	1994	Lowland mesotrophic slow-moving canal	Extant

1. A 'site' is taken to mean a *continuous, still or slow-moving* water body; thus a canal population extending over 25 km or more is regarded as a single site, whilst two lakes linked by a fast-flowing stream would be listed separately. The names of the sites where one or more populations or subpopulations were sampled (see Table 3) are shown in italics.

2. 'Lowland' and 'upland' sites are defined by their *catchment altitudes*, so that Llyn Padarn at c. 100 m altitude is regarded as an 'upland' lake because of its primarily unenclosed catchment extending above 350m height, whilst Llyn Glasfryn at c. 130 m altitude remains wholly within a 'lowland' catchment.

TABLE 4. CONTINUED

Vice county and site name	Grid reference	Status	First record	Latest record	Habitat	Status
MONTGOMERY (47)						
Montgomery Canal	SO/12.94/ – SJ/26.20	SSSI	1933	1997	Lowland mesotrophic slow-moving canal	Extant
Llyn Coch-hwyad	SH/92.11		1993	1997	Upland oligotrophic lake	Extant
Llyn Gwyddior	SH/93.07		1993	1997	Upland oligotrophic lake	Extant
Llyn Bugeilyn	SN/82.92	SSSI	1962	1995	Upland oligotrophic lake	Extant
Llyn Ebyr	SN/97.88	SSSI	1988	1988	Lowland mesotrophic lake	Unknown
CARDIGAN (46)						
Llyn-yr-Oerfa	SN/72.79		1893	1893	Upland oligotrophic lake	Probably extinct
Llyn Eiddwen	SN/60.67	NNR	1893	1994	Upland oligotrophic lake	Extant
Llyn Fanod	SN/60.64	SSSI	1893	1997	Upland oligotrophic lake	Extant
Llyn Teifi	SN/78.67	SSSI	1893	1997	Upland oligotrophic lake	Extant
Llyn Hir	SN/78.67	SSSI	1989	1997	Upland oligotrophic lake	Extant
Llyn Egnant	SN/79.67	SSSI	1893	1996	Upland oligotrophic lake	Extant
Llyn-y-Gorlan	SN/78.66	SSSI	1893	1996	Upland oligotrophic lake	Extant
Llyn Gynon	SN/79.64	SSSI	1893	1994	Upland oligotrophic lake	Extant
Afon Teifi	SN/67.62	SSSI/ NNR	1924	1997	Upland mesotrophic slow-moving river	Extant
RADNOR (43)						
Llyn Cerrig-llwydion isaf	SN/84.69	SSSI	1995	1997	Upland oligotrophic lake	Extant
Llyn Cerrig-llwydion uchaf	SN/84.69	SSSI/ NNR	1997	1997	Upland oligotrophic lake	Extant
GLAMORGAN (41)						
Singleton	SS/62.91		1840	1840	Lowland, site uncertain	Probably extinct
Crymlyn Bog	SS/69.94	SSSI/ NNR	1840	1840	Lowland oligotrophic fen	Probably extinct
PEMBROKE (45)						
Porthmelgan stream	SM/73.28	SSSI	1944	1944	Former heathland pond	Probably extinct
Dowrog Pool	SM/77.27	SSSI	1905	1997	Heathland pool	Extant
Penlan Farm reservoir	SM/74.25		1981	1982	Modified heathland pond	Unknown
Ramsey Ponds	SM/70.23	SSSI	1925	1997	Heathland pond	Extant
Houghton Farm reservoir	SM/98.07		1982	1982	Lowland eutrophic pond	Probably extinct

or particularly threatened habitats (rivers and heathland ponds) should also be conserved where possible. The lake and pool habitats are delicately balanced and face a variety of threats, including eutrophication, acidification by acid rain and run-off from extensive conifer plantations, reservoir construction, pollution and disturbance, especially by powered boats and other recreational uses. At the 48 sites where it has been recorded in Wales (Table 4), *L. natans* is certainly or probably still present (recorded in 1992 or later) in 28, of uncertain present status in six, and believed lost in 14 (five of these losses having taken place since 1980).

By far the most severe decline has been in lowland sites. Ten out of 17 recorded populations (59%) have been lost here, as opposed to only four of the 31 upland populations (11%). The true ratio is likely to be worse, since in lowland sites *Luronium natans* is comparatively easy to detect, and there were certainly more lowland sites for the species in the past, which were not recorded individually (e.g. Davies 1813), whereas recent searches in upland lakes, where *L. natans* is often hard to detect, have yielded a number of rediscoveries and also some new sites.

The selection of sites and species for conservation has tended to rely on records of range and abundance (measured, all too often, in terms of 10 km square distribution). This study shows not only how changeable these data can be, but also what other levels of significance are being overlooked. In order to conserve the species as a whole we need to consider more than just its overall numbers and where it occurs: we need to see the relationships within and between these data – a population (if not a metapopulation) level of analysis. If, in this sense, the canal sites for *Luronium natans* do not quite have their former priority, they still have considerable ecological significance. One consequence of this study is to show the relationship between an apparently remote and isolated upland locality for the species and a newly-created artificial lowland site. The rapid expansion into the canal system demonstrates the possibility of recovery for this species in the lowlands, and the importance of low-nutrient (now mainly upland) refuge sites. A strategy for the conservation and recovery of *L. natans* needs to integrate maintenance of refugia with the restoration of natural lowland habitat. It is to be hoped that future population management will take account of both processes, within a framework of applied genetic research.

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REFERENCES

Baker, J. G. (1885). *The Flora of the English Lake District*. George Bell, London.

Benoit, P. & Richards, M. (1963). *A contribution to a Flora of Merioneth*, 2nd ed. West Wales Naturalists' Trust, Haverfordwest.

Björkvist, I. (1961). *Luronium natans* (L.) Raf. återfunnen i Skåne. *Botaniska notiser* **114**: 365–367.

Briggs, J. D., ed. (1988). *Montgomery Canal ecological survey, Survey Report 1985–1988*. Montgomery Canal Ecological Survey, Canal Wharf, Llanmynech.

Briggs, J. (1996). Canals – Wildlife value and restoration issues. *British wildlife* **7**: 365–377.

Chater, A. O. (1990). Recording in v.c. 46 during 1988 and 1989. *Botanical Society of the British Isles, Welsh bulletin* **49**: 9–12.

Clapham, A.R., Tutin, T.G. & Warburg, E.F. (1962). *Flora of the British Isles*, 2nd ed. Cambridge University Press, Cambridge.

Davies, H. (1813). *Welsh botanology*. W. Marchant, London.

Driscoll, R. J. (1992). A further note on Floating Water-plantain, *Luronium natans*, in Norfolk. *Transactions of the Norfolk and Norwich Naturalists' Society* **29**: 184.

Ellis, R. G. (1983). *Flowering plants of Wales*. National Museum of Wales, Cardiff.

Felsenstein, J. (1993). *PHYLIP – Phylogeny Inference Package (Version 3.5c)*. University of Washington, Seattle.

Ferguson, C. M. (1991). *The distribution of the Floating Water-plantain (*Luronium natans* (L.) Raf.) in Great Britain and Ireland*. 40 pp. B.Sc. thesis, Loughborough Institute of Technology.

Ferguson, C., Briggs, J. & Willby, N. (1998). Floating Water-plantain in Britain – under-recorded and overlooked? *British wildlife* **9**: 298–303.

Fritz, O. (1989). Flytsvalting, *Luronium natans*, funnen i Halland 1988. *Svensk botanisk tidskrift* **83**: 135–136.

Griffith, J. E. (1895). *The Flora of Anglesey and Carnarvonshire*. Nixon & Jarvis, Bangor.

Gutch, J. W. G. (1842). A list of plants met with in the neighbourhood of Swansea, Glamorganshire. *Phytologist* **1**: 180–187.

Halliday, G. (1978). *Flowering plants and ferns of Cumbria*. University of Lancaster, Lancaster.

Halliday, G. (1997). *A Flora of Cumbria*. Centre for North-West Regional Studies, University of Lancaster, Lancaster.

HANSPACH, D. & KRAUSCH, H. D. (1987). Zur Verbreitung und Ökologie von *Luronium natans* (L.) Raf. in der DDR. *Limnologica (Berlin)* **18**: 167–175.

HODGSON, W. (1898). *Flora of Cumberland*. W. Meals, Carlisle.

HOOKER, W. J. (1831). *The British Flora*. Longman, Rees, Orme, Brown & Green, London.

HYDE, H. A. & WADE, A. E. (1957). *Welsh flowering plants*, 2nd ed. National Museum of Wales, Cardiff.

JONES, R. A. & RICH, T. C. G. (1998). *Luronium natans / Baldellia ranunculoides / Alisma*, in RICH, T. C. G., JERMY, A. C. & CAREY, J. L., eds. *Plant crib 1998*, pp. 314–317. Botanical Society of the British Isles, London.

KAY, Q. O. N. & JOHN, R. F. (1995). *The conservation of scarce and declining plant species in lowland Wales: population genetics, demographic ecology and recommendations for future conservation in 32 species of lowland grassland and related habitats*. Science Report no. 110, Countryside Council for Wales, Bangor.

LACK, A. J. & KAY, Q. O. N. (1986). Phosphoglucose isomerase (EC 5.3.1.9) isozymes in diploid and tetraploid *Polygala* species: evidence for gene duplication and diversification. *Heredity* **56**: 111–118.

LOCKTON, A. & WHILD, S. (1997). *Rare plants of Shropshire: a Red Data Book of vascular plants*. Shropshire Flora Group, Shrewsbury.

LOUSLEY, J. E. (1970). The influence of transport on a changing flora, in PERRING, F., ed. *The Flora of a changing Britain* (B.S.B.I Conference Report no. 11). E. W. Classey, Faringdon.

MURPHY, K. J. & EATON, J. W. (1983). Effects of pleasure-boat traffic on macrophyte growth in canals. *Journal of applied ecology* **20**: 713–729.

NEI, M. (1972). Genetic distances between populations. *American naturalist* **106**: 385–398.

PERRING, F. H. & FARRELL, L. (1983). *British Red Data books: 1. vascular plants*, 2nd ed. Royal Society for Nature Conservation, Lincoln.

PERRING, F. H. & WALTERS, S. M. (1976). *Atlas of the British Flora*, 2nd ed. EP Publishing, East Ardley.

PRESTON, C. D. (1994). *Luronium natans* (L.) Raf., in STEWART, A., PEARMAN, D. A. & PRESTON, C. D., eds. *Scarce plants in Britain*, pp. 247–248. JNCC, Peterborough.

PRESTON, C. D. & CROFT, J. M. (1997). *Aquatic plants in Britain and Ireland*. Harley Books, Colchester.

PRESTON, C. D. & HILL, M. O. (1997). The geographical relationships of British and Irish vascular plants. *Botanical journal of the Linnean Society* **124**: 1–120.

RICH, T. C. G., KAY, G. M. & KIRSCHNER, J. (1995). Floating water-plantain *Luronium natans* (L.) Raf. (Alismataceae) present in Ireland. *Irish Naturalists' journal* **25**: 140–145.

RIDLEY, H. N. (1930). *The dispersal of plants throughout the world*. L. Reeve, Ashford.

RODRIGUEZ-OUBINA, J. & ORTIZ, S. (1991). *Luronium natans* (Alismataceae) in the Iberian peninsula. *Willdenowia* **21**: 77–80.

ROELOFS, J. G. M. (1983). Impact of acidification and eutrophication on macrophyte communities in soft waters in the Netherlands. I. Field investigations. *Aquatic botany* **17**: 139–155.

ROSE, F. (1983). *The wild flower key*. Warne, London.

SALTER, J. H. (1935). *The flowering plants and ferns of Cardiganshire*. University of Wales Press, Cardiff.

SHAW, C. R. & PRASAD, R. (1970). Starch gel electrophoresis of enzymes – a compilation of recipes. *Biochemical genetics* **4**: 297–320.

SHIELDS, C. R., ORTON, T. J., & STUBER, C. W. (1983). An outline of general resource needs and procedures for the electrophoretic separation of active enzymes from plant tissues, in TANKSLEY, S. D. & ORTON, T. J., eds. *Isozymes in plant genetics and breeding, Part A*, pp. 443–468. Elsevier, Amsterdam.

SMITH, J. E. (1828). *The English Flora*, 2nd ed. Longman, Rees, Orme, Brown and Green, London.

TRIEST, L., ed. (1991). Isozymes in water plants. *Opera botanica Belgica* **4**: 1–259. National Botanic Garden of Belgium, Meise.

TRIEST, L. & ROELANDT, B. (1991). Isozymes in diploid and polyploid *Alisma* species (Alismataceae), in TRIEST, L., ed. Isozymes in water plants. *Opera botanica Belgica* **4**, pp. 27–36. National Botanic Garden of Belgium, Meise.

TRIEST, L. & VUILLE, F.-L. (1991). Isozyme variation in several seed collections and hybrids of *Baldellia* (Alismataceae), in TRIEST, L., ed. Isozymes in water plants. *Opera botanica Belgica* **4**, pp. 37–48. National Botanic Garden of Belgium, Meise.

TRUEMAN, I., MORTON, A. & WAINWRIGHT, M. (1995). *The Flora of Montgomeryshire*. Montgomeryshire Field Society and Montgomeryshire Wildlife Trust, Welshpool.

TURNER, D. & DILLWYN, L. W. (1805). *The botanist's guide through England and Wales*. Phillips and Fardon, London.

WILLBY, N. J. & EATON, J. W. (1993). The distribution, ecology and conservation of *Luronium natans* (L.) Raf. in Britain. *Journal of aquatic plant management* **31**: 70–78.

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