POPULATION GENETICS OF SOME SPECIES OF *POTAMOGETON* L.

Thesis submitted for the degree of
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by

Peter Michael Hollingsworth BSc (Wolverhampton)
Department of Botany
University of Leicester

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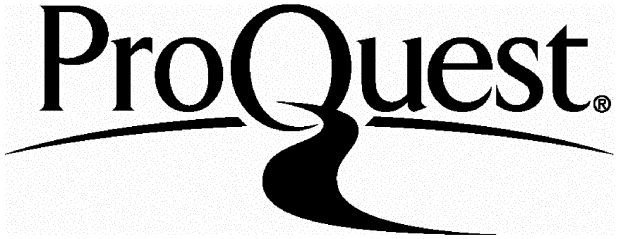

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ABSTRACT

Selected *Potamogeton* taxa were studied to establish the levels of gene flow within species, and to test the identity of putative hybrids.

390 individuals from twelve British populations of the anemophilous aquatic *Potamogeton coloratus* were analysed using starch gel electrophoresis of isozymes. Low levels of variability were found, with evidence of considerable inbreeding and / or clonal spread. Only two of the populations sampled are polymorphic; both inhabit sites with a long post-glacial history as wetlands. Populations of recent origin, as well as some of older vintage, contain only a single multi-locus isozyme genotype. Evidence for a duplicated IDH locus is presented. A further 647 individuals of *Potamogeton coloratus* from 60 ditches in the Gordano Valley, Somerset were analysed for variation at two polymorphic PGM loci. High levels of partitioning of genetic variation between ditches was observed with F_{ST}=0.575.

A population of pondweeds from the River Stour in Dorset intermediate in morphology between *Potamogeton natans* L. and *P.nodosus* Poir. is shown by means of isozyme evidence to be the hybrid *P.* x *schreberi* G.Fisch. It is represented by a single multi-enzyme phenotype which, together with its sterility, suggests it reproduces vegetatively and may well constitute a single clone. It is genetically distinct from the morphologically similar hybrid between *P.lucens* L. and *P.natans* (*P.* x *fluitans* Roth).

Genetic variation in *Potamogeton pectinatus* and *P.filiformis* was studied using isozymes. The overall levels of variability are similar to some other well-studied hydrophilous species, as well as to the average for terrestrial clonal species. Variation is shown to be distributed more between than within populations. Isozyme evidence supports the hypothesis that plants morphologically intermediate between *Potamogeton pectinatus* and *P. filiformis* are hybrids of these two species. Variation in enzyme banding patterns suggests that this hybrid has arisen on at least eleven occasions.

In a review of molecular population genetic studies of aquatic plants, no significant difference was found between the levels of genetic diversity in aquatic plants compared to that found in clonal terrestrial plants. Many populations of aquatic plants do however show high levels of subdivision between populations.

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POPULATION GENETICS OF SOME SPECIES OF *POTAMOGETON* L.

PREFACE

The purpose of this study was to use molecular markers to study hybridisation and population genetic structure of selected *Potamogeton* species.

Chapter 1 is an introduction, briefly describing the genus and the techniques available for population genetic and biosystematic studies. Chapter 2 presents a discussion on *Potamogeton* cytology. Chapters 3 and 4 discuss gene flow within and between populations of *P. coloratus*. Chapter 5 details a study using molecular techniques to investigate hybridisation between *P. natans* and *P. nodosus*. Chapters 6 and 7 describe studies investigating intra- and inter-specific gene flow in the subgenus *Coleogeton*. Chapter 8 is a review of the work described here, set in the context of other published studies on aquatic plants. Appendix 1 contains an 'Isozyme Lab. Guide' which was compiled during the course of these studies.

The chapters in this thesis are mainly manuscripts of papers submitted for publication. An expanded version (including information on *Ruppia*) of the Cytology section (chapter 2- Hollingsworth 1995) is currently in press as part of a BSBI handbook entitled '*Pondweeds of Great Britain and Ireland*' (Preston 1995a). Chapter 3 (Hollingsworth *et al.* 1995a) has been submitted to 'Plant Systematics and Evolution' . Chapter 4 (Hollingsworth *et al.* 1995b) will be submitted to 'Aquatic Botany'. Chapter 5 (Hollingsworth *et al.* 1995c) has been accepted by the 'Botanical Journal of the Linnean Society'. Chapters 6 and 7 (Hollingsworth *et al.* 1995d, e) will be submitted to the 'Nordic Journal of Botany', and chapter 8 (Hollingsworth *et al.* 1995f) to 'Aquatic Botany'. As the work is presented as a collection of papers rather than as a traditional thesis, there is a certain amount of unavoidable repetition of introductory material as each paper (chapter) needs to be complete in its own right.

Nomenclature for the British members of the Potamogetonaceae follows Stace (1991). For all other species mentioned, I use the nomenclature given (unless otherwise stated) in the publication in question.

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CHAPTER 1

INTRODUCTION

Plants adapted to the aquatic habitat occur across a taxonomically wide range of groups including algae, pteridophytes and angiosperms. Among angiosperms, aquatic plants are found in a diverse array of monocot and dicot families, many of which are considered to represent numerous independent invasions of the aquatic habitat (Philbrick 1988). The genus at the centre of the present work, *Potamogeton,* belongs in the Potamogetonaceae, one of an assemblage of families in the monocot subclass Alismatidae (Cronqulst 1981). This subclass includes a high proportion of aquatic species, and shows a remarkable diversity of floral and vegetative characters (Tomlinson 1982). It has been suggested that the Alismatidae represent an ancient lineage of monocots, and their similarity to the dicot order Nympaeales has led to suggestions that they may have evolved from a group of dicots similar to this (Cronquist 1981), with some support for this coming from a *rbc* L sequence phylogeny constructed by Chase *et al.* (1993). This view, however, is not without its critics, and Dahlgren *et al.* (1985) noted that life in an aquatic environment could possibly give rise to similarities due to convergent evolution, and no clear consensus of opinion exists.

Potamogeton

The family Potamogetonaceae contains two genera, *Potamogeton* L. and *Groenlandia* Gay. *Groenlandia* is a monotypic genus, the only species being *G. densa* (L.) Fourr. *Potamogeton* in contrast is estimated to contain 80-90 species worldwide (Wiegleb 1988), although as yet there remains some uncertainty due to the absence of a recent world monograph (Preston 1995a). The word *Potamogeton* is derived from the Greek *Potamos* - meaning river and *Geton* - meaning neighbour (Stearn 1973).

Potamogeton is a widespread genus, which occurs in all the major areas of the world except the cold deserts of the Arctic and Antarctic, and hot deserts elsewhere (Preston 1995a). All of the species are primarily aquatic and some can form dense, monospecific stands and are considered weeds

(Sculthorpe 1967). The genus contains narrow-leaved, broad-leaved and heterophyllous species and, as a group, *Potamogeton* has a certain notoriety for the species being difficult to identify. This stems from three main reasons, firstly their aquatic habitat discourages casual observations, and some effort and discomfort is often required to collect material. Secondly the differences between species are often subtle and in some cases require microscopic examinations of structures such as leaf sheaths, precluding field identifications. Finally the extensive degree of phenotypic plasticity shown by individual plants can cause confusion when an environmentally induced phenotypically different 'form' of a species is encountered.

There has been a number of studies regarding the taxonomy of *Potamogeton* since its description by Linnaeus in 1753. The publications of Ascherson & Graebner (1907), Fernald (1932), Hagström (1916), Haynes (1974, 1985), Ogden (1943), Sauvageau (1893-1894), St. John (1925) and Wiegleb (1988) have all made significant contributions to the present knowledge of the genus. Hagström (1916) recognised two subgenera, five sections, 26 subsections, 138 species and a large number of hybrids in *Potamogeton* ; he did not, however, present a key. This led St John (1925) to comment "in order to identify a *Potamogeton* , it is necessary to read every page of this work and practically memorise them".

In the British Isles, Alfred Fryer devoted much of his life's work to a study of *Potamogeton* , and co-authored with Arthur Bennett a monograph entitled *'The Potamogetons (Pond Weeds) of the British Isles'* (1915). It was Fryer (1890) who first appreciated the extent of hybridisation in *Potamogeton* (Preston 1988) which, coupled with his understanding of the difference between 'temporary states' and 'permanent varieties' (ie: the difference between phenotypic and genetic forms) (Fryer & Bennett 1915), led to a particularly valuable contribution to the taxonomic treatment of the genus.

Another British student of the genus was J. E. Dandy who, along with Sir George Taylor, published a whole series of papers (Dandy 1937, 1970, 1975, 1980; Dandy & Taylor 1938a,b,c, 1939a, b,c,d,e,f,g,h, 1940a,b,c, 1941, 1942a,b, 1944a,b, 1946, 1957; Taylor 1949) which helped further to

elucidate relationships among the different *Potamogeton* taxa. Dandy (1975, 1980) recognised 21 British species of *Potamogeton* along with 25 hybrids. More recent studies of British *Potamogeton* have been carried out by C. D. Preston and are documented in '*Pondweeds of Great Britain and Ireland*' (Preston 1995a) and references therein, with the evidence for a 26th hybrid given by Preston 1995b).

The primary subdivision in the genus *Potamogeton* is into two subgenera: *Potamogeton* and *Coleogeton* Reichb. The main distinguishing characteristics of subgenus *Coleogeton* are the presence of a leaf sheath, translucent ligule and a flexuous peduncle (Preston 1995a). Differences in chromosome number (Hollingsworth 1995 - chapter 2) and pollen morphology (Sorsa 1988) also add further support to the taxonomic separation of the two subgenera.

The majority of the species of the genus *Potamogeton* are contained within the subgenus *Potamogeton,* which has three recognised sections represented in the British Isles with the main features of each group, taken from Preston (1995a), given below. Section *Potamogeton* consists of rhizomatous plants with terete stems; most species have broad submersed leaves, and many have coriaceous floating leaves. Turions are not formed, with vegetative perennation being achieved by elongated buds on the rhizome, or more rarely, by buds on short shoots in the lower leaf axils. The section *Graminifolii* consists of non-rhizomatous plants with often (although not always) compressed stems, linear submersed leaves, and no floating leaves. Members of this section regularly produce turions. *P. epihydrus* is a somewhat anomalous member of this group in European terms (although some North American species have a similar combination of characters), in that it is a rhizomatous plant, with compressed lower stems, linear submersed leaves, broad floating leaves, and relatively unspecialised turions. Section *Batrachoseris* (of which *P. crispus* L. is the sole representative) consists of plants with compressed stems with a groove running down the broader side, markedly serrate leaves and fruits with a beak which is at least half as long as the rest of the fruit. Turions are regularly formed.

Wiegleb (1988) recognised eleven species in the "*P. pectinatus* group" (i.e. subgenus *Coleogeton*) with only three of these being found in Europe. One of these, *P. vaginatus* Turcz., is the only *Potamogeton* species found in Europe that does not occur in the British Isles. The three European species occur in two sections of subgenus *Coleogeton,* with *P. filiformis* Pers. placed in section *Connati,* and *P. pectinatus* L. and *P. vaginatus* Turcz. placed in section *Convoluti* by Hagström (1916). A summary of the major taxonomic divisions among species of the Potamogetonaceae is shown in Fig. 1.

Evolution in *Potamogeton*

Hagström (1916) considered that the subgenera *Coleogeton* and *Potamogeton* diverged at an early stage. Les & Sheridan (1990) outlined three possible evolutionary scenarios to account for the present morphological diversity among *Potamogeton* species:

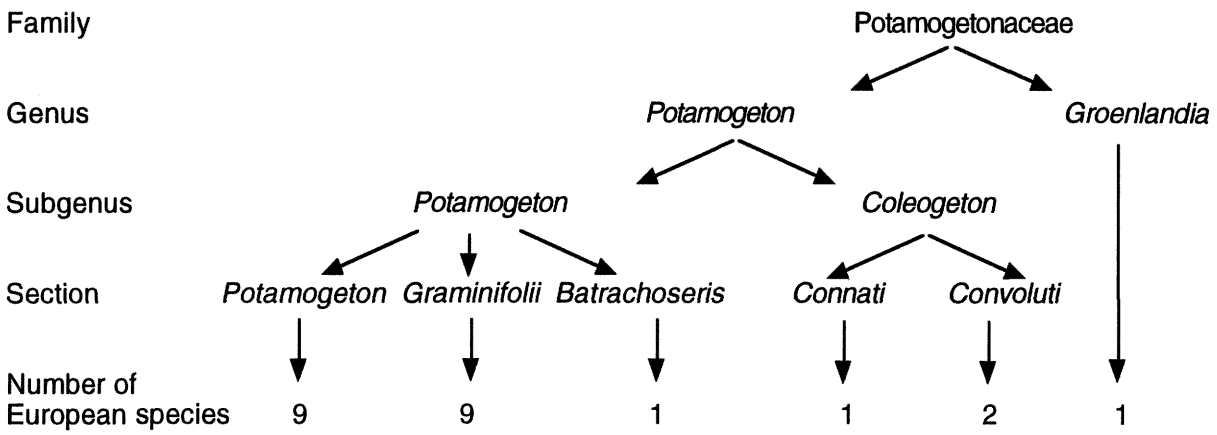
1. Linear submersed leaved species gave rise to broad submersed leaved species and heterophyllous (submersed and floating) leaved species.

2. Broad submersed leaved species gave rise to linear submersed leaved species and heterophyllous species.

3. Heterophyllous species gave rise to broad leaved species and linear leaved species.

Les and Sheridan (1990) used Hagström's (1916) morphological data and analysed it using modern cladistic methods. They concluded that the data were most consistent with the second suggestion, that the ancestral species were broad leaved species. They suggested that subgenus *Coleogeton* represents a highly specialised group derived from section *Graminifolii.* However, until further research is carried out this represents merely a tentative hypothesis.

Fig. 1 Outline of classification of European Potamogetonaceae



As well as the confusion over the broad evolutionary history of *Potamogeton*, there still remain many questions relating to the finer scale problems of gene flow within and between populations and species. Hybrids between all of the British sections of the subgenus *Potamogeton* have been reported, and hybrids have also been recorded within the subgenus *Coleogeton* but none has been found between subgenera.

Preston (1995a) noted that in Britain and Ireland, *Potamogeton* is not remarkable for the number of hybrids recorded, but for their ability to persist and spread vegetatively once they are established. Almost all of the *Potamogeton* hybrids recorded appear to be completely sterile, with *P.* x *zizii* being the only one that sometimes produces numerous fruit. The viability of these fruits remains to be proven, as does that of the occasional fruit found on plants of *P.* x *fluitans* and *P.* x *sudermanicus.* However, the long term persistence of putative hybrids at sites where either one or both of the parental species is absent (Dandy & Taylor 1946), demonstrates that vegetative means of reproduction can be effective.

In a genus like *Potamogeton* , where individuals can show extensive phenotypic plasticity, and where hybrids between morphologically similar species have been described, the identity of hybrids can sometimes be challenged (Les & Philbrick 1993). Furthermore, even in the case where the identity of the hybrid is unambiguous, it is still not clear how many times such a hybrid has arisen.

Some aspects of the population biology of *Potamogeton* species as yet remain unclear, with many showing adaptations to vegetative as well as sexual reproduction. Vegetative spread by specialised structures such as turions, and unspecialised structures such as rhizomes and stem fragments, can be observed in a number of *Potamogeton* species. It still, however, remains to be tested which reproductive mode (vegetative versus sexual), if any, predominates in a particular species. Certainly, seedlings of *Potamogeton* species, have only rarely been seen in the wild (Preston 1995a) Thus it is not clear to what extent gene flow within a population occurs, whether taxa are inbreeding or outbreeding, whether populations

consist of ramets of a single genet, or whether multiclonal or sexual populations are the norm. On a broader scale, the degree of isolation of populations is also unclear. On the one hand populations of aquatic plants in habitats such as lakes are often considered as 'aquatic islands in a terrestrial sea' (Laushman 1993), and as such are potentially isolated from one another. And on the other, the speed by which *Elodea canadensis* Michx. colonised the waterways of Britain (Richards 1986) implies that levels of dispersal between populations can be high.

PROJECT AIMS

1. To establish the levels of gene flow within and between populations of selected *Potamogeton* taxa.

2. To test the identity of putative hybrids, and to determine how often they have arisen.

Approach

Most studies on the taxonomy and evolution of *Potamogeton* have involved comparisons of vegetative and reproductive structures, including stem anatomy (Wiegleb 1990), pollen morphology (Sorsa 1988) and fruit morphology (Aalto 1970). However, for questions pertaining to population biology and hybridisation, it is desirable to have further lines of evidence to compare and contrast with morphological and anatomical data.

Molecular Markers

One method of gaining biosystematic and population genetic information is to use molecular markers. Molecular markers (if correctly chosen) have the advantage that, as well as detecting variation which is often not morphologically evident, they are (unlike many phenotypic characters) not environmentally malleable and thus present ideal tools to study the levels of genetic exchange between individuals, populations and species (Avise 1994).

Chromosomes

While not molecular markers as such, chromosomes have been included in this section as they represent microscopic genetic characters and perhaps share more in common with molecular markers than they do with anatomical or morphological characters.

The chromosome number of a plant is of interest to taxonomists for several reasons (Stace 1989). It provides a character which can help to distinguish one species from another, and which can be used to test the homogeneity of taxa whose circumscription has been arrived at by the study of macroscopic characters. If differences in chromosome number occur within a species, they can indicate breeding barriers within populations of that species. In a group in which hybrids occur, there is the additional prospect of confirming the identity of hybrids between parents of differing chromosome number. Chromosome numbers also can provide information about the affinities of species in a genus, and suggest possible evolutionary relationships. Studies of chromosome numbers in *Potamogeton* had started before the end of the 19th century, and the documentation of the chromosome number of *P.foliosus* Raf. (Wiegand 1899) probably represents one of the earliest chromosome counts. A detailed review of the current knowledge on *Potamogeton* chromosome numbers is presented in Hollingsworth (1995 - chapter 2).

Flavonoids

Compounds such as flavonoids and other secondary metabolites have provided much useful systematic data (Crawford 1978, Giannasi 1978, Gornall *et al.* 1979). Bohm (1987) reviewed the levels of intraspecific flavonoid variation in plants, and cited many instances where complex arrays of pigments are known within a species. However, ontogenetic and environmentally induced variation, coupled with uncertainties in many cases regarding the exact nature of their inheritance, has reduced the applicability of this approach in population studies.

Isozymes

Isozymes are by far the most significant class of protein markers for

population biologists. Smithies (1955) described starch gel electrophoresis which, coupled with the report of histochemical staining of enzymes on gels (Hunter & Markert 1957), laid the foundations for the technique of starch gel electrophoresis of isozymes. It was not until the 1960s, however, when studies by Harris (1966) and Lewontin & Hubby (1966) demonstrated the simple co-dominant Mendelian inheritance of allelic forms of isozymes, that the 'protein electrophoresis revolution' really began.

Electrophoresis of isozymes offers a relatively simple and cheap method of quickly analysing large numbers of individuals to gain allelic data, on which inferences of gene flow can be made (Schaal *et al.* 1991). In the 30 or so years following the discovery of starch gel electrophoresis of isozymes, literally thousands of studies have been published on animals (Harris & Hopkinson 1976), fungi (Micales 1986) and plants (Soltis & Soltis 1989).

In plants, isozyme studies have been used to address a number of problems including assessment of levels of genetic variation within and between populations (Ellstrand & Roose 1987, Hamrick 1989, Hamrick & Godt 1989, Loveless & Hamrick 1984, Silander 1985); analysis of mating systems (Brown *et al.* 1989, Ritland 1983); investigations into interspecific hybridisation (Crawford 1989, Wang *et al.* 1990) and studies attempting to elucidate the evolutionary origin of polyploid species (Ashton & Abbott 1992, Raybould *et al.* 1991a & b; Roose & Gottlieb 1976). Several reviews covering applications of isozyme techniques to these botanical questions, have been published in recent years, with excellent summaries and bibliographies presented in Soltis & Soltis (1989).

Several studies (Hamrick *et al.* 1979, Hamrick 1989, Hamrick & Godt 1989, Loveless & Hamrick 1984) have reviewed evidence from isozyme data relating to the determinants of levels of genetic variation in plants. Although a wide range of factors appear to affect the levels of variation (e.g. reproductive biology, seed dispersal mechanisms, phenology, life cycle, geographical range, successional stage, population size, population density, and population spatial distribution), Hamrick (1989) concluded that a combination of the mating system with the pollination mechanism provided

the best means of predicting the distribution of variation, with annual selfing species having more than 50% of their variation stored among, as opposed to within, populations whereas predominantly outcrossed wind pollinated species have less than 10% of their variation distributed among populations.

Critics of isozymes as molecular markers in population and biosystematic studies express concern that they could be affected by epistatic and pleiotropic interactions, that they may not be selectively neutral, and that their use can lead to under-estimates of the levels of genetic diversity. Over the past three decades, numerous studies have demonstrated the simple Mendelian inheritance of a considerable number of isozymes, while comparatively few studies have reported problems of the nature suggested above (Wendel & Weeden 1989). There have been occasional reports which challenge the value of isozymes as population genetic and biosystematic markers on the grounds that selection can occur for specific isoforms (Koehn & Hilbish 1987; Watt, 1983) and non-genetic variation in banding patterns can occur (Ryan *et al.* 1991), although with careful experimental design many of the potential pitfalls of this technique can be greatly reduced or eliminated.

DNA

Nuclear and organelle DNA can now be analysed using a number of techniques predominantly based around Southern hybridisations (Southern 1975) and PCR - the polymerised chain reaction (Mullis *et al.* 1986). In angiosperms, nuclear markers are typically inherited in a bi-parental fashion, and organelle markers are typically (but not exclusively) maternally inherited. Thus different classes of marker that show different evolutionary rates can be selected as appropriate for the study in question (Avise 1994). In plants these markers will usually be from either the nuclear or chloroplast genomes. Mitochondrial DNA markers have not been employed in botany to anything like the extent they have in animal biology, primarily due to the great variation in size and the extent of recombination of mitochondrial molecules within individuals (Palmer 1992).

Some DNA markers can show powerful discrimination between closely

related individuals, such as minisatellite fingerprints (Jeffreys *et al.* 1985, 1987; Burke *et al.* 1991; Nybom *et al.* 1990), microsatellite fingerprints (Bruford & Wayne 1993) and RAPDs (Williams *et al.* 1990). Analysis of variation between populations has typically (although not exclusively) been assessed using variation in multi-gene families, such as length and sequence variation in both transcribed and non transcribed spacer regions of ribosomal RNA genes (Saghai-Maroof *et al.* 1984, Schaal *et al.* 1987). Differentiation between widely separated populations and studies of genetic variation between species have also used ribosomal markers (Doyle *et al.* 1985, Liston *et al.* 1990), although the more slowly evolving chloroplast DNA markers are also often suitable if appropriate regions can be found (Ferris *et al.* 1993, Wang & Szmidt 1990, Palmer 1987).

For this study I have chosen isozymes as being the most appropriate tool, for reasons of efficiency, cost, and the fact that co-dominant inheritance allows gels to be read like a genetic book without having to perform additional analyses. The efficiency of this technique has allowed the examination of genetic variation in more than 2000 individuals in this study.

CHAPTER 2

CHROMOSOME NUMBERS OF THE BRITISH POTAMOGETONACEAE

The Potamogetonaceae is by far the largest flowering plant family in the British Isles for which there are no published chromosome numbers based on native material (R.J. Gornall pers. comm.). The following unpublished and tentative counts of *Potamogeton* have been obtained from British material by me.

P. coloratus	2n=*c.*26	Gordano Valley, Somerset	ST/43.73
P. natans	2n=*c.*52	Arne, Dorset	SY/95.86
P. pectinatus	2n=*c.*78	Welches Dam, Cambridgeshire	TL/46.85
P. perfoliatus	2n=*c.*52	Block Fen, Cambridgeshire	TL/43.83
P. polygonifolius	2n=28	Hartland Moor, Dorset	SY/96.85
P. x *nitens*	2n=*c.*52	Block Fen, Cambridgeshire	TL/43.83
P. x *salicifolius*	2n=*c.*52	Welches Dam,Cambridgeshire	TL/48.85

Chromosome numbers have been published for all the British and Irish species from material collected outside our area. These numbers are listed in Table 1. The haploid chromosome number is indicated by n, the diploid number by 2n. The counts given in the first column are taken from Les (1983), whose criterion for inclusion was the most commonly reported number (Les & Sheridan 1990). The number of reports on which this figure is based is given in brackets after the count. Counts in the second column are deviations from those reported in Les' review, with a code letter for the reference to the source of the deviant count. The code letters are explained below the list. *Potamogeton berchtoldii* and *P. pusillus* are regarded as synonymous by Les (1983) and *Groenlandia densa* is not included within the scope of his paper; counts for these species are taken from Palmgren (1939). There do not appear to be any published counts of the *Potamogeton* hybrids which are recorded in Britain and Ireland.

Table1.Chromosome numbers in British members of the Potamogetonaceae

Potamogeton		
Subgenus *Potamogeton*		
Section *Potamogeton*		
P. natans	2n=52 (8)	n=21 (A), 2n=*c.*42 (B)
P. nodosus	2n=52 (3)	
P. lucens	2n=52 (2)	
P. gramineus	2n=52 (8)	
P. polygonifolius	2n=52 (2)	n=13 (C), 2n=26 (D), 2n=28 (E)
P. coloratus	2n=26 (1)	
P. alpinus	2n=52 (6)	2n=26 (F)
P. praelongus	2n=52 (6)	
P. perfoliatus	2n=52 (12)	n=7 (G), n=*c.*24 (H), 2n=26 (B), 2n=*c.*40 (B), 2n=78 (I,J)
Section *Graminifolii*		
P. epihydrus	2n=26 (2)	
P. friesii	2n=26 (2)	
P. rutilus	2n=26 (1)	
P. pusillus	2n=26	2n=28 (K)
P. obtusifolius	2n=26 (1)	
P. berchtoldii	2n=26	
P. trichoides	2n=26 (3)	
P. compressus	2n=26 (3)	
P. acutifolius	2n=26 (2)	
Section *Batrachoseris*		
P. crispus	2n=52 (9)	2n=26 (L,M), 2n=50,56 (N), 2n=52, some cells 36 (O), 2n=78, some cells 72 (O)
Subgenus *Coleogeton*		
P. filiformis	2n=78 (6)	2n=*c.*66 (C)
P. pectinatus	2n=78 (9)	2n=42 (N), 2n=*c.*66 (J),2n=70,71,73, 74,75,76,77,79,80,81,82,83,84,85, 86,87 (P), 2n=86 (Q)
Groenlandia		
G. densa	2n=30	

Key to reference codes: A: Stern (1961); B: Probatova & Sokolovskaya (1984); C: Palmgren (1939); D: Ficini *et al.*, (1980); E: Hollingsworth (see above); F: Löve & Kjellqvist (1973); G: Takusagawa (1939); H: Wisniewska (1931); I: Arohonka (1982); J: Probatova & Sokolovskaya (1986); K: Harada (1956); L: Bhattacharya & Ghosh (1978); M: Ghosh & Bhattacharya (1980); N: Misra (1972); O: Sharma & Chatterjee (1967); P: Kalkman & van Wijk (1984); Q: Yurtsev *et al.* (1975).

All chromosome counts of *Potamogeton* should be treated with some caution. *Potamogeton* chromosomes are difficult to work with. They vary in size from small to very small (>1μm), and usually lack any distinguishing morphological characters; this means that they are not only difficult to count but also makes it difficult to distinguish 'A-chromosomes' (which are included in the chromosome number) from super-numerary chromosomes (traditionally excluded) (Kalkman & van Wijk 1984). Obtaining accurate karyotype data is consequently fraught with difficulty.

The technical difficulties of counting *Potamogeton* chromosomes make it hard to discriminate between genuine cytological phenomena, such as aneuploidy (the gain or loss of individual chromosomes), and experimental errors. Few students admit to the difficulties involved in *Potamogeton* cytology, and most do not indicate that there is any possibility that some counts may not be accurate. Kalkman & van Wijk (1984), however, provided useful information on the range of variation in the chromosome number of *P. pectinatus*. They reported a range of chromosome numbers in each of the 15 populations they studied, with the accepted number of the species, 2n=78, being the mode of 17 different counts. It is not clear from their paper whether the variation in chromosome number in each population occurred within or between individuals.

Within the genus *Potamogeton,* the most frequently reported numbers correlate quite well with the delimitation of the sections based on morphology and anatomy. The members of subgenus *Coleogeton* have the same base number as those in subgenus *Potamogeton,* but at a higher ploidy level. The lack of systematic variation in modal chromosome numbers within sections reduces the possibility of obtaining useful cytological evidence on the identity of putative hybrids.

Les (1983) reviewed the chromosome numbers of all species of *Potamogeton* and suggested that the diploid number for the genus is 2n=14. This has only been reported twice from the genus (Hollingsworth *et al.* 1995a - chapter 3), but five species characterised (on Les' criterion) as tetraploids with 2n=28 occur in eastern Asia and two are found in North

America. This number has also been found recently for a British population of *P. polygonifolius*. Les interpreted chromosome numbers which are multiples of 13 as being derived by aneuploidy from plants with 2n=14 followed by polyploidy. This reduction from 14 to 13 may have happened only once, or may have occurred on several occasions independently. The interpretation put forward by Les (1983) has been challenged by Wiegleb (1988), who regarded the ancestral chromosome number as 2n=26. Les & Sheridan (1990) argue that phylogeny should be used to clarify chromosomal trends rather than *vice versa*, but at the moment there seems little prospect that either can be clarified without a lot of basic research.

The use of chromosome numbers to interpret the evolutionary history of the species of *Potamogeton* is bedevilled both by uncertainties about the accuracy of many counts, and by the difficulties posed by aneuploidy. Aneuploidy has almost certainly occurred in the genus and can be used to construct any number of scenarios to link numbers based on multiples of 7, 13 and (for *Groenlandia*) 15.

CHAPTER 3

GENETIC VARIABILITY IN BRITISH POPULATIONS OF *POTAMOGETON COLORATUS* (POTAMOGETONACEAE)

ABSTRACT

The partitioning of genetic variability within and between British populations of the anemophilous aquatic *Potamogeton coloratus* is investigated by isozyme analysis. Low levels of variability are found, with evidence of considerable inbreeding and / or clonal spread. Only two populations are polymorphic; both inhabit sites with a long post-glacial history as wetlands. Populations of recent origin, as well as some of older vintage, contain only a single multi-locus isozyme genotype. Evidence for a duplicated IDH locus is presented.

INTRODUCTION

A considerable body of evidence from isozyme studies has shown that characteristics of the breeding system and life history are key factors determining genetic variability both within and between populations of plant species (Loveless & Hamrick 1984). There have been, however, remarkably few empirical studies aimed at analysing the partitioning of variation within and between populations of aquatic macrophytes (Les 1991). Most of these studies have recorded only low levels of isozyme variation, especially within populations (Les 1988; Triest 1991e). This is consistent with several features of many aquatic vascular plants that are likely to reduce or restrict genetic variability. These include a) infrequent flowering and seed production; b) hydrophilous pollination leading to reduced pollen-mediated gene flow; and c) clonal spread. Long distance dispersal of the diaspores of water plants may sometimes lead to founder effects and genetic drift, although at other times it may increase genetic variability by promoting immigration.

There have been very few studies which deal with wind-pollinated aquatic macrophytes. Anemophily in terrestrial species is often associated with xenogamy (Richards 1986) and typically produces considerable levels of

genetic variability within populations, with differentiation between populations being less marked (Loveless & Hamrick 1984). In a detailed investigation of the anemophilous *Myriophyllum alterniflorum* DC. in Britain (chiefly in western Scotland), Harris *et al.* (1992) observed extensive isozyme variation within and between populations. Many populations were in Hardy-Weinberg equilibrium and differentiation among them was strong Harris *et al.* ascribed these findings to the plant's abundant flowering, its anemophilous pollination syndrome, and its long post-glacial history in the study sites in western Scotland, which are close together and variable in water chemistry. A second study of wind-pollinated aquatic taxa was conducted by Hettiarachchi & Triest (1991), who examined the isozymes of 188 populations of 18 *Potamogeton* species, nearly all of which were anemophilous. They observed a range of levels of genetic variability but, as the emphasis of the work was taxonomic, detailed information on variation within populations was not presented.

Further studies of anemophilous aquatics are needed to examine the expectation that such a pollination syndrome necessarily affects the genetic structuring of populations. The purpose of the present work, therefore, is to investigate the population genetic structure in Britain of *Potamogeton coloratus* Hornem., a wind-pollinated aquatic vascular plant species which flowers and fruits freely, has no specialised means of vegetative reproduction other than rhizomatous spread, and which occurs in both newly created sites (where it is a recent immigrant) and in much older sites (where it may have a long post-glacial history). As with *Myriophyllum*, such a species might be expected to show high levels of variability, at least within old populations, and a low degree of differentiation between them.

Distribution, habitat and reproductive biology.

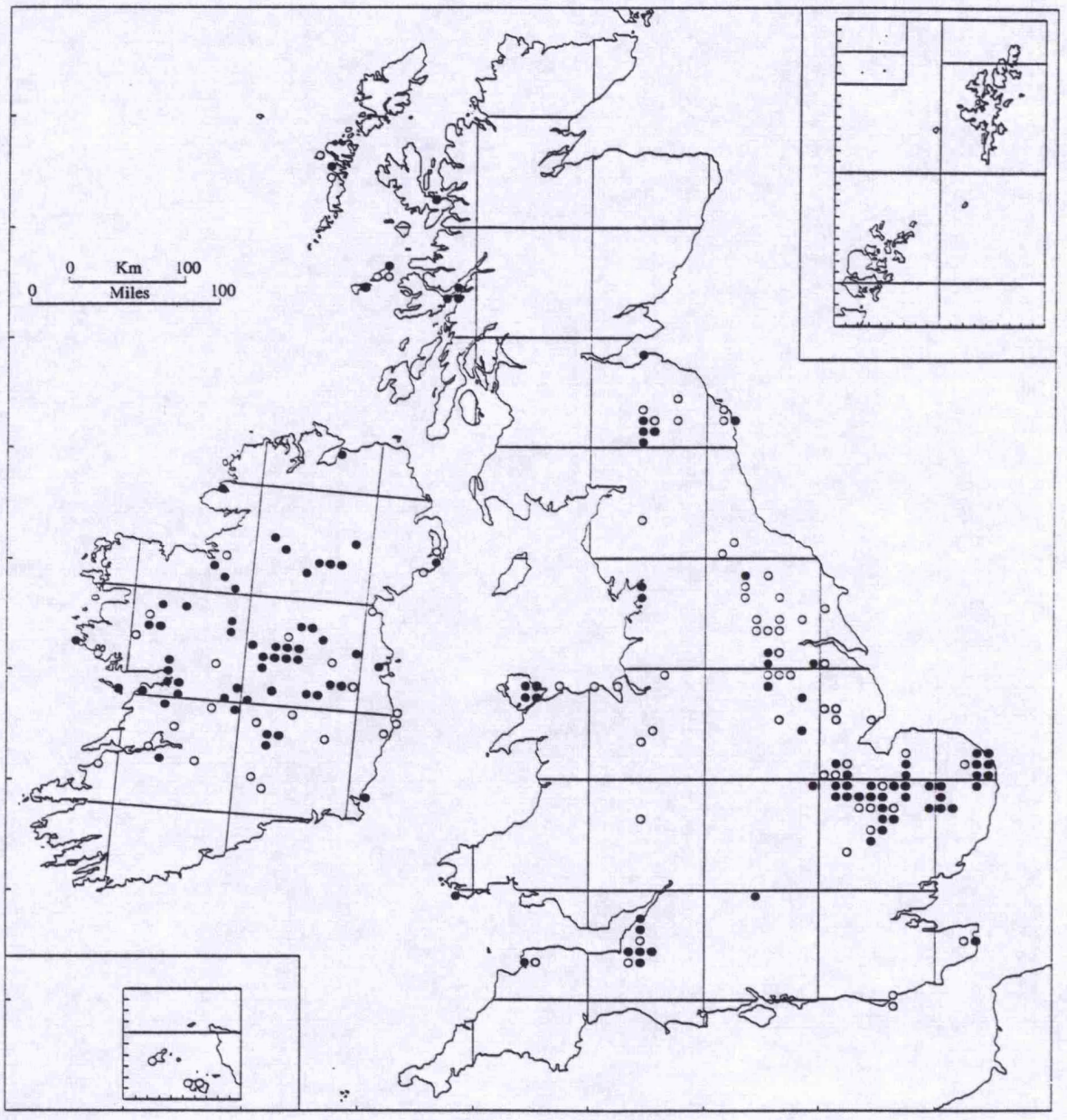
Potamogeton coloratus is a broad-leaved member of *Potamogeton* subgenus *Potamogeton*. It is closely related to the widespread calcifuge species *P.polygonifolius* Pourr. The submersed leaves are narrowly to broadly elliptical or oblanceolate, whereas the floating leaves tend to be ovate or circular. With the exception of this marked ontogenetic variation, the species shows rather little morphological variation.

P.coloratus is virtually confined to Europe, where it is found from the Mediterranean, including N. Africa and SW. Turkey, northwards to Scotland and southern Scandinavia (Hultén & Fries 1986, Wiegleb 1989). In Britain it has a disjunct distribution, occurring primarily in the east from Kent to East Lothian but also at scattered western sites from N. Devon to the Monach Isles in the Outer Hebrides (Fig. 2). It is a native species that is known to have been present in the temperate periods of the Hoxnian and Ipswichian interglacials and is also recorded as fossil seeds in the Flandrian (Godwin 1975).

In its ecology, *P.coloratus* is a plant of shallow, base- rich but nutrient-poor water. Typical habitats include fenland ditches and pools, slow-flowing streams and the edges of sheltered lakes over limestone or at the landward edge of calcareous sand dunes. Most of these are semi-natural habitats and, in England, *P.coloratus* is often found in relict areas of fenland. In many of these sites it has a long recorded history. The species is, however, also found as a colonist of disused clay-pits. At Fremington in N. Devon, for instance, it was recorded in a disused clay-pit in 1955 (Wallace 1956), but by 1989 the pit had reverted to swampy woodland and no suitable habitat remained. There are currently sizable populations in disused brick-pits in the Peterborough area.

P.coloratus is a rhizomatous perennial. Unlike many species of *Potamogeton* it overwinters as bright green submersed leaves. Although the main flowering period is in the summer, plants can be found in flower at some sites even in the winter. The inflorescences are held above the water level at anthesis; the flowers are protogynous and mature in an acropetal sequence. They are usually wind-pollinated (Cook 1988), but plants in cultivation which have been forced by flooding to produce inflorescences underwater have flowered, released pollen and fruited, demonstrating that under unusual circumstances under-water self-pollination is also possible in this species. Plants usually set abundant, well-formed fruit, both in the wild and in cultivation. Isolated plants of *P.coloratus* cultivated in a glasshouse at Leicester fruited well, indicating that they are self-compatible and autogamous.

Figure 2. Distribution of *Potamogeton coloratus* in Britain. Dots indicate the presence of the species in a 10 km square from 1970 onwards; circles indicate earlier records.



The species can survive unfavourable periods, such as summer drought, as buds on the rhizome (Fryer & Bennett 1915). It lacks specialised organs for vegetative dispersal, but can spread by growth of the rhizome. It can probably also become established from detached fragments of stems or rhizomes.

Cytologically the species is probably diploid, with a count of 2n=26 having been reported (Palmgren 1939); a count of 2n = ca 26 was also made by us for the population in the Gordano Valley studied here (Table 2).

MATERIALS AND METHODS

Materials

390 plants from 12 populations were collected from the wild (Table 2) between January 1992 and November 1993. Sites were selected to include wetlands with a long post-glacial history as well as water bodies of a much more recent vintage. At least 30 plants were gathered from each population, except for Braunton Burrows, Hacklinge and Little Wilbraham, where small populations demanded an appropriately reduced sample size. Sampling strategy varied according to the population density at each site, but a minimum sampling interval of 2m was maintained throughout the study in an effort to avoid collecting material emanating from the same rhizome. Selected rooted cuttings were grown on in buckets of water in a glasshouse and reanalysed 6 months later to test for environmentally induced isozyme variation (Ryan *et al.* 1991).

Methods

Standard horizontal electrophoresis of isozymes using 12% starch gels was carried out as described by Wendal & Weeden (1989). Two gel/electrode buffer systems were used: a) Lithium borate ($LiBO_3$) system. Electrode buffer, pH 8.1: 1 litre dH_20, 11.9g H_3BO_3, 1.2g LiOH. Gel buffer, pH 8.3: 100 ml $LiBO_3$ electrode buffer, 900ml dH_20, 5.45g Trizma base, 1.2g citric acid. b) Morpholine citrate (MC) system. Electrode buffer, pH 8.0: 983ml dH_20, 17ml N-(3-aminopropyl)-morpholine, 8.4g citric acid. Gel buffer: 960ml dH_20, 40ml MC electrode buffer.

Table 2. Details of collection localities of *Potamogeton coloratus*, with location of voucher specimens.

Site	Sample size	Nature of site	Date of 1st record	Habitat
N.Devon, v.c.4 Braunton Burrows, SS4.3 (LTR)	25	Semi-natural	1882	ditch
N.Somerset, v.c.6 Gordano Valley, ST4.7 (LTR)*	69	Semi-natural	1849	ditch
E.Kent, v.c.15 Hacklinge, TR3.5 (LTR)	30	Semi-natural	1946**	ditch
Cambs., v.c.29 Wicken Fen, TL5.7 (LTR)	30	Semi-natural	1859	ditch
Cambs., v.c.29 Chippenham Fen, TL6.6 (LTR)	30	Semi-natural	1884	ditch
Cambs., v.c.29 Little Wilbraham, TL5.5 (LTR)	8	Semi-natural	1878	ditch
Cambs., v.c.29 Stanground, Peterborough, TL2.9 (CGE)	30	Artificial	1988	stream by brick pit
Hunts., v.c.31 Orton Goldhay, Peterborough, TL1.9 (CGE)	53	Artificial	1972	brick pit
Hunts., v.c.31 Old Fletton, Peterborough, TL1.9 (CGE)	30	Artificial	1953	brick pit
Pembs., v.c.45 Castlemartin Corse, SR8.9 (LTR)	30	Semi-natural	1851	ditch
N.E.Yorks, v.c.62 Thornton-le-Street, SE3.8 (CGE)	30	Artificial	1882	ornamental lake
Tiree, v.c.103 Loch Riaghin, NM0.4 (LTR)	30	Natural	1989	loch

* 2n = ca 26, root-tip squash, voucher HOLLINGSWORTH & GORNALL, 5 April 1994 (LTR)
** Recorded from a nearby site in 1837

Young, actively growing leaves were dabbed dry of water, and, using approximately 25mm^2 of leaf tissue, a crude protein homogenate was extracted by mechanical grinding of the sample at 4^{0}C in 2 drops of the following extraction buffer: 50ml $LiBO_3$ gel buffer, 37mg KCl, 10mg $MgCl_2$, 18mg EDTA Na_4, 25mg polyvinylpolypyrrolidone, 0.5ml Triton X-100, 1.25ml β -mercaptoethanol. This extract was then soaked onto wicks cut from Whatman 3MM filter paper and loaded into the gel. For the lithium borate system, electrophoresis was carried out at a constant voltage of 240V and a maximum current of 70mA until the running front had migrated 8cm from the origin. For the morpholine citrate system, a constant current of 40mA and a maximum voltage of 200V was used.

Nine anodally migrating enzyme systems were studied:
Alcohol dehydrogenase (ADH): 50ml 0.1M Tris-HCl pH 8.0, 2.5ml EtOH, 25mg ATP, 10mg nicotinamide adenine dinucleotide (NAD),10mg 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), 3mg phenazine methosulphate (PMS), 1ml dH_20.

Aspartate aminotransferase (AAT): 50ml 0.1M Tris-HCl pH 8.5, 18mg α-ketoglutaric acid, 65mg aspartic acid, 250mg polyvinylpyrrolidone 40,000 (PVP-40T), 50mg EDTA Na_2, 710mg Na_2HPO_4, 1mg pyridoxal-5-phosphate, 200mg fast blue BB salt.

Glucose-6-phosphate dehydrogenase (G6PDH): 50ml 0.1M Tris-HCl pH 7.5, 50mg glucose-6-phosphate, 10mg nicotinamide adenine dinucleotide phosphate (NADP), 15mg MTT, 3mg PMS, 0.5ml 10% $MgCl_2$.

Glucose-6-phosphate isomerase (GPI): 50ml 0.1M Tris-HCl pH 8.0, 40mg fructose-6-phosphate, 30 units glucose-6-phosphate dehydrogenase, 10mg NADP, 15mg MTT, 3mg PMS, 0.5ml 10% $MgCl_2$.

Isocitrate dehydrogenase (IDH): 50ml 0.1M Tris-HCl pH 8.0, 100mg isocitric acid, 10mg NADP, 15mg MTT, 3mg PMS, 0.5ml 10% $MgCl_2$.

Lactate dehydrogenase (LDH): 50ml 0.1M Tris-HCl pH 8.0, 100mg lactic acid, 10mg NAD, 10mg MTT, 3mg PMS, 1ml dH_20.

Phosphogluconate dehydrogenase (PGD): 50ml 0.1M Tris-HCl pH 8.0, 50mg 6-phosphogluconic acid, 50mg $MgCl_2$, 10mg NADP, 15mg MTT, 3mg PMS, 0.5ml 10% $MgCl_2$.

Phosphoglucomutase (PGM): 50ml 0.1M Tris-HCl pH 7.5, 100mg glucose-1-phosphate, 25mg ATP, 35 units glucose-6-phosphate dehydrogenase, 10mg NADP, 15mg MTT, 3mg PMS, 0.5ml 10% $MgCl_2$.
Shikimate dehydrogenase (SKD): 50ml 0.1M Tris-HCl pH 8.0, 50mg shikimic acid, 10mg NADP, 15mg MTT, 3mg PMS, 0.5ml 10% $MgCl_2$.

GPI was prepared as an agar overlay as described by Wendel & Weeden (1989).

l5mg NADP per 350ml was added to the gel and the negative electrode tank buffers when analysing IDH, G6PDH, PGD, and SKD. IDH was resolved using the morpholine citrate buffer system, and all other enzymes were resolved on the lithium borate system.

Interpretation of enzyme banding patterns in terms of gene and allele frequencies was based on subunit structure and subcellular compartmentalization (Gottlieb 1981, 1982; Weeden & Wendel 1989), and on the isozyme profile of two families, each of 15 seedlings, raised from two isolated, self-pollinated plants.

Data analysis

The following measures of genetic variation were calculated for each population: a) the proportion of loci that are polymorphic (P); b) mean number of alleles per locus (A); and c) mean observed heterozygosity per locus (Ho). Estimates of breeding behaviour were based on comparisons with data expected under Hardy-Weinberg equilibrium. Deviations from expectations were assessed by calculating Wright's fixation index, F = 1-H/2pqN, where H is the observed number of heterozygotes and 2pqN is the expected number (Wright 1943, 1951).

Genetic variation within and among the twelve populations was assessed using Wright's F statistics, FIS, FIT and FST (Wright 1965, 1978; Nei 1977), calculated using BIOSYS-1 (Swofford & Selander 1981). The statistics are related by the formula 1-FIT = (1-FIS)(1-FST). FIS and FIT are the correlations between uniting gametes in individuals relative to populations (individuals

from a given site) and the total population (all individuals from all sites) respectively. F_{IS} was calculated as the average of F across all populations, weighted according to the allele frequencies in each (Kirby 1975; Nei 1977). F_{ST} is the correlation between uniting gametes within populations and can be used as a measure of differentiation between populations. It was calculated from the formula, $F_{ST} = s^2/p(1-p)$, where s^2 is the variance in allele frequency, and p and 1-p are mean allele frequencies. Mean F_{ST} values per locus are weighted averages over alleles. A chi-square test was used to test the statistical significance of F_{ST} values: $X^2 = 2NF_{ST}(k-1)$, with (k-1)(j-1) degrees of freedom, where N is the total sample size, k is the number of alleles for the locus, and j is the number of populations studied (Workman & Niswander 1970).

Genetic identity values (Nei 1972) were calculated for pairwise comparisons of the twelve populations, and the results subjected to a cluster analysis using UPGMA.

RESULTS

Isozyme loci

A total of 14 gene loci were identified: AAT-1, AAT-2, AAT-3, G6PDH-1, GPI-1, GPI-2, IDH-1, IDH-2, LDH-1, PGD-1, PGD-2, PGM-1, PGM-2, SKD-1. All migrated anodally.

For each of LDH and SKD a single band was produced, which I have interpreted as the product of a single homozygote locus. Each locus was monomorphic among all samples analysed.

G6PDH, GPI and PGD were each represented by two bands, although in G6PDH one of the bands was often faint and not consistently scorable and was consequently excluded from the analysis. The two-banded patterns are consistent with expression by two homozygote loci. The loci were monomorphic among all samples analysed.

AAT appeared as three asymmetrical bands. I have attributed this pattern to three homozygote loci. Again the loci were monomorphic among all

samples.

PGM showed variation between and within populations. A total of four bands was seen, corresponding to diallelic variation at each of two gene loci (Figs. 3 & 4). PGM-1 and PGM-2 were the only polymorphic loci, allele frequencies for which are given in Table 3.

IDH was represented by a classic heterozygote three-banded pattern in all individuals surveyed. No segregation was observed among the progeny of the two self-pollinated plants, indicating fixed heterozygosity. Examination of isozyme profiles from pollen also showed the three-banded pattern. Since IDH is a dimeric enzyme, the presence in pollen of three rather than two bands indicates that the locus is duplicated in *P.coloratus*.

ADH was variable in its expression; it always produced at least one band, but additional bands were inconsistent in their occurrence. Since it could not be scored reliably, this enzyme system was omitted from the analysis.

Amount of genetic variability

Genetic variability in the twelve populations is summarised in Table 3. In ten of the populations all 14 loci were monomorphic. The proportion of loci that are polymorphic (P) averaged over all populations was 0.018; the mean number of alleles per locus (A), averaged over populations, was 1.02; the mean observed heterozygosity per locus (Ho), averaged over populations, was 0.001; and the mean expected heterozygosity per locus under Hardy-Weinberg (Hs), averaged over populations was 0.007. Of the two variable populations, measures from Gordano Valley gave P = 0.143, A = 1.14, Ho = 0.013, and Hs = 0.043; and those from Wicken Fen gave: P = 0.071, A = 1.07, Ho = 0.002, and Hs = 0.036.

Fig. 3 Isozyme banding patterns for PGM

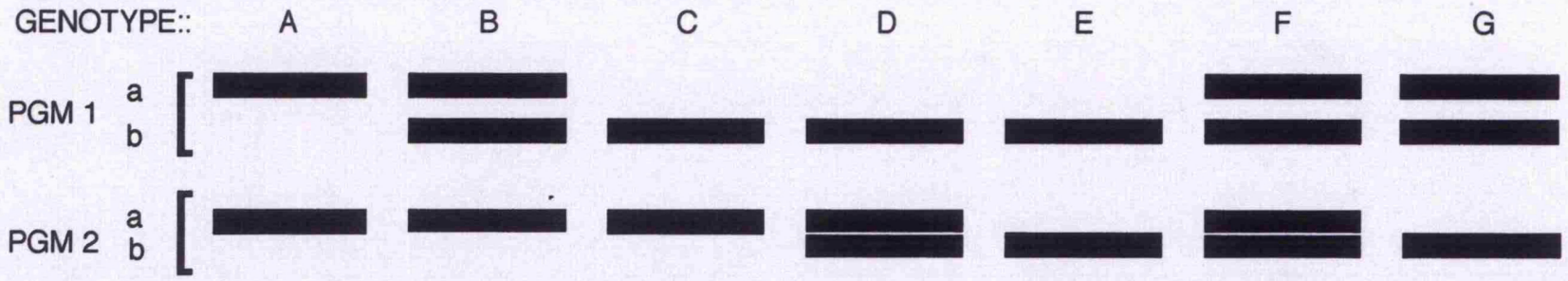


Fig. 4. Photograph showing variation at PGM1, with all of the individuals shown being monomorphic for the 'a' allele for PGM2. From left to right, lane 1 = genotype C, lane 2 = genotype B, lanes 3 -10 = genotype A, lanes 11-14 = genotype C. Letters indicate the genotype codes given in Fig. 3.

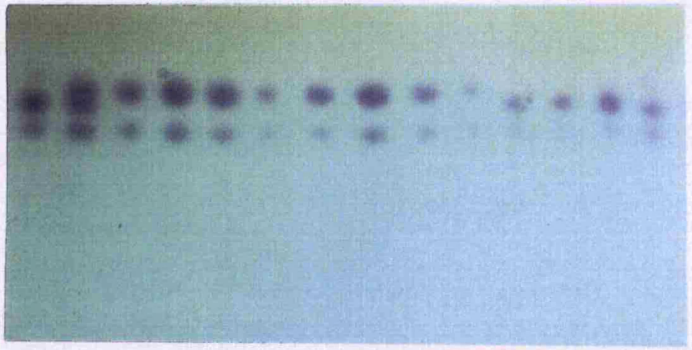


Table 3. Allele frequencies for the two polymorphic loci observed in *Potamogeton coloratus*, together with statistics estimating levels of genetic variability (see text for details).

	PGM-1		PGM-2		P	A	Ho	Hs
	a	b	a	b				
Braunton Burrows	0.0	1.000	1.000	0.0	0.0	1.0	0.0	0.0
Gordano Valley	0.449	0.551	0.949	0.051	0.143	1.14	0.013	0.043
Hacklinge	0.0	1.000	1.000	0.0	0.0	1.0	0.0	0.0
Wicken Fen	0.0	1.000	0.450	0.550	0.071	1.07	0.002	0.036
Chippenham Fen	0.0	1.000	1.000	0.0	0.0	1.0	0.0	0.0
Little Wilbraham	0.0	1.000	1.000	0.0	0.0	1.0	0.0	0.0
Stanground	0.0	1.000	1.000	0.0	0.0	1.0	0.0	0.0
Orton Goldhay	0.0	1.000	1.000	0.0	0.0	1.0	0.0	0.0
Old Fletton	0.0	1.000	1.000	0.0	0.0	1.0	0.0	0.0
Castlemartin Corse	0.0	1.000	0.0	1.000	0.0	1.0	0.0	0.0
Thornton-le-Street	0.0	1.000	1.000	0.0	0.0	1.0	0.0	0.0
Loch Riaghin	0.0	1.000	1.000	0.0	0.0	1.0	0.0	0.0
Mean					0.018	1.02	0.001	0.007

Breeding system

Tests for Hardy-Weinberg equilibrium were conducted on the polymorphic populations of Gordano Valley and Wicken Fen, using the inbreeding coefficient or fixation index of Wright (1943, 1951). The results are summarised in Table 4, where significance levels were calculated by comparing observed genotype frequencies with those expected under panmixia. Both PGM-1 and PGM-2 showed significant deviations from the values expected; in particular, there were marked deficiencies of heterozygotes at both loci at Gordano Valley, and at PGM-2 at Wicken Fen (PGM-1 was invariant at this locality).

Partitioning of genetic variability.

The partitioning of genetic variability within and between populations of *P.coloratus*, as estimated from F statistics (Wright 1965, 1978; Nei 1977), is summarised in Table 5. The mean FIT was 0.939, with substantial contributions being made to this value by both FIS and FST. The high value of the latter is indicative of significant genetic differentiation among populations at PGM-1 and PGM-2.

Overall similarity between populations as measured by Nei's genetic identity in pairwise comparisons gave values ranging from 0.92 (Gordano Valley and Castlemartin Corse) to 1.00 (all other pairs not including the previous two populations or Wicken Fen). Cluster analysis (UPGMA) produced a phenogram in which two groups were distinguished: a group containing the populations at Castlemartin Corse and Wicken Fen, and a group linking the population in the Gordano Valley with the remaining nine others.

A total of seven multi-locus (14-loci) isozyme genotypes (effectively equivalent to PGM genotypes) was observed, the geographical distribution of which is summarised in Table 6 and Fig. 5. Ten of the twelve populations consisted of a single 14- locusisozyme genotype.

Table 4. Inbreeding coefficients (F) for each polymorphic locus in the populations of *Potamogeton coloratus* at Gordano Valley and Wicken Fen.

Locus	Gordano Valley	Wicken Fen
PGM-1	0.649***	(invariant)
PGM-2	0.850***	0.933***
Mean	0.682	0.933

*** $P < 0.001$

Table 5. F statistics at polymorphic loci in *Potamogeton coloratus* from the British Isles.

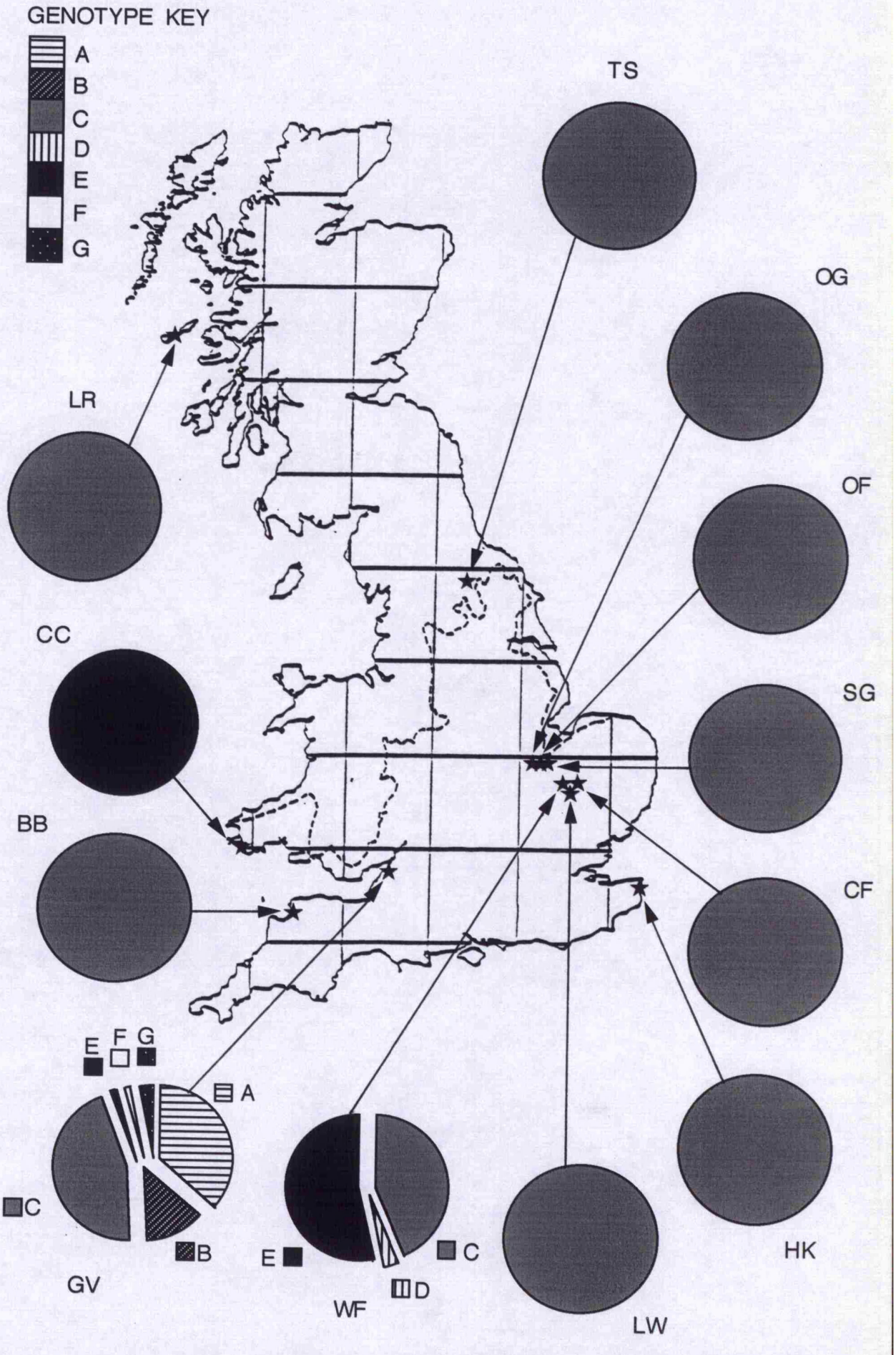
Locus	Fis	Fst	Fit
PGM-1	0.649	0.428***	0.799
PGM-2	0.919	0.787***	0.983
Mean	0.796	0.702***	0.939

*** $P < 0.001$

Table 6. Distribution of PGM genotypes in *Potamogeton coloratus*

Site	No. of PGM genotypes (see Fig. 2)							N
	A	B	C	D	E	F	G	
Braunton Burrows	0	0	25	0	0	0	0	25
Gordano Valley	25	9	31	0	1	1	2	69
Hacklinge	0	0	25	0	0	0	0	25
Wicken Fen	0	0	13	1	16	0	0	30
Chippenham Fen	0	0	30	0	0	0	0	30
Little Wilbraham	0	0	8	0	0	0	0	8
Stanground	0	0	30	0	0	0	0	30
Orton Goldhay	0	0	53	0	0	0	0	53
Old Fletton	0	0	30	0	0	0	0	30
Castlemartin Corse	0	0	0	0	30	0	0	30
Thornton-le-Street	0	0	30	0	0	0	0	30
Loch Riaghin	0	0	30	0	0	0	0	30
Total	25	9	305	1	47	1	2	390
%	6.4	2.3	78.2	0.3	12.0	0.3	0.5100	

Figure 5. Distribution of multi-locus genotypes of *Potamogeton coloratus* in Britain. Genotypes: see Fig. 3 for codes. Populations: BB, Braunton Burrows; CC, Castlemartin Corse; CF, Chippenham Fen; SG, Stanground; GV, Gordano Valley; HK, Hacklinge; LR, Loch Riaghin; LW, Little Wilbraham; OF, Old Fletton; OG, Orton Goldhay; TS, Thornton-le-Street; WF, Wicken Fen.
---------- Indicates the presumed southerly limit of the Devensian glaciation (Godwin 1975).


GENOTYPE KEY
A
B
C
D
E
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G
TS
OG
LR
OF
CC
SG
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E F G
A
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B
GV
E
C
D
WF
HK
LW

DISCUSSION

The flowers of *Potamogeton coloratus* are protogynous, anemophilous and, being produced in abundance, they regularly give rise to large quantities of fruit. As with many aquatic plants, I presume that the species has at least the potential to be dispersed readily by water and waterfowl (Sculthorpe 1967). I expected, therefore, to find high levels of genetic variation within populations and a high level of differentiation between them, as was the case in the similarly endowed *Myriophyllum alterniflorum* (Harris *et al.* 1992). The results, however, are quite different.

Amount of genetic variability

The levels of P, A and H found are low. In the only other isozyme study of *P.coloratus* (a single population from Belgium), Hettiarachchi & Triest (1991) reported P = 0.18, A = 1.2, and Ho = 0.059, based on a survey of 17 putative loci (9 peroxidases, 1 malic enzyme, 3 xanthine dehydrogenases, 2 glutamate dehydrogenases and 2 superoxide dismutases). They did not report, however, the sample size analysed. Their value of P is identical to ours, but their values of A and Ho are rather higher (cf. Table 3). Studies of many other aquatic species apparently also reveal lower levels of variability as measured by these statistics than is found in equivalent terrestrial species (Triest 1991e).

Breeding system

In the only two populations which could be tested for Hardy-Weinberg equilibrium, Gordano Valley and Wicken Fen, the high values of F (mean F = 0.682, 0.933 respectively; Table 4) indicate that the species at these localities is predominantly inbreeding, despite its protogynous floral development and anemophilous pollination syndrome. It should however be borne in mind that the occurrence of extensive clonal growth could well distort statistical estimations of breeding system, particularly if some clones with certain allelic combinations fair better than others due to selection or stochastic events. This could be especially significant in a study such as this where only a few variable loci were detected.

Philbrick & Anderson (1987) reported that two other aerial-flowering species of *Potamogeton* (*P.epihydrus* Raf. and *P.spirillus* Tuckerm), despite having high pollen/ovule ratios and therefore expected to be xenogamous, were in fact autogamous. Nevertheless, the F-values cited above and the occurrence of two inter-specific hybrids, with *P.gramineus* L. and *P.berchtoldii* Fieber (Dandy 1975), indicate that outcrossing does sometimes occur in *P. coloratus*.

Inter-population differentiation

Analysis of inter- population differentiation using Wright's F statistics revealed a high mean value of the overall inbreeding coefficient, (F_{IT} = 0.939), which can be attributed both to high levels of genetic subdivision among populations (mean F_{ST} = 0.702) and to a high frequency of inbreeding within populations (mean F_{IS} = 0.796).

With two exceptions, each population is composed of a single multilocus isozyme genotype, a pattern of differentiation consistent with predominant inbreeding and / or clonal spread. The fact that there are only seven multilocus isozyme genotypes and that they are distributed unevenly in Britain (Fig. 5) suggest that populations have been founded by a small number of individuals, and that restricted gene flow, inbreeding and / or clonal spread have preserved the specific allelic combinations found therein. Given the high values of F_{ST}, the high genetic identities (I) between populations may seem surprising, but merely reflect the fact that only polymorphic loci contribute to F_{ST} whereas all loci, including monomorphic ones, contribute to I.

In Britain, *P.coloratus* is not only at the north- western edge of its total range, but it is also scarce here. In this context it is interesting to find that one particular multilocus isozyme genotype ('C' in Fig. 3) characterises 75% of the populations and about 78% of the plants (Table 6). The depauperate gene pool of the species in Britain could be explained on the basis of a genetic bottleneck, or series of bottlenecks, following the Devensian glaciation, accompanied by recolonisation by relatively few founders. Severe bottlenecks would account for the fact that all but two of the

populations in our sample are monomorphic. The two polymorphic populations are at Gordano Valley and Wicken Fen, ancient wetland sites, which are south of the limit of the Devensian glaciation. *P.coloratus* might conceivably have a long post-glacial, and even pre-glacial, history at these sites, although, regrettably, there is no fossil evidence on this point. I also accept that due to the absence of comparable data from other parts of the species range I cannot say whether *P. coloratus* as a species is rather invariable, whether individual populations tend to be uniform elsewhere, or whether British populations are truly genetically depauperate. It may well be that population bottlenecks regularly occur due to the shallow waters in which *P. coloratus* grows being dredged or becoming overgrown. However in the context of conservation of the species in Britain, the polymorphic populations of *P.coloratus* in the Gordano Valley and at Wicken Fen assume a special significance.

The three other ancient wetland sites south of the Devensian glacial boundary (Castlemartin Corse, Braunton Burrows and Chippenham Fen) have monomorphic populations, the first, uniquely in our sample, for genotype 'E', and the last two for genotype 'C'. I have no reason for suspecting one particular cause over another for this monomorphism, whether it be founder effects , bottlenecks, or even inbreeding and drift. Founder effects, however, would best explain the monomorphic nature of the populations at the five 'new' sites in Cambridgeshire and Huntingdonshire, and possibly also the two sites north of the Devensian glacial limit (Thornton-le-Street and Loch Riaghin).

It is curious that only one genotype ('C') appears to have been consistently dispersed to the 'new' sites, and exists to the exclusion of others in some of the ancient ones. Thus one might have thought that Wicken Fen, might have acted as a source of genotype 'E' for the 'new' sites in Cambridgeshire and Huntingdonshire, some 10-35 km away.

Gene duplication and ploidy level

There are two hypotheses regarding the chromosome base number in the genus *Potamogeton*, in which the lowest widespread haploid numbers are n

= 14 and n = 13. One view is that n = 7 is the basic number, from which most species have been derived by polyploidy, leaving only a few relics (now extinct?) with the original number (Goldblatt 1979; Les 1983). The evidence for this rests largely on only two counts of 2n = 14, one from *P. foliosus* Raf. and one from *P.perfoliatus* L. (Wiegand 1899; Takusagawa 1939). Alternatively, Stern (1961) and Haynes (1974) proposed that n = 14 should be regarded as the basic number, from which n = 13 has been derived by aneuploidy, and that polyploid series have been established on these two numbers. On this interpretation the reports of n = 7 should, presumably, be regarded as errors, either of counting or of identification.

Gottlieb (1981, 1982) has pointed out that the number of isozymes in a species can be used to indicate the number of copies of a genome present, and hence distinguish between aneuploidy and polyploidy. From our data I can say that *P.coloratus*, which has the chromosome number 2n = 26, has the conserved number of isozymes expected of a diploid in eight of the nine enzyme systems examined. Similarly, evidence from SOD isozymes led Hettiarachchi & Triest (1991) to suggest tentatively that 2n=26 or 28 is the diploid condition. In view of this, the discovery of a duplicated locus of the IDH enzyme is interesting and may indicate an origin through unequal crossing over or translocation between non-homologous chromosomes, rather than through polyploidy. Whether the duplication can be used as a phylogenetic marker shared by other species within the genus remains to be seen. IDH is also known to be coded by duplicated genes in *Zea mays* L. (Poaceae) (Goodman *et al.* 1980), various cultivars of *Glycine max* (L.) Merr. (Fabaceae) (Yong *et al.* 1981) and species of *Layia* (Compositae) (Gottlieb 1987).

CHAPTER 4

THE PARTITIONING OF GENETIC VARIATION WITHIN A POPULATION OF *POTAMOGETON COLORATUS* (POTAMOGETONACEAE)

ABSTRACT

647 individuals of *Potamogeton coloratus* from 60 ditches in the Gordano Valley, Somerset were analysed for variation at two polymorphic PGM loci using starch gel electrophoresis of isozymes. A total of eight genotypes were detected, and high levels of partitioning of genetic variation between ditches were observed with F_{ST}=0.575. 33 of the 60 ditches are polymorphic, with 19 of these showing significant deviations from Hardy-Weinberg equilibrium at at least one locus. Strong evidence for clonal growth was observed.

INTRODUCTION

In an extensive study of genetic variation in *Potamogeton coloratus* Hornem. in the British Isles, 390 individuals from twelve populations were examined using isozyme analysis (Hollingsworth *et al.* 1995a - chapter 3). Of the twelve populations, nine were monomorphic for the same multi-locus genotype, and only 2 populations showed any intra-population variation. Only two loci (PGM-1 and PGM-2) out of 14 examined showed any variation. One of the variable populations, Wicken Fen (Cambridgeshire) showed variation only at PGM-2, a diallelic locus at which both homozygotes and the heterozygote were detected. The second variable population was in the Gordano Valley (Somerset) and this showed diallelic variation at both PGM loci with a total of seven genotypes detected.

The Gordano Valley is a wedge shaped area of land about 1.5 km wide and 7.5 km long, surrounded by low lying hills. The plant ecology, and the late- and post-glacial history of the valley have been summarised by Willis & Jefferies (1959) and Jefferies *et al.* (1968). An extensive network of managed drainage ditches occurs within the Gordano Valley nature reserve and it is in these that *P. coloratus* grows. Preliminary observations from isozyme analysis of this population from the earlier study showed that there appeared to be considerable partitioning of the genetic variation between

these drainage ditches. It was thus decided to examine this population in greater detail to investigate its genetic structure, using alleles at the two polymorphic PGM loci as markers.

MATERIALS AND METHODS

Materials

Single leaf samples were collected from a total of 647 individuals from 60 separate ditches in the Gordano Valley (ST/4.7) in 1994. Sampling reflected local population density, with the number of samples collected from a ditch representing the number of plants present. A minimum distance of 2m between sampled sites was maintained to try to avoid collecting leaves emanating from the same rhizome. All ditches within the reserve where *P. coloratus* was growing were visited, with almost every clump sampled. The location from where each leaf was collected was recorded to the nearest metre and plotted on a 1:5000 scale map (Fig. 6 - in folder in back cover). Ditches were named according to their approximate polar orientation and the field they are in. Thus ditch 3CS is the ditch running along the southern edge of field 3C.

Methods

Material was returned to the laboratory, stored at 4^{0}C and analysed within 5 days of collection. All samples were analysed for variation at the two polymorphic PGM loci using the protocol described by Hollingsworth *et al.* (1995a - chapter 3).

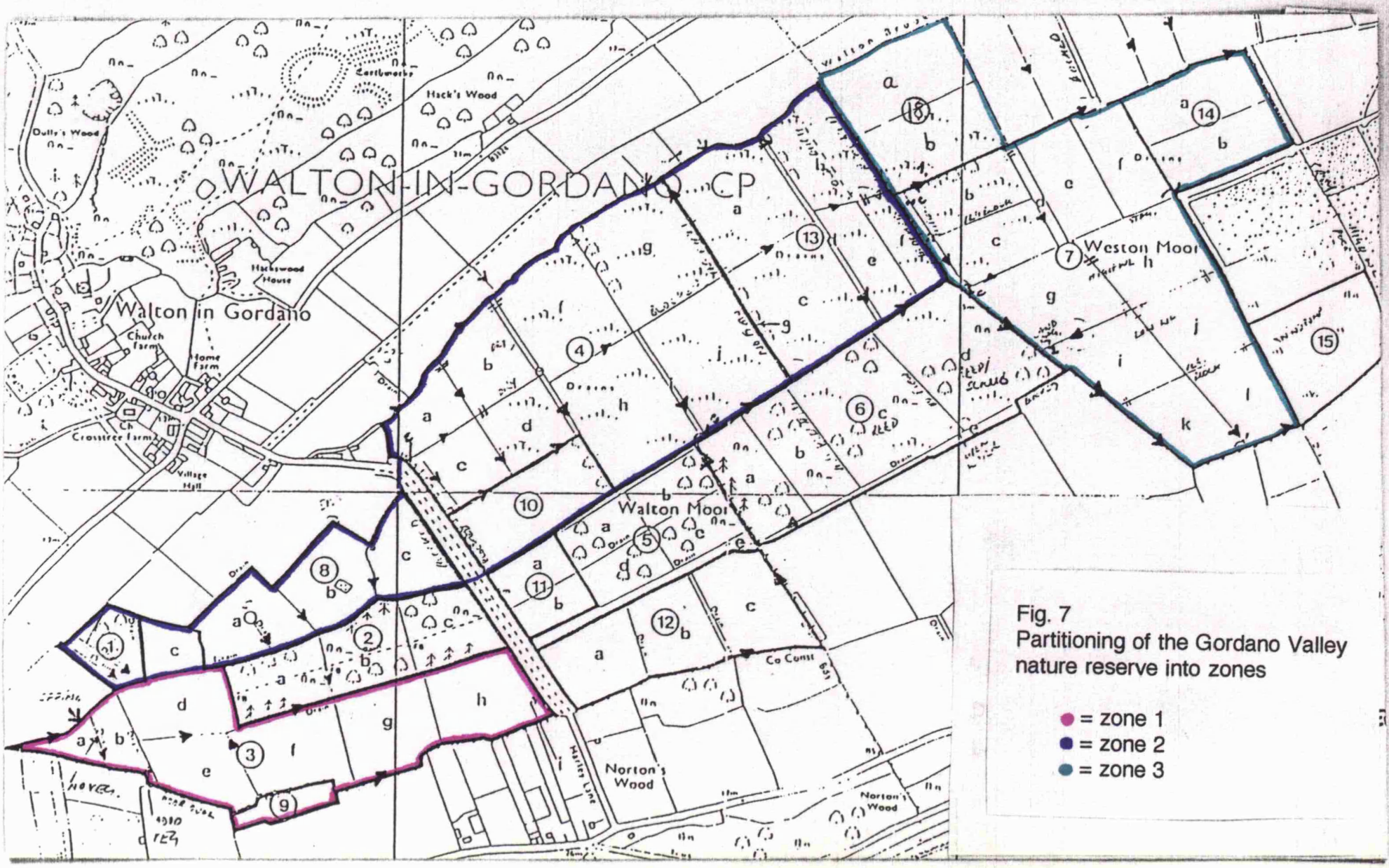
Data Analysis

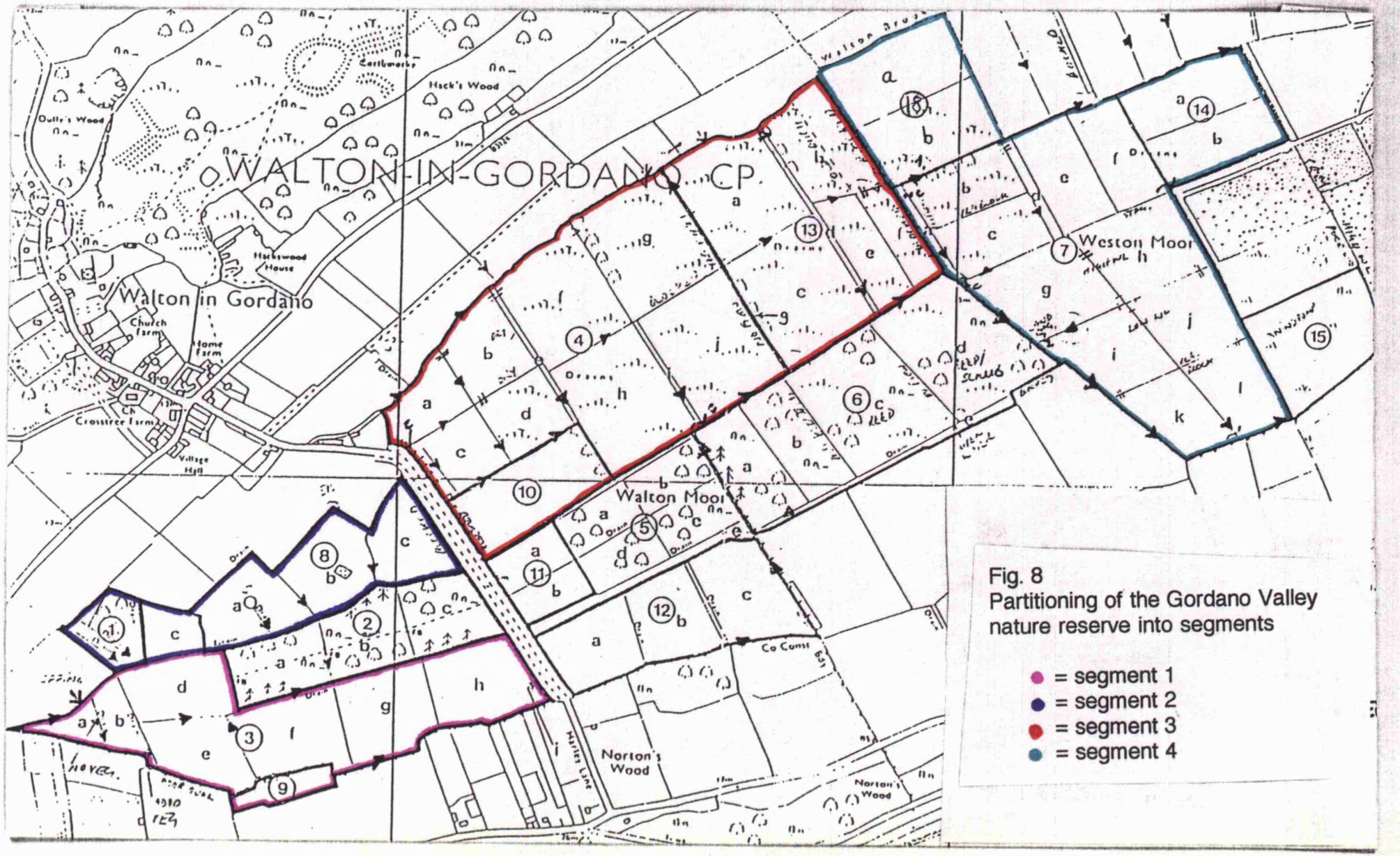
Estimates of breeding behaviour were made based on comparisons with data expected under Hardy-Weinberg equilibrium using Levene's (1949) correction for small sample sizes. Data analysis on genetic variation within and between the ditches was carried out using Wright's F statistics, F_{IS}, F_{IT} and F_{ST} (Wright 1965, 1978, Nei 1977).

To examine the effects of physical barriers to gene flow such as the woods and the road at the site, a hierarchical analysis using F-statistics was carried out. Data were pooled from ditches into segments and then into zones. The

boundaries of the segments and zones are shown on the map (Fig. 7 & 8), with the zones corresponding to areas separated by woods, and a further division into two segments being made for zone 2 where it is bisected by the road. (Thus segments = zones for zones 1 and 3 which are not divided by a road.)

All statistics were calculated using BIOSYS-1 (Swofford & Selander 1981).


WALTON-IN-GORDANO CP
Walton in Gordano
Weston Moor
Walton Moor
Norton's Wood
Co Const
Hack's Wood
Hackwood House
Church Farm
Home Farm
Crosstree Farm
Village Hall
Earthworks
Dulls Wood
Fig. 7
Partitioning of the Gordano Valley nature reserve into zones
= zone 1
= zone 2
= zone 3


WALTON-IN-GORDANO CP
Walton in Gordano
Weston Moor
Walton Moor
Norton's Wood
Hack's Wood
Church Farm
Home Farm
Crossfree Farm
Village Hall
Co Const
Fig. 8
Partitioning of the Gordano Valley nature reserve into segments
= segment 1
= segment 2
= segment 3
= segment 4

RESULTS

The distribution of the alleles in the different ditches is shown in Fig. 6 and Fig. 9, with the observed genotype frequencies for each locus given in Table 7. Although both alleles for PGM-1 were relatively common, for PGM-2 by far the most common genotype was 'aa', with only 3 'bb' and 12 'ab' genotypes detected out of the 647 plants analysed (Fig. 9).

A total of eight multilocus genotypes was observed out of a possible nine combinations of the two alleles at the two loci examined (PGM-1 'ab', PGM-2 'bb' was the only genotype not recovered).

Of the 60 ditches sampled, 33 (55%) were polymorphic, with 19 (58%) of these showing significant deviations from Hardy-Weinberg equilibrium at at least one locus (Tables 8 & 9). All of these deviations from Hardy-Weinberg were due to an excess of homozygotes except for one ditch, 4GE, which showed a excess of heterozygotes for PGM-1. All zones and fields showed significant deviations from Hardy-Weinberg equilibrium at both loci with an excess of homozygotes noted in all cases.

A general increase in genetic variation was noted to the north and east of the site, i.e. down the valley. Analysis of population genetic structure using Wright's F-statistics is presented in Table 10 for all levels of the hierarchy. When ditches are considered as 'populations', the mean overall inbreeding coefficient (F_{IT}=0.749) receives a greater contribution from subdivision between ditches (F_{ST}=0.575), than from inbreeding within ditches (F_{IS}=0.408). However when the data from ditches is pooled and segments or zones are considered as 'populations' the situation is reversed, with the greater contribution being from inbreeding within zones or segments (Table 10). This is predictable as levels of subdivision between ditches (F_{ST}) is high, thus grouping these ditches together into segments and zones, and basing expectations of random mating on their pooled allele frequencies will naturally lead to the deviations from panmixia evident from Table 9.

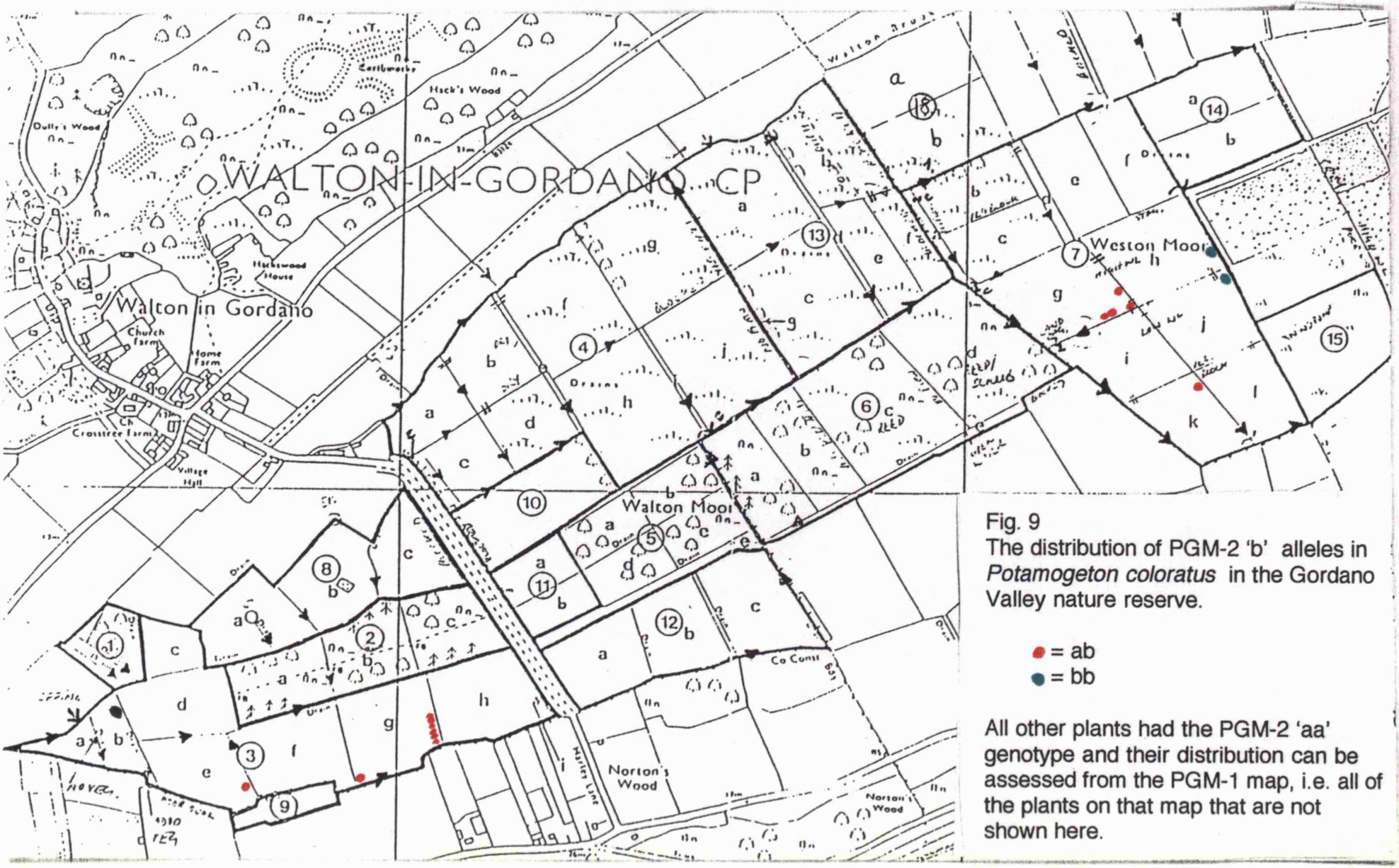


Fig. 9
The distribution of PGM-2 'b' alleles in *Potamogeton coloratus* in the Gordano Valley nature reserve.

● = ab
● = bb

All other plants had the PGM-2 'aa' genotype and their distribution can be assessed from the PGM-1 map, i.e. all of the plants on that map that are not shown here.

The values for F_{IT}, F_{IS} and F_{ST} for the hierarchical levels of segments and zones are very similar to each other suggesting that the splitting of zone 2 by the road has little effect on gene flow. Although the levels of subdivision between segments and between zones are lower than those between ditches, all of the values for mean F_{ST} are greater than the 0.25 value which Wright (1978) suggested indicates *very great* genetic differentiation.

Lower values of all three F-statistics are were found for PGM-2 than for PGM-1, with this being attributed to the rarity of the PGM-2 'b' allele.

Table 7. Genotype Frequencies of Individual Ditches

DITCH 3AS
PGM-1 BB:12
PGM-2 AA:12
DITCH 3EW
PGM-1 BB:08
PGM-2 AA:08
DITCH 3DW
PGM-1 BB:12
PGM-2 AA:11 BB:01
DITCH 3BS
PGM-1 BB:04
PGM-2 AA:04
DITCH 3BW
PGM-1 BB:21
PGM-2 AA:21
DITCH 3CS
PGM-1 BB:01
PGM-2 AA:01
DITCH 3DS
PGM-1 BB:17
PGM-2 AA:17
DITCH 3EE
PGM-1 BB:03
PGM-2 AA:02 AB:01
DITCH 3ES
PGM-1 BB:21
PGM-2 AA:21
DITCH 3FS
PGM-1 BB:01
PGM-2 AA:01
DITCH 3GE
PGM-1 BB:08
PGM-2 AA:03 AB:05
DITCH 3GN
PGM-1 BB:08
PGM-2 AA:08
DITCH 3GS
PGM-1 BB:07
PGM-2 AA:06 AB:01
DITCH 3GW
PGM-1 BB:24
PGM-2 AA:24
DITCH 3HE
PGM-1 BB:06
PGM-2 AA:06
DITCH 4AS
PGM-1 AA:25
PGM-2 AA:25
DITCH 4BS
PGM-1 AA:05 AB:01 BB:05
PGM-2 AA:11
DITCH 4BW
PGM-1 AA:19 AB:01
PGM-2 AA:20
DITCH 4CS
PGM-1 AA:18 AB:03 BB:02
PGM-2 AA:23
DITCH 4DS
PGM-1 AA:17
PGM-2 AA:17
DITCH 4DW
PGM-1 AA:20
PGM-2 AA:20
DITCH 4FS
PGM-1 AA:07
PGM-2 AA:07
DITCH 4FW
PGM-1 AA:05
PGM-2 AA:05
DITCH 4GE
PGM-1 AB:11 BB:03
PGM-2 AA:14
DITCH 4HW
PGM-1 AA:04 AB:01
PGM-2 AA:05
DITCH 4JE
PGM-1 AA:03 AB:01 BB:08
PGM-2 AA:12
DITCH 4JW
PGM-1 AA:04
PGM-2 AA:04
DITCH 7BE
PGM-1 AA:02 AB:01 BB:07
PGM-2 AA:10
DITCH 7BN
PGM-1 AA:01 BB:02
PGM-2 AA:03
DITCH 7CE
PGM-1 AA:02 BB:06
PGM-2 AA:08
DITCH 7DE
PGM-1 BB:02
PGM-2 AA:02
DITCH 7EE
PGM-1 AA:01 AB:01 BB:21
PGM-2 AA:23
DITCH 7EN
PGM-1 AA:02 BB:02
PGM-2 AA:04
DITCH 14BW
PGM-1 AA:01 BB:12
PGM-2 AA:13
DITCH 14AW
PGM-1 AA:02 AB:02 BB:09
PGM-2 AA:13
DITCH 7GN
PGM-1 BB:26
PGM-2 AA:26
DITCH 7GS
PGM-1 AA:16 AB:15 BB:04
PGM-2 AA:32 AB:03
DITCH 7HE
PGM-1 AA:02 BB:03
PGM-2 AA:03 AB:01 BB:01
DITCH 7ES
PGM-1 AA:02 AB:04 BB:11
PGM-2 AA:17
DITCH 7FS
PGM-1 AB:01 BB:04
PGM-2 AA:05
DITCH 7HS
PGM-1 AA:01 AB:05 BB:06
PGM-2 AA:12
DITCH 7HW
PGM-1 AA:05 AB:01 BB:04
PGM-2 AA:09 AB:01
DITCH 7IS
PGM-1 AA:04 AB:08 BB:11
PGM-2 AA:23
DITCH 7JE
PGM-1 AA:02 AB:03
PGM-2 AA:04 BB:01
DITCH 7JS
PGM-1 AB:02 BB:02
PGM-2 AA:04
DITCH 7JW
PGM-1 AA:03 AB:01 BB:03
PGM-2 AA:07
DITCH 7LE
PGM-1 AA:02
PGM-2 AA:02
DITCH 7LW
PGM-1 AA:02 BB:03
PGM-2 AA:04 AB:01
DITCH 8AS
PGM-1 BB:03
PGM-2 AA:03
DITCH 8BS
PGM-1 BB:01
PGM-2 AA:01
DITCH 8BW
PGM-1 BB:06
PGM-2 AA:06
DITCH 8CE
PGM-1 AA:04 AB:09 BB:06
PGM-2 AA:19
DITCH 8CS
PGM-1 BB:01
PGM-2 AA:01
DITCH 8CW
PGM-1 AA:14
PGM-2 AA:14
DITCH 13AE
PGM-1 AA:01 BB:01
PGM-2 AA:02
DITCH 13AS
PGM-1 AA:18 AB:07 BB:06
PGM-2 AA:31
DITCH 13CE
PGM-1 AB:01 BB:01
PGM-2 AA:02
DITCH 13EN
PGM-1 AA:07
PGM-2 AA:07
DITCH 13FE
PGM-1 AA:07 AB:03 BB:02
PGM-2 AA:12
DITCH 13FN
PGM-1 AA:01
PGM-2 AA:01

Table 8. Genetic variability at two loci for all ditches

Population	Sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic[1]	Mean heterozygosity Direct count	HdyWbg expected[2]
1. DITCH 3AS	12.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
2. DITCH 3EW	8.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
3. DITCH 3DW	12.0	1.5	50.0	.000	.080*
	(.0)	(.5)		(.000)	(.080)
4. DITCH 3BS	4.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
5. DITCH 3BW	21.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
6. DITCH 3CS	1.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
7. DITCH 3DS	17.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
8. DITCH 3EE	3.0	1.5	50.0	.167	.167
	(.0)	(.5)		(.167)	(.167)
9. DITCH 3ES	21.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
10. DITCH 3FS	1.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
11. DITCH 3GE	8.0	1.5	50.0	.313	.229
	(.0)	(.5)		(.313)	(.229)
12. DITCH 3GN	8.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
13. DITCH 3GS	7.0	1.5	50.0	.071	.071
	(.0)	(.5)		(.071)	(.071)
14. DITCH 3GW	24.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
15. DITCH 3HE	6.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
16. DITCH 4AS	25.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
17. DITCH 4BS	11.0	1.5	50.0	.045	.262*
	(.0)	(.5)		(.045)	(.262)
18. DITCH 4BW	20.0	1.5	50.0	.025	.025
	(.0)	(.5)		(.025)	(.025)
19. DITCH 4CS	23.0	1.5	50.0	.065	.132*
	(.0)	(.5)		(.065)	(.132)
20. DITCH 4DS	17.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
21. DITCH 4DW	20.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
22. DITCH 4FS	7.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
23. DITCH 4FW	5.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
24. DITCH 4GE	14.0	1.5	50.0	.393	.247*
	(.0)	(.5)		(.393)	(.247)
25. DITCH 4HW	5.0	1.5	50.0	.100	.100
	(.0)	(.5)		(.100)	(.100)
26. DITCH 4JE	12.0	1.5	50.0	.042	.216*
	(.0)	(.5)		(.042)	(.216)
27. DITCH 4JW	4.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)

Table 8 cont.

Population	Sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic[1]	Mean heterozygosity Direct count	HdyWbg expected[2]
28. DITCH 7BE	10.0	1.5	50.0	.050	.197*
	(.0)	(.5)		(.050)	(.197)
29. DITCH 7BN	3.0	1.5	50.0	.000	.267*
	(.0)	(.5)		(.000)	(.267)
30. DITCH 7CE	8.0	1.5	50.0	.000	.200*
	(.0)	(.5)		(.000)	(.200)
31. DITCH 7DE	2.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
32. DITCH 7EE	23.0	1.5	50.0	.022	.062*
	(.0)	(.5)		(.022)	(.062)
33. DITCH 7EN	4.0	1.5	50.0	.000	.286*
	(.0)	(.5)		(.000)	(.286)
34. DITCH 14BW	13.0	1.5	50.0	.000	.074*
	(.0)	(.5)		(.000)	(.074)
35. DITCH 14AW	13.0	1.5	50.0	.077	.185*
	(.0)	(.5)		(.077)	(.185)
36. DITCH 7GN	26.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
37. DITCH 7GS	35.0	2.0	100.0	.257	.265
	(.0)	(.0)		(.171)	(.182)
38. DITCH 7HE	5.0	2.0	100.0	.100	.500*x
	(.0)	(.0)		(.100)	(.033)
39. DITCH 7ES	17.0	1.5	50.0	.118	.185
	(.0)	(.5)		(.118)	(.185)
40. DITCH 7FS	5.0	1.5	50.0	.100	.100
	(.0)	(.5)		(.100)	(.100)
41. DITCH 7HS	12.0	1.5	50.0	.208	.216
	(.0)	(.5)		(.208)	(.216)
42. DITCH 7HW	10.0	2.0	100.0	.100	.311*x
	(.0)	(.0)		(.000)	(.211)
43. DITCH 7IS	23.0	1.5	50.0	.174	.232
	(.0)	(.5)		(.174)	(.232)
44. DITCH 7JE	5.0	2.0	100.0	.300	.411*x
	(.0)	(.0)		(.300)	(.056)
45. DITCH 7JS	4.0	1.5	50.0	.250	.214
	(.0)	(.5)		(.250)	(.214)
46. DITCH 7JW	7.0	1.5	50.0	.071	.269*
	(.0)	(.5)		(.071)	(.269)
47. DITCH 7LE	2.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
48. DITCH 7LW	5.0	2.0	100.0	.100	.367*x
	(.0)	(.0)		(.100)	(.167)
49. DITCH 8AS	3.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
50. DITCH 8BS	1.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
51. DITCH 8BW	6.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
52. DITCH 8CE	19.0	1.5	50.0	.237	.254
	(.0)	(.5)		(.237)	(.254)
53. DITCH 8CS	1.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
54. DITCH 8CW	14.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
55. DITCH 13AE	2.0	1.5	50.0	.000	.333*
	(.0)	(.5)		(.000)	(.333)

Table 8 cont.

Population	Sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic[1]	Mean heterozygosity Direct count	HdyWbg expected[2]
56. DITCH 13AS	31.0	1.5	50.0	.113	.216*
	(.0)	(.5)		(.113)	(.216)
57. DITCH 13CE	2.0	1.5	50.0	.250	.250
	(.0)	(.5)		(.250)	(.250)
58. DITCH 13EN	7.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)
59. DITCH 13FE	12.0	1.5	50.0	.125	.216
	(.0)	(.5)		(.125)	(.216)
60. DITCH 13FN	1.0	1.0	.0	.000	.000
	(.0)	(.0)		(.000)	(.000)

[1] A locus is considered polymorphic if more than one allele was detected

[2] Unbiased estimate (see Nei 1977)

* Significant deviation from Hardy Weinberg Equilibrium P<0.05 for all polymorphic loci

*X Significant deviation from Hardy Weinberg Equilibrium P<0.05 for one out of two polymorphic loci

(Standard errors in parentheses)

Table 9 Genetic variability for all hierarchical levels

Genetic variability at two loci for all segments

Population	Sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic[1]	Mean heterozygosity Direct count	HdyWbg expected[2]
1. Segment 1	152.0	1.5	50.0	.023	.029**
	(.0)	(.5)		(.023)	(.029)
2. Segment 2	45.0	1.5	50.0	.100	.253**
	(.0)	(.5)		(.100)	(.253)
3. Segment 3	218.0	1.5	50.0	.067	.157**
	(.0)	(.5)		(.067)	(.157)
4. Segment 4	232.0	2.0	100.0	.108	.236**
	(.0)	(.0)		(.082)	(.193)

Genetic variability at two loci for all zones

Population	Sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic[1]	Mean heterozygosity Direct count	HdyWbg expected[2]
1. ZONE 1	152.0	1.5	50.0	.023	.029**
	(.0)	(.5)		(.023)	(.029)
2. ZONE 2	263.0	1.5	50.0	.072	.186**
	(.0)	(.5)		(.072)	(.186)
3. ZONE 3	232.0	2.0	100.0	.108	.236**
	(.0)	(.0)		(.082)	(.193)

[1] A locus is considered polymorphic if more than one allele was detected

[2] Unbiased estimate (see Nei 1977)

* Significant deviation from Hardy Weinberg Equilibrium P<0.05 for all polymorphic loci

*X Significant deviation from Hardy Weinberg Equilibrium P<0.05 for one out of two polymorphic loci

(Standard errors in parentheses)

Table 10 F-statistics

F-statistics for individual alleles by ditch

LOCUS: PGM-1

Allele	F(IS)	F(IT)	F(ST)
A	.449	.785	.610
B	.449	.785	.610
Mean	.449	.785	.610

LOCUS: PGM-2

Allele	F(IS)	F(IT)	F(ST)
A	.193	.350	.195
B	.193	.350	.195
Mean	.193	.350	.195

Summary of F-statistics at all loci

Locus	F(IS)	F(IT)	F(ST)
PGM-1	.449	.785	.610
PGM-2	.193	.350	.195
Mean	.408	.749	.575

F-statistics for individual alleles by segment

LOCUS: PGM-1

Allele	F(IS)	F(IT)	F(ST)
A	.579	.729	.355
B	.579	.729	.355
Mean	.579	.729	.355

LOCUS: PGM-2

Allele	F(IS)	F(IT)	F(ST)
A	.278	.288	.014
B	.278	.288	.014
Mean	.278	.288	.014

Summary of F-statistics at all loci

Locus	F(IS)	F(IT)	F(ST)
PGM-1	.579	.729	.355
PGM-2	.278	.288	.014
Mean	.557	.707	.338

F-statistics for individual alleles by zone

LOCUS: PGM-1

Allele	F(IS)	F(IT)	F(ST)
A	.582	.757	.417
B	.582	.757	.417
Mean	.582	.757	.417

LOCUS: PGM-2

Allele	F(IS)	F(IT)	F(ST)
A	.278	.285	.009
B	.278	.285	.009
Mean	.278	.285	.009

Summary of F-statistics at all loci

Locus	F(IS)	F(IT)	F(ST)
PGM-1	.582	.757	.417
PGM-2	.278	.285	.009
Mean	.549	.724	.389

Summary of mean F-statistics at all loci by hierarchical level

Level	F(IS)	F(IT)	F(ST)
Ditch	.408	.749	.575
Segment	.557	.707	.338
Zone	.549	.724	.389

DISCUSSION

Variation within ditches

Strong evidence for clonal growth within ditches is evident from the map with the neighbour of a given plant usually being of the same genotype. Whilst this is not conclusive when dealing with homozygotes, where self pollination and limited dispersal of seeds would give a similar pattern, in the case of unbroken lines of heterozygotes such as that observed in ditch 4GE, the evidence is compelling. The overall mean value of F_{IS} is 0.408 which, coupled with the deviations from Hardy-Weinberg equilibrium in many ditches, suggests that inbreeding occurs within ditches. Some caution is necessary in the interpretation of these statistics as it is not clear to what extent clonal growth will have distorted the values.

Variation between ditches

From the maps (Fig. 6 & 9) and the F_{ST} values it is clear that a high level of partitioning of genetic variation is present in the population of *P. coloratus* in the Gordano Valley. It is remarkable, considering the close proximity of ditches to one another, that two adjacent ditches can remain so genetically different, eg: ditches 8BW and 8CW.

The cause of the pattern in the partitioning of the variation remains unclear. The distribution of the PGM-2 genotypes appears to be more random than that of PGM-1, with the rare PGM-2 'b' allele occurring at scattered locations across the site. It could be the case that the distribution of PGM-2 alleles is random, whereas different PGM-1 alleles (or alleles for a gene closely linked to them) are being subjected to selective pressures. However given the apparent homogeneity of the habitat within the site, and the fact that in many ditches both alleles coexist, this explanation is somewhat unsatisfactory.

Another possible explanation is that the pattern is generated by genetic drift. The ditches at the site are managed, being cleared and dredged every 10-15 years. These clearing events, while almost certainly not completely destroying the plants in a ditch (seed banks and vegetative tissue such as rhizomes could well survive), could well result in a bottleneck. When a ditch has been cleared, it does, however, create areas suitable for recolonisation

from plant material from elsewhere or from the seed bank.

It is interesting that the greatest genotypic diversity in ditches occurs in the north-east of the site, which is also the direction in which the prevailing winds and the water flows (S.Wilson pers comm. 1994). If ditches at the top of the valley (SW) lost genetic diversity due to bottlenecks and subsequent drift, the chance of recolonisation by genetically different individuals or pollen from other parts of the site against the prevailing wind and water flow may be unlikely. However, if ditches further down the valley (NE) lost genetic diversity, they perhaps stand a greater chance of having variation introduced from different genotypes by the prevailing wind and water flow. For this model to work, it requires that chance drift results in different ditches at the top of the valley becoming monomorphic for different alleles. This does appear to be the case, and I suggest that the variation noted in field 7 (PGM-1 'a' & 'b') could be explained by immigration from sites such as fields 3 (PGM-1 'b') and 4 (PGM-1 'a'). Seeds presumably float down the valley on a regular basis, and could enter the seed bank in ditches downstream of their starting point. When conditions are suitable (e.g. after a ditch is cleared), some of these seeds may germinate and become established. Thus it could be that while gene flow between ditches is generally low, clearing of ditches (which provides increased areas for colonisation) could result in a directional gene flow down the valley.

In the absence of further data I am unwilling to support one hypothesis over the other, and feel that further work is required before any conclusions can be made. Most importantly, the use of more markers would help distinguish between selection and drift. The relative rarity of the PGM-2 'b' allele means that only one locus (PGM-1) provided widespread variable markers. One obvious approach would be to use RAPD markers (Williams *et al.* 1990) which could also increase the accuracy of estimation of the extent of clonal growth. I am also awaiting detailed information on the management of the site from English Nature, as the frequency and the methods of clearing the ditches are likely to affect the population biology of the constituent species (Beltman 1984, Wade & Edwards 1980). Of particular interest is the order in which ditches are cleared, as the chances of plant material being transferred

between ditches by dredging implements could be high.

Sampling Implications

Clearly the partitioning of genetic variation within a site has implications for sampling strategy. If samples are only taken from a small area of a site, a misrepresentative picture could be obtained. While it is obviously impractical to sample to the levels of this study if a number of populations are to be examined, careful sampling at sites such as the Gordano Valley is to be recommended. The previous investigation into the genetic diversity of this population (Hollingsworth *et al.* 1995a - chapter 3) detected six multi-locus genotypes, and only eight genotypes were recovered in the additional 647 plants analysed here. Thus the 69 plants in the previous study (which were collected from ten widely spaced ditches) appear to have been a reasonably representative sample.

There have been relatively few comparable studies that have made a detailed examination of the partitioning of genetic variation within populations of clonal plant species. McClintock & Waterway (1993) examined clonal diversity in *Carex lasiocarpa* and *C. pellita* populations They found greater genetic diversity within a population among plants growing in open water than they did among plants growing in dense established stands. They suggested that seedling recruitment may be more likely in open water than in a dense stand which could consist of a single successful clone. This situation appears to represent a parallel to that of the Gordano Valley, where colonisation events (either by vegetative or seed material) are probably more significant after the clearing of ditches when new pioneer habitats are formed.

Laushman (1993) summarised detailed studies on a population of *Zostera marina* L. and a population of *Vallisneria americana* Michx. The *Zostera marina* population showed a high level of substructure with GST=0.295 (GST is equivalent to a multilocus FST), and geographical partitioning of two PGM alleles noted. The *Vallisneria americana* population showed an "increase in sexuality" (increased flowering?) and an increase in " females / males in deeper waters", but GST values were not given. Silander (1979) studied

clone structure in a population of *Spartina patens* (Aiton) Mulh., and here also considerable substructuring of the population was found.

I agree with Silander (1979) in emphasising the need for fine scale sampling in clonal populations. In habitats such as lakes where limited physical barriers are present to impede the dispersal of vegetative fragments and seeds, population substructuring may be less evident. However in sites such as the Gordano valley where ditches are separated by fields and woods, examination of a number of individuals from a number of different ditches is essential. Furthermore, pooling of data from different subpopulations (i.e. ditches) within a site can lead to distorted estimates of the breeding system, and in such situations further sampling may be necessary to determine the actual extent of population substructure.

CHAPTER 5

ISOZYME EVIDENCE FOR HYBRIDISATION BETWEEN *POTAMOGETON NATANS* AND *P. NODOSUS* (POTAMOGETONACEAE) IN BRITAIN

ABSTRACT

A population of pondweeds from the River Stour in Dorset intermediate in morphology between *Potamogeton natans* L. and *P.nodosus* Poir. is shown by means of isozyme evidence to be the hybrid *P.* x *schreberi* G.Fisch. It is represented by a single multi-enzyme phenotype which, together with its sterility, suggests it reproduces vegetatively and may well constitute a single clone. It is genetically distinct from the morphologically similar hybrid between *P.lucens* L. and *P.natans* (*P.* x *fluitans* Roth).

INTRODUCTION

The widespread occurrence of hybrids in the genus *Potamogeton* was first postulated in the late 19th century, and since then has become generally accepted (Ascherson & Graebner, 1907; Hagström, 1916; Dandy, 1975). Wiegleb (1988) listed 43 taxa which he regarded as sufficiently well known to be accepted as hybrids, excluding many more which have been suggested on evidence that (by implication) he considered insufficient. In the British Isles, where Wiegleb (1988) considered that hybrids may be particularly frequent, Dandy (1975) recognised 25. He cited 16 of the 21 species recorded in the area as a parent of at least one hybrid.

The evidence for the hybrid nature of most taxa comes from their morphological and anatomical intermediacy, coupled in some cases with their sterility. In the case of hybrids between two distinctive and dissimilar species, the evidence of morphological intermediacy and sterility can be compelling: *P.x cooperi* (Fryer) Fryer, the putative hybrid between *P.crispus* L. and *P.perfoliatus* L., provides an example. Even if a plant is a hybrid between dissimilar species, however, it may be difficult to establish the exact parentage if there are two or more morphologically similar species which might plausibly be one of the parents. It is even more difficult to be certain of the hybrid identity of plants which appear to be intermediate between two

closely related species. In these circumstances morphological intermediacy and sterility may not, of themselves, be reliable guides to hybridity. According to Les & Philbrick (1993) this is especially true of aquatic plants where developmental and phenotypic plasticity, as well as aneuploidy, are confounding effects.

Despite the difficulties involved in recognising hybrids and determining their parentage from morphological and anatomical studies, there have been few attempts to use evidence from other fields. The practical difficulties of growing and crossing *Potamogeton* species have presumably dissuaded botanists from trying to synthesise hybrids artificially. There have, however, been a few attempts to test the identity of putative hybrids by the investigation of their phenolic chemistry. Only one of these has produced convincing evidence for the hybridity of the taxon under investigation. A study of *P.strictifolius* A.Benn., *P.zosteriformis* Fern. and their putative hybrid, *P.x longiligulatus* Fern. in Michigan revealed an additive pattern of spots in two-dimensional chromatograms of phenolic extracts of *P.* x *longiligulatus* (Haynes & Williams, 1975). This, together with its morphological intermediacy and almost complete sterility, was interpreted as good evidence of its hybrid origin. However, a similar study of plants from New York, which are intermediate in morphology between *P.hillii* Morong and *P.zosteriformis*, showed no such additive phenolic spot pattern (Hellquist & Hilton, 1983). Since the intermediate plants also showed considerable fertility, they were treated as a distinct species, *P.ogdenii* Hellquist & Hilton. In a third study of phenolic chemistry, in which the compounds were identified, Hagström's (1916) theory of the hybrid origin of *P.richardsonii* (A.Benn.) Rydb. was rejected because its flavonoid profile could not be explained as a combination of those of its putative parents, *P.perfoliatus* and *P.praelongus* Wulfen (Roberts & Haynes, 1986).

There have been even fewer attempts to use isozyme markers to investigate putative *Potamogeton* hybrids. Hettiarachchi & Triest (1991) conducted an isozyme survey of 190 populations of 19 species of *Potamogeton*, which included *P.gramineus* L., *P.lucens* L. and their putative hybrid, *P.* x *zizii* Roth. They suggested that their data indicate a close relationship between

P.lucens and *P.x zizii*, but did not comment on the possible parental role of *P.gramineus*. The second, and to our knowledge the only other, isozyme investigation of hybridisation in *Potamogeton* involved *P.natans* L. and *P.illinoensis* Morong as putative parents. In a study of morphologically intermediate plants from Wisconsin "electrophoretic evidence provided no indication of hybridisation" (Les & Sheridan unpublished data, cited in Les & Philbrick, 1993).

It is against this largely negative background that I present the results of an isozyme study designed to test the hypothesis that a newly discovered British population of *P.* x *schreberi* G.Fisch. originated as a hybrid between *P.natans* and *P.nodosus* Poir., and is distinct from the morphologically similar hybrid between *P.natans* and *P.lucens* (*P.* x *fluitans* Roth).

Potamogeton x *schreberi* and *P.* x *fluitans* in Britain

Potamogeton natans is the most frequent *Potamogeton* species in lowland Britain, found in many different water bodies (including rivers, streams, lakes and ponds) and tolerant of a wide range of water chemistries. By contrast, *P.nodosus* is one of the rarest and most restricted species, known only from a few lowland calcareous rivers in southern England, including the River Stour in Dorset. In 1992 a *Potamogeton* population was discovered in the R.Stour which was identified as *P.* x *schreberi*, the hybrid between *P.natans* and *P.nodosus* (Preston, 1995b). The identification was based on the morphology of the plants, which was intermediate between that of the putative parents, and their resemblance to material from Germany and Switzerland which had previously been ascribed to this hybrid.

Potamogeton x *schreberi* is found as occasional colonies in a stretch of the R.Stour at least 4 km long near Marnhull; within a colony plants may be frequent or locally abundant. Neither of its putative parents nor any other broad-leaved *Potamogeton* species have been found with *P.* x *schreberi*, although *P.nodosus* is locally frequent downstream, the nearest known population being some 10 km from the hybrid. There may be an ecological explanation for the separation of *P.nodosus* and *P.* x *schreberi*, as the substrate where the hybrid occurs is soft clay, whereas downstream

P.nodosus characteristically grows on gravel. Although there are no reliable recent records of *P.natans* from the R.Stour, it was collected in 1865 from the river at Durweston, which lies on the stretch where *P.nodosus* occurs. Les & Philbrick (1993) commented on the large number of suspected *Potamogeton* hybrids reported from localities where either one or both parents do not occur, suggesting that such cases are worth special investigation.

Both *Potamogeton natans* and *P.nodosus* are rhizomatous, wind-pollinated perennials with a commonly reported tetraploid chromosome number of 2n=52 (Les, 1983). (There are, however, also two reports of 2n=42 for *P.natans* (reviewed in Hollingsworth 1995 - chapter 2)), although material from Dorset (Arne, near Wareham) studied by us yielded an approximate count of 2n = 52.) *P.natans* usually flowers and fruits freely, although flowers may not be produced or fruits may fail to develop on plants which grow in deep or rapidly flowing water. *P.nodosus* also flowers in its British localities, its flowering time overlapping with that of *P.natans*, but mature fruit rarely, if ever, develops. Fruiting plants have been reported by Druce (1929) and Good (1969), but no mature fruits have been found on herbarium specimens collected in Britain or on plants growing in the wild (C D. Preston, pers. comm.). However, British plants will fruit if cultivated in standing water in small containers (Fryer 1899; N.T.H.Holmes, pers. comm.). Plants of *P.nodosus* produce short stolons terminated by an ovoid bud in the leaf axils towards the end of the growing season; these are easily detached and probably constitute a means of vegetative propagation. *P.natans* normally has no special means of vegetative reproduction or dispersal. *P.* x *schreberi* appears to be sterile, but produces axillary stolons similar to those of *P.nodosus*. Like many aquatics, both it and *P.natans* may also be able to become established from fragments of stem or rhizome which become detached and washed away.

Potamogeton x *schreberi* is similar in appearance to two other hybrids of *P.natans*: *P.* x *sparganiifolius* Fries (*P.natans* x *P.gramineus*) and *P.* x *fluitans* (*P.natans* x *P.lucens*). *P.* x *sparganiifolius* has a predominantly northern and western distribution in the British Isles, which reflects the

distribution of the rarer parent, *P.gramineus*. It is unlikely to occur in Dorset, where *P.gramineus* has not been reliably recorded, and it is not considered further in this paper. *P.lucens*, however, is locally frequent in Dorset, and grows in abundance in the same stretch of the R.Stour in which *P.nodosus* is found. The putative hybrid between *P.lucens* and *P.natans* (*P.* x *fluitans*), which resembles *P.* x *schreberi*, has not been recorded from the R.Stour. It is, however, found in a major tributary, the Moors River, some 35 km to the east, a site where it was first collected in 1879 (Dandy & Taylor, 1939g). Here it grows in great abundance in a stretch of river over 6 km long, often with *P.natans*. The population of *P.* x *fluitans* in the Moors River may not be totally sterile: a few well-formed fruits can sometimes be found on the old inflorescences, but I do not know if they are viable. In addition to any reproduction by seed which may occur, *P.* x *fluitans* almost certainly spreads vegetatively by small, easily detached lateral branches and perhaps by fragments of stem or rhizome broken off during times of rapid water flow. In addition to the Moors River population, *P.* x *fluitans* is currently known from two localities in East Anglia. The East Anglian plants differ from those in the Moors River in being closer to *P.natans* in their morphology, and they do not appear to produce well-formed fruit.

MATERIAL AND METHODS

Materials

Single leaves of *P.nodosus, P.* x *schreberi, P.natans, P.* x *fluitans* and *P.lucens* were collected during 1993 and 1994 from the localities given in Table 11. Since the plants identified as *P.* x *schreberi* occur in Britain only in the R.Stour, sampling of the putative parents and allied taxa was concentrated chiefly in that vicinity, although I have also included material of *P.natans* from Austria and Scotland which was available. Samples were taken at least 2m apart to reduce the chance of sampling the same ramet. The leaves were placed in plastic bags and taken back to the laboratory for isozyme analysis. Voucher specimens for each population were deposited in CGE.

Table 11. Species, localities and sample sizes (N) of material studied.

Species and locality	Grid Ref.	N
P.nodosus Poir.		
River Stour (Dorset, v.c.9): total 41 plants		
- Hayward Bridge, Child Okeford	ST82.12	5
- Durweston	ST85.09	20
- Shillingstone	ST82.11	14
- near Alders Coppice	ST83.10	2
River Loddon (Berkshire, v.c.22): total 8 plants		
- Arborfield Bridge	SU74.67	6
- Sheepbridge	SU72.65	2
P.x schreberi G.Fisch.		
River Stour (Dorset, v.c.9): total 36 plants		
- Fifehead Mill Bridge	ST76.20	1
- N. of Marnhull	ST76.19	4
- Marnhull	ST76.18	28
- S. of Marnhull	ST76.17	3
P.natans L.		
Moors River (S.Hampshire, v.c.11): total 34 plants		
- tributary	SU10.00	2
- St Leonard's Peat South	SU10.00	16
- Troublefield reserve	SZ12.97	14
- S. of Hurn	SZ12.96	2
Wareham (ditches) (Dorset, v.c.9)	SY92.87	32
Tiree, Loch Riaghain (Mid Ebudes, v.c.103)	NM03.47	2
Austria, prov. Tirol, Landeck (pond)	-	30
P. x *fluitans* Roth		
Moors River (S.Hampshire, v.c.11): total 35 plants		
- St Leonard's Peat South	SU10.00	28
- Troublefield reserve	SZ12.97	5
- S. of Hurn	SZ12.96	2
P.lucens L.		
River Piddle, Wareham (Dorset, v.c.9)	SY48.79	6
River Avon, Christchurch (S.Hampshire, v.c.11)	SZ15.94	30

Methods

Standard horizontal electrophoresis of isozymes using starch gels was carried out as described by Wendel & Weeden (1989), using the lithium borate ($LiBO_3$) gel/electrode buffer system. Electrode buffer, pH 8.1: 1 litre dH_20, 11.9g H_3BO_3, 1.2g LiOH. Gel buffer, pH 8.3: 100 ml $LiBO_3$ electrode buffer, 900ml dH_20, 5.45g Trizma base, 1.2g citric acid.

Approximately $25mm^2$ of young, actively growing leaf tissue was dabbed dry of water, and mechanically ground at 4^0C in 2 drops of the following extraction buffer: 50ml $LiBO_3$ gel buffer, 37mg KCl, 10mg $MgCl_2$, 18mg EDTA Na_4, 25mg polyvinylpolypyrrolidone, 0.5ml Triton X-100, 1.25ml ß-mercaptoethanol. The resulting crude protein homogenate was then soaked onto wicks cut from Whatman 3MM filter paper and loaded into a 12% starch gel. Electrophoresis was carried out at a constant voltage of 240V and a maximum current of 70mA until the running front had migrated 8cm from the origin. Eight enzyme systems were stained as follows.

Aspartate aminotransferase (AAT): 50ml 0.1M Tris-HCl pH 8.5, 18mg α–ketoglutaric acid, 65mg aspartic acid, 250mg polyvinylpyrrolidone 40000 (PVP-40T), 50mg EDTA Na_2, 710mg Na_2HPO_4, 1mg pyridoxal-5-phosphate, 200mg fast blue BB salt.

Aldolase (ALD): 50ml 0.1M Tris-HCl pH 8.0, 200mg fructose-1,6-diphosphate, 75mg arsenic acid, 150 units glyceraldehyde-3-phosphate dehydrogenase, 10mg nicotinamide adenine dinucleotide (NAD), 10mg 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), 3mg phenazine methosulphate (PMS), 1ml dH_20.

Glucose-6-phosphate dehydrogenase (G6PDH): 50ml 0.1M Tris-HCl pH 7.5, 50mg glucose-6-phosphate, 10mg nicotinamide adenine dinucleotide phosphate (NADP), 15mg MTT, 3mg PMS, 0.5ml 10% $MgCl_2$.

Glucose-6-phosphate isomerase (GPI): 50ml 0.1M Tris-HCl pH 8.0, 40mg fructose-6-phosphate, 30 units glucose-6-phosphate dehydrogenase, 10mg NADP, 15mg MTT, 3mg PMS, 0.5ml 10% $MgCl_2$.

Isocitrate dehydrogenase (IDH): 50ml 0.1M Tris-HCl pH 8.0, 100mg isocitric acid, 10mg NADP, 15mg MTT, 3mg PMS, 0.5ml 10% $MgCl_2$.

Phosphogluconate dehydrogenase (PGD): 50ml 0.1M Tris-HCl pH 8.0, 50mg 6-phosphogluconic acid, 50mg $MgCl_2$, 10mg NADP, 15mg MTT, 3mg PMS, 0.5ml 10% $MgCl_2$.
Phosphoglucomutase (PGM): 50ml 0.1M Tris-HCl pH 7.5, 100mg glucose-1-phosphate, 25mg ATP, 35 units glucose-6-phosphate dehydrogenase, 10mg NADP, 15mg MTT, 3mg PMS, 0.5ml 10% $MgCl_2$.
Shikimate dehydrogenase (SKD): 50ml 0.1M Tris-HCl pH 8.0, 50mg shikimic acid, 10mg NADP, 15mg MTT, 3mg PMS, 0.5ml 10% $MgCl_2$.

Staining solutions for ALD and GPI were prepared as agar overlays as described by Wendel & Weeden (1989). 15mg NADP per 350ml was added to the gel and the negative electrode tank buffers when analyzing IDH, G6PDH, PGD, and SKD.

A comparison was made of the isozyme banding patterns of floating and submerged leaves in two heterophyllous taxa (*P.natans* and *P.* x *fluitans*) to check for intra-individual variation. I found none.

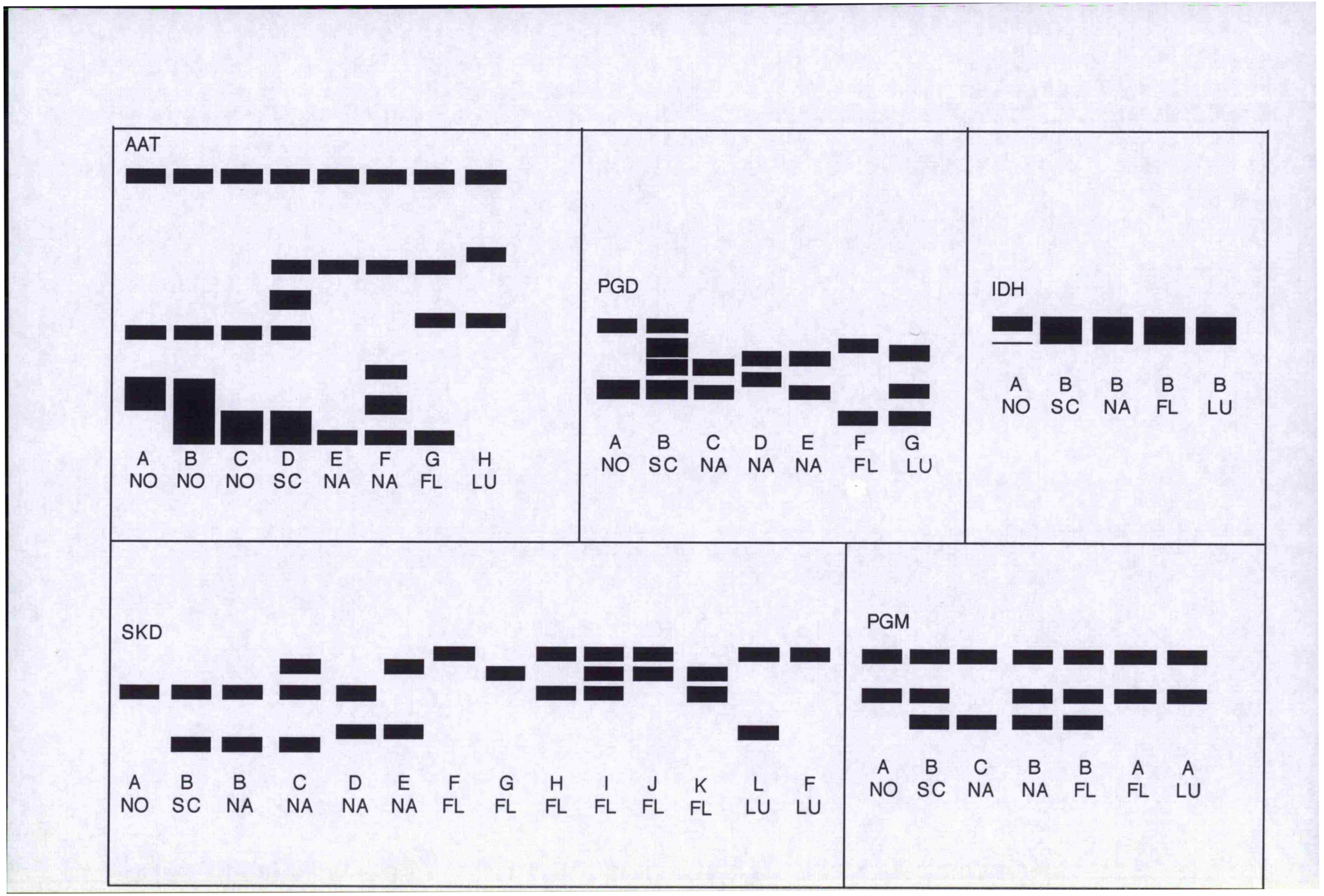
RESULTS

All eight enzyme systems examined generated banding patterns that migrated anodally. Interpretation of these was based on knowledge of sub-unit structure and subcellular compartmentalisation (Weeden & Wendel 1989). Since all taxa in the study are tetraploid, I expected at least some evidence of enzyme multiplicity. This was observed in the case of IDH, although it was not possible to determine whether this involved multiple isozymes or more than two allozymes, partly because the multiple bands produced were not fully resolved (Fig. 10). AAT gave patterns consistent with three loci (the lower two of which were polymorphic) in all taxa except *P.* x *fluitans*, in which four bands were seen (Fig. 10). The interpretation of banding patterns at the putative AAT-3 locus requires some explanation. The patterns shown by *P.natans* reflect diallelic variation, with the single-banded phenotype (pattern 'E') representing a homozygote and the clearly resolved triple-banded phenotype (pattern 'F') representing a heterozygote (Fig. 10). The patterns in *P.nodosus* could also reflect diallelic variation in a

similar fashion, but crucially the bands in the heterozygote (pattern 'B', Fig. 10) are unresolved; furthermore, although the bands in patterns 'A' and 'C' (Fig. 10) could represent single alleles, they are broader than is usual in this situation. I therefore interpret pattern 'B' as being the product of three or four alleles, i.e. plants with this heterozygous phenotype exhibit allozyme multiplicity, as might be expected in a tetraploid. I interpret patterns 'A' and 'C' as being heterozygous for different subsets of the alleles in pattern 'B'.

GPI produced the same two invariant bands, corresponding to two putative loci, in all taxa. ALD and G6PDH behaved similarly, although the lower bands were faint and not consistently scorable and thus were omitted from the analysis. The patterns in PGD were also consistent with two loci, both of which showed variation, except for *P.lucens* in which a three-banded phenotype was found (Fig. 10). PGM followed a 2-locus model throughout, with diallelic variation seen in the lower one (Fig. 10). The one- to three-banded patterns produced by SKD, though consistent, did not prove to be readily interpretable in terms of numbers of loci (Fig. 10).

Fig. 10. Isozyme phenotypes for AAT, PGD, IDH, SKD and PGM. Letters of the alphabet designate individual phenotypes for each isozyme. FL = *Potamogeton* x *fluitans*, LU = *P.lucens*, NA = *P.natans*, NO = *P.nodosus*, SC = *P.* x *schreberi.*


AAT
A NO
B NO
C NO
D SC
E NA
F NA
G FL
H LU
PGD
A NO
B SC
C NA
D NA
E NA
F FL
G LU
IDH
A NO
B SC
B NA
B FL
B LU
SKD
A NO
B SC
B NA
C NA
D NA
E NA
F FL
G FL
H FL
I FL
J FL
K FL
L LU
F LU
PGM
A NO
B SC
C NA
B NA
B FL
A FL
A LU

Variation within taxa

A summary of the frequency of the single-enzyme phenotypes observed in each taxon is given in Table 12. Three multi-enzyme phenotypes were detected in *P.nodosus*, based on variation only in AAT. The most common was 'B' (Fig. 10), which characterised all individuals from the River Stour and five from the River Loddon. In the latter river, variation was confined to the site at Arborfield Bridge, where I found individuals apparently segregating for AAT-3: two with a 'C' heterozygote phenotype, one with an 'A' heterozygote phenotype and three with a 'B' tri- or tetra-allelic heterozygote phenotype (Fig. 10).

Eleven multi-enzyme phenotypes were detected in *P.natans*, based on variation in AAT, PGD, PGM and SKD. Populations ranged from being monomorphic (Wareham and Landeck) to highly polymorphic (seven multi-enzyme phenotypes from 16 plants at St Leonard's Peat). The Austrian population at Landeck had the same multi-enzyme phenotype as that of twelve plants sampled from various places in the Moors River in Britain.

Two multi-enzyme phenotypes were found in *P.lucens*, based on variation in SKD, with each phenotype characteristic of a different river.

In *P.* x *schreberi*, all individuals consisted of a single multi-enzyme phenotype, whereas in *P.* x *fluitans* nine multi-enzyme phenotypes were detected, with variation occurring in PGM and SKD.

Table 12. Distribution and frequency of isozyme phenotypes within and between populations for all polymorphic enzyme systems. Letters refer to the phenotypes depicted in Fig. 11.

Species & locality		N	Isozyme phenotype				
			AAT	IDH	PGD	PGM	SKD
P.nodosus							
River Stour	- Child Okeford	5	B	A	A	A	A
	- Durweston	20	B	A	A	A	A
	- Shillingstone	14	B	A	A	A	A
	- near Alders Coppice	2	B	A	A	A	A
River Loddon	- Arborfield Bridge	1	A	A	A	A	A
		3	B	A	A	A	A
		2	C	A	A	A	A
	- Sheepbridge	2	B	A	A	A	A
P.x schreberi							
River Stour	- Fifehead Mill Bridge	1	D	B	B	B	B
	- N. of Marnhull	4	D	B	B	B	B
	- Marnhull	28	D	B	B	B	B
	- S. of Marnhull	3	D	B	B	B	B
P.natans							
Moors River	- tributary	1	E	B	E	C	B
		1	E	B	E	C	C
	- St Leonard's Peat South	5	E	B	E	C	B
		3	E	B	D	C	B
		3	E	B	C	C	B
		2	E	B	E	B	B
		1	E	B	E	C	C
		1	E	B	E	C	D
		1	E	B	D	C	E
	- Troublefield reserve	8	E	B	E	B	B
		2	E	B	C	B	B
		4	E	B	E	C	B
	- S. of Hurn	2	E	B	E	C	B
Wareham (ditches)		32	F	B	E	C	C
Tiree, Loch Riaghain		1	F	B	E	C	C
		1	F	B	D	C	C
Austria, Landeck (pond)		30	E	B	E	C	B
P.x fluitans							
Moors River	- St Leonard's Peat South	1	G	B	F	A	F
		1	G	B	F	B	F
		9	G	B	F	B	J
		8	G	B	F	B	G
		2	G	B	F	B	H
		1	G	B	F	A	G
		4	G	B	F	B	I
		1	G	B	F	B	K
		1	G	B	F	A	J
	- Troublefield reserve	2	G	B	F	B	J
		1	G	B	F	B	G
		2	G	B	F	B	H
	- S. of Hurn	2	G	B	F	B	H
P.lucens							
River Piddle, Wareham		6	H	B	G	A	L
River Avon, Christchurch		30	H	B	G	A	F

Evidence for hybridisation between *P.nodosus* and *P.natans*

The most informative enzyme systems were PGD and AAT, which are dimers. PGD behaved as though it were encoded by two loci, each with two alleles. *P.nodosus* and *P.natans* had no alleles in common at PGD-1 and for which *P.* x *schreberi* showed an additive profile, composed of a triple-banded, heterozygote pattern, consistent with a cross between *P.nodosus* (phenotype 'A') and *P.natans* (phenotype 'C') (Fig. 10). Similarly, the putative AAT-2 locus distinguished both parents, with the hybrid displaying an additive, triple-banded, heterozygote pattern (Fig. 10 & 11).

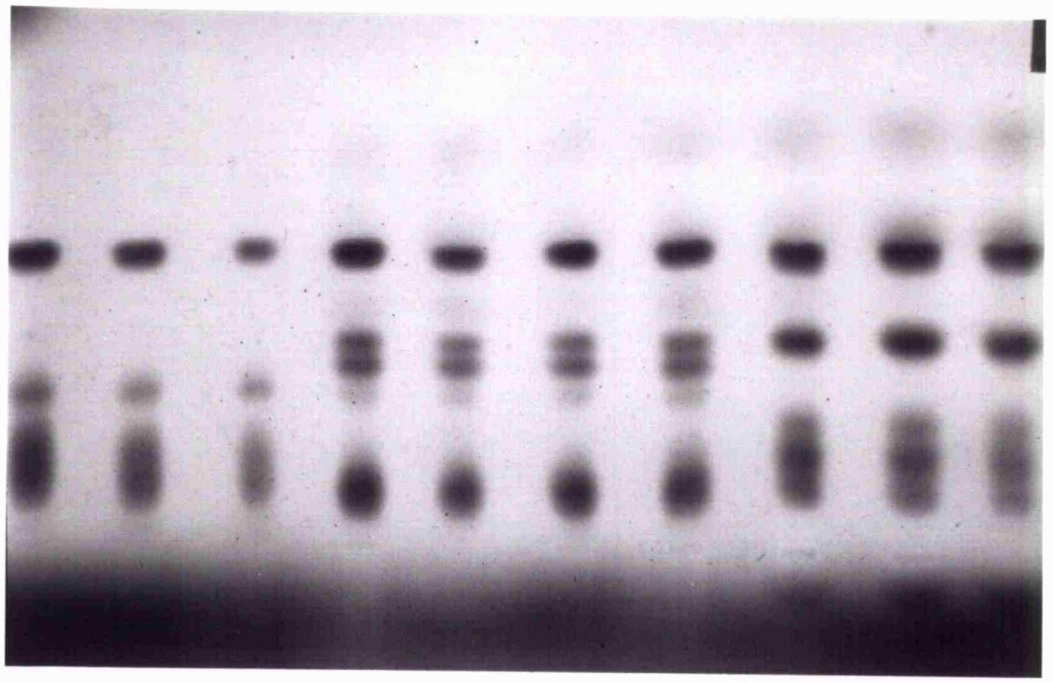
Following our preferred interpretation, inheritance at the putative AAT-3 locus in *P.* x *schreberi* involves a slow allele from *P.natans* and an intermediate allele from *P.nodosus*, producing the heterozygote phenotype shown in Fig. 11 as a dark blob.

The enzyme systems IDH, PGM and SKD distinguished the parents to the extent that *P.nodosus* presented a subset of the bands seen in *P.natans* ; the putative hybrid, *P.* x *schreberi*, displayed banding patterns which were consistent with additive inheritance but which were also possessed by some or all plants of *P.natans* (Fig. 10).

The least informative enzyme systems were ALD, G6PDH and GPI. None of these distinguished *P.natans* from *P.nodosus* ; both species, as well as their putative hybrid, produced the same monomorphic banding patterns for all the enzymes.

In summary, all bands produced by *P.* x *schreberi* were explicable on the basis of inheritance from *P.nodosus* and *P.natans*. Furthermore, a possible role for *P.* x *fluitans* or *P.lucens* in the origin of *P.* x *schreberi* can be ruled out by a comparison of the isozyme banding patterns of AAT, PGD and SKD in *P.* x *schreberi* with those in *P.* x *fluitans* and *P.lucens*.

Fig. 11 Starch gel showing AAT isozyme phenotypes for *Potamogeton nodosus*, *P.* x *schreberi* and *P.natans*. Putative loci are numbered in sequence with the most anodally migrating (towards the top of the picture) designated 'AAT-1', etc. Lanes 1-3: *P.nodosus*, with a single band at AAT-1, a single band at AAT-2 and a minimum of five bands (not fully resolved) at AAT-3. Lanes 4-7: *P.* x *schreberi*, with a single band at AAT-1, a triple band at AAT-2, and a minimum of three closely-spaced bands at AAT-3. Lanes 8-10: *P.natans*, with a single band at AAT-1, a single band at AAT-2 and a triple band at AAT-3. The two parents are fixed for different alleles at AAT-2, which are inherited additively and form a heterodimer in the putative hybrid, generating a three banded phenotype. The different intensities of the AAT-2 bands in *P.nodosus* and *P.natans* are also reflected in this pattern. The AAT-3 locus apparently segregates in both parents (Fig. 10), with the hybrid inheriting an intermediate allele from *P.nodosus* and the slow allele from *P.natans*.



DISCUSSION

The isozyme data presented here provide strong evidence in support of a hybrid origin for those plants in the River Stour known as *P.* x *schreberi* that have a morphological phenotype intermediate between *P.nodosus* and *P.natans*. This is the first example of isozymes having been used successfully to demonstrate the parentage of a putative *Potamogeton* hybrid. That the approach might be more widely applicable in the genus is indicated by the fact that I have also obtained evidence of isozyme additivity in the putative hybrid between *P.pectinatus* L. and *P.filiformis* Pers. (Hollingsworth *et al.* 1995e - chapter 7).

The fact that only a single multi-enzyme phenotype of *P.* x *schreberi* was found over a 4km stretch of the river indicates either that it has had multiple origins from the same parental genotypes or, more likely, that it is a single clone, owing its spread to vegetative reproduction by means of axillary stolons or rhizomatous growth. At any rate, the potential of *P.* x *schreberi* to vary is limited by the fact that it has not been observed to set any fruit.

Clonal spread of *P.natans* at Wareham and of *P.nodosus* in the River Stour is also implied by the isozyme data. Particularly informative in this respect is the AAT enzyme system, in which these populations are apparently composed entirely of heterozygotes at the putative AAT-3 locus (Figs. 10 & 11). Banding patterns consistent with segregation at AAT-3 were observed at other localities but not at Wareham or the R.Stour. This lack of segregation is strongly suggestive of clonal spread. It might have been predicted for *P.nodosus*, which rarely, if ever, fruits in Britain, but it is surprising to find it in the large population of *P.natans* at this site which flowers freely, producing clouds of pollen followed later by abundant fruit set. Given the apparent absence of segregants in the adult population, however, it seems not unreasonable to conclude that seedling recruitment is zero and that all spread is by vegetative means. The growth of *P.lucens* may be similarly clonal, at least at Christchurch where a sample of 30 plants revealed only a single multi-enzyme phenotype, although there are no heterozygote-like patterns to be used in evidence.

Finally, some discussion of the limited data on *P.lucens* and its putative hybrid with *P.natans, P.* x *fluitans*, is needed. *P.* x *fluitans* has a novel band not found in either *P.natans* or *P.lucens* for both SKD and PGD. Furthermore, all individuals of *P.lucens* have an AAT band, two PGD bands and an SKD band which are shared with *P.natans* but are not present in any of the samples of *P.* x *fluitans*. The absence of bands expected in *P.* x *fluitans* could perhaps be explained by the fact that it is one of the few *Potamogeton* hybrids that sets the occasional fruit, and therefore may be represented by recombinant or backcrossed individuals. The finding of nine multi-enzyme phenotypes in this hybrid is consistent with its partial fertility. Bands unexpectedly present in *P.* x *fluitans* may represent a sampling artefact, and a wider survey, particularly of *P.lucens*, may resolve this discrepancy.

CHAPTER 6

GENETIC VARIATION IN TWO HYDROPHILOUS SPECIES OF *POTAMOGETON* (POTAMOGETONACEAE)

ABSTRACT

Genetic variation in *Potamogeton pectinatus* and *P.filiformis* was studied by means of isozymes. The overall levels of variability are similar to some other well-studied hydrophilous species, as well as to the average for terrestrial clonal species. Variation is shown to be distributed more between than within populations, with mean values of Simpson's D=0.18 (*P. pectinatus*) and D=0.49 (*P. filiformis*). This partitioning of variability is attributed to three main factors. 1) Clonal growth (as measured by the frequency of multi-enzyme phenotypes) is shown to be a major factor in both species, although more common in *P.pectinatus*. 2) Geographical isolation is indicated as an important factor in regulating gene flow, at least in *P.filiformis*; the data suggest that dispersal is mainly by seed rather than by vegetative means. 3) Limited sexual reproduction, as mediated by reduced seedling recruitment rather than by infrequent flowering, is shown to be a likely contributor to the partitioning of variability in *P.pectinatus*. I found no evidence of genetic differentiation between brackish and freshwater populations of *P.pectinatus*.

INTRODUCTION

The predominance of vegetative reproduction in aquatic macrophytes is well documented (Sculthorpe 1967; Grace 1993), and it has been predicted that levels of genetic diversity within populations of such plants may be lower than in their terrestrial counterparts due to the extent of clonal reproduction (Hutchinson 1975; Triest 1991e). Furthermore, it has been argued that water-pollinated angiosperms may also show levels of genetic variability lower than those found in plants with other pollination syndromes, owing to inefficient pollen transfer and the strict limits imposed on pollen dispersal by the aquatic medium (Les 1988). Evidence that this may be true has come from studies of seagrasses (McMillan 1991), *Ceratophyllum* (Les 1986), and *Zannichellia* (Triest & Vanhecke 1991) in which little or no isozyme variation was found either within or between populations. Although there is now electrophoretic data on more than 100 taxa of aquatic macrophytes, much of

which has been gathered or reviewed by Triest (1991a), there are remarkably few studies that have set out to analyse the partitioning of variability within and between populations (Les 1991), and even fewer that deal adequately with hydrophilous species (Laushman 1993). Harris *et al.* (1992) pointed out that many of the studies of aquatic plants have had a taxonomic rather than a population genetic focus, and as such have not dealt adequately with variation at the levels of individual and population.

The present work provides isozyme data relating to variation within and between populations of *Potamogeton pectinatus* L. and *P.filiformis* Pers. two closely related species of pondweed in subgenus *Coleogeton* Reichb. which share a rhizomatous growth habit and a hydrophilous pollination syndrome. The two are believed to hybridise, and this study also provides the necessary background to an investigation of the putative hybrids (Hollingsworth *et al.* 1995e - chapter 7). The focus of the investigation is chiefly in Britain, although I have also included populations from Sweden, Crete and Argentina for comparison. Argentinian plants were formerly regarded as a variety of *P.pectinatus* (var. *striatus* (Hagström 1916)), but are now treated as a separate species, *P.striatus* Ruiz et Pavon (Tur 1982). A single Swedish population of *P.vaginatus* Turcz., the third European member of subgenus *Coleogeton*, is also included for comparison.

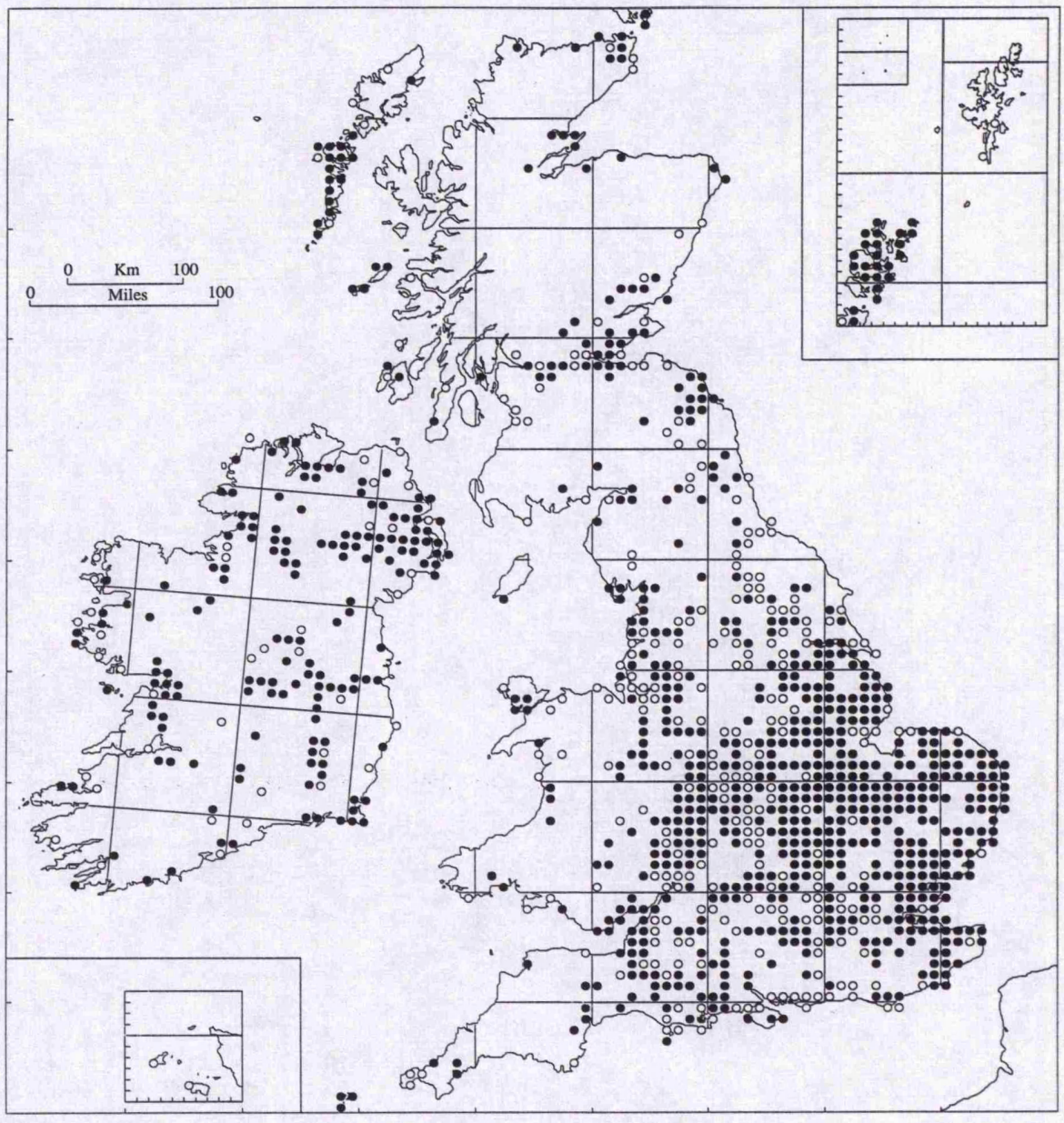
Distribution and habitat of *P. pectinatus* and *P. filiformis*

P. pectinatus is a phenotypically plastic aquatic macrophyte with a world-wide distribution ((Hultén & Fries 1986; Wiegleb 1988) and a correspondingly large ecological and altitudinal range. It can be a serious weed, forming dense stands which may impede irrigation, navigation or drainage channels (Pieterse & Murphy 1990), and is often the only species to survive at sites after herbicide treatment (van der Bliek *et al.* 1982). It has consequently been the subject of many studies of its physiology, ecology and life history (Anderson 1978; Hodgson 1966; Jupp & Spence 1977; Kautsky 1987; Madsen & Adams 1989; Spencer 1986, 1987, Spencer & Anderson 1987; van der Bijl *et al.* 1989; van Wijk 1983, 1988, 1989a,b,c; van Wijk *et al.* 1988; Yeo 1965). In Britain it is a widespread, native species, and is often abundant in eutrophic, lowland fresh and brackish waters (Fig. 12a),

including lakes, ponds, rivers, streams, canals, ditches and flooded sand and gravel pits.

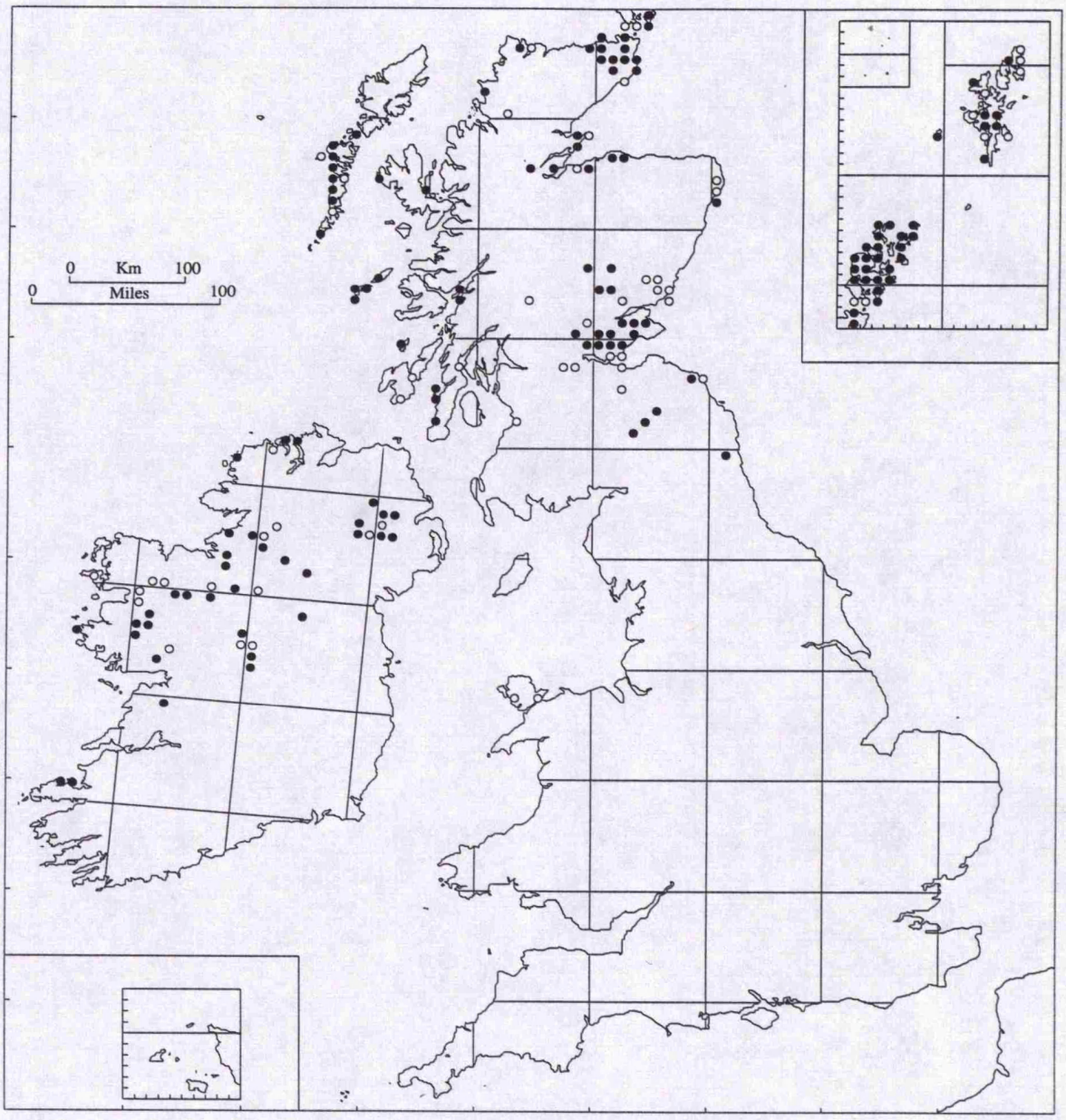
In contrast to *P. pectinatus, P. filiformis* has a circumboreal distribution, extending southwards in Eurasia to Spain, the Alps and the Himalaya, and in North America to Pennsylvania and Arizona (Hultén & Fries 1986). In Britain it is virtually confined to Scotland (Fig. 12b), where it usually grows in open vegetation at the edge of eutrophic, brackish or highly calcareous but nutrient-poor lochs at low altitudes. It is also recorded from ditches, streams and rivers. As a species with a relatively restricted world distribution and little significance to man, it has not received much ecological study.

Fig. 12a Distribution map of *P. pectinatus* in the British Isles.



Closed circles indicate post-1970 records, open circles represent pre-1970 records

Fig. 12b Distribution map of *P. filiformis* in the British Isles.



Closed circles indicate post-1970 records, open circles represent pre-1970 records

Reproductive biology

There are few published observations on the reproductive biology of *P. pectinatus* in Britain. However, the following conclusions, drawn from studies in the Netherlands (van Wijk 1988, 1989a,b) and Switzerland (Guo & Cook 1989), appear from our casual observations to be equally applicable to British plants.

Some populations of *P. pectinatus* are perennial, over-wintering as leafy shoots, whereas others are functionally (although not genetically) annual, regenerating each spring from tubers formed on the rhizome during the previous growing season. The flowers are hermaphrodite and borne on a flexuous peduncle which normally floats on the surface of the water at anthesis. The pollen is hydrophobous, and is released onto the water surface where pollination takes place. Plants are self-compatible. Inflorescences which do not reach the surface of the water can be pollinated under the water, presumably by bubble-mediated self-pollination as was suggested for some other *Potamogeton* species (Philbrick 1988). In standing waters populations often flower and set seed, although the number of seeds set may be considerably less than the potential maximum. Guo & Cook (1989) found that at a Swiss site seed was set by 25% of the flowers which reached the surface of the water at anthesis, and by only 4% of those which flowered under water. In flowing water flowering may be initiated but the flowers may not mature (van Wijk 1988); even if they do attain anthesis, seed is rarely if ever set in faster-flowing streams and rivers.

Vegetative reproduction in *P. pectinatus* is much more important in established populations than reproduction by seed. Tubers are produced on the rhizomes by all populations, and small tubers sometimes develop in the leaf axils of senescent plants. Plants can also regenerate from detached shoots which have survived the winter floating at the edge of a lake in an apparently moribund condition.

There are no detailed studies of the reproductive biology of *P. filiformis*, but it appears to be essentially similar to that of *P. pectinatus*. Plants flower freely in shallow water, and the inflorescences usually float at anthesis. The fruits

of *P. filiformis* are smaller than those of *P. pectinatus* and casual observations suggest that a higher proportion of the flowers may set seed. Plants which flower underwater are also capable of fruiting. Like *P.pectinatus, P. filiformis* also reproduces vegetatively from tubers on the rhizome.

Cytology

Chromosome counts of 2n = 78 have been reported for both *P.pectinatus* and *P.filiformis*, including an approximate one from British material of *P.pectinatus* from Cambridgeshire (Hollingsworth 1995 - chapter 2). These suggest that the species are hexaploid derived from a putative base number of x = 13. Aneuploid variation has been observed, however, within *P.pectinatus* (Kalkman & van Wijk 1984).

Genetic variation

To our knowledge there has been no investigation of genetic variation in *P.filiformis*, but there have been studies of *P.pectinatus*. Van Wijk *et al.* (1988) cultivated material under standard conditions and found that populations differed in characters such as total biomass, growth form, tuber morphology, the time taken to initiate tuber production and salt tolerance. Van Wijk *et al.* (1988) also examined isozyme variation in ten populations from the Netherlands and France. The two enzyme systems, SOD and PER, that proved to be resolvable and variable showed that variation was considerable between populations but was very low or absent within them. Further isozyme data on material from Hungary, Egypt and South Africa, although not fully resolvable, also showed differences between the populations. Overall, the data provided no evidence of any readily interpretable groupings and van Wijk suggested that the sampling of more populations from a wider geographical range, together with the use of more polymorphic enzyme systems, would be needed to understand the patterns of variation in this species.

Following a preliminary investigation of isozyme polymorphism in *P.pectinatus* (Hettiarachchi & Triest 1986), Hettiarachchi & Triest (1991) presented the results of a formidable study of the genus *Potamogeton*,

involving 188 populations of 18 species. Among these were 70 populations of *P.pectinatus*, originating from the USA, Egypt and Europe (Austria, Belgium, Finland, France, Hungary, Luxembourg, Netherlands, Sweden and one British population, from Woodwalton Fen, Cambridgeshire). Ten enzyme systems were examined: GDH, ME, PER, SOD and XDH in leaf material from all plants; AAT, GDH, GPI, ME, PGM, SKD, SOD and XDH in tubers where available; and ADH, GDH, ME and SOD in achenes where available. Some 57% (text) to 63% (table) of the populations were found to be monomorphic, but it is not possible to tell from the published report which these were. Cluster analysis of genetic distances between the populations revealed a major split between those from freshwater and those from brackish-water. The analysis also revealed that the freshwater populations formed well-defined geographical groups consisting of populations from northern Europe, western continental Europe, European Alps, Hungary, Britain, Egypt and the USA.

MATERIALS & METHODS

Materials

447 individuals of *P. pectinatus* from 26 populations, 270 individuals of *P.filiformis* from 13 populations, and 31 individuals of *P.striatus* and 24 of *P.vaginatus*, each from one population, were sampled from the localities given in Table 13. Sample sites were chosen to include a range of habitats and localities within Britain, although a certain emphasis was placed on the Inner and Outer Hebrides because of the occurrence there of *P.* x *suecicus*, the putative hybrid between *P.pectinatus* and *P.filiformis*. A sampling interval of at least 2m between plants was maintained to reduce the chance of collecting shoots growing from the same rhizome. Rooted fragments were placed into sealed plastic bags and returned to the laboratory for cultivation and analysis.

Table 13. Localities from which samples of *P.pectinatus* (P), *P.filiformis* (F), *P.vaginatus* (V) and *P.striatus* (S) were taken. Details of habitat are given in parentheses when not obvious.

Locality	Vice-county	Grid ref.	Species
Britain:			
River Stour	Dorset, v.c.9		
- Marnhull		ST71	P
- Fifehead Miil		ST72	P
- Hamoon		ST81	P
River Loddon, Arborifield Bridge	Berks., v.c.22	SU76	P
Babingley River, Castle Rising	W.Norfolk, v.c.28	TF62	P
Snettisham (brackish pool)	W.Norfolk, v.c.28	TF63	P
Newton Fen, North Level	Cambs., v.c.29		
- Main Drain		TF41	P
- N.of bridge (ditch)		TF41	P
- S.of bridge (ditch)		TF41	P
River Torne, Wroot	N.Lincs., v.c.54	SE70	P
Groby Pool	Leics., v.c.55	SK50	P
Chesterfield Canal, Gringley	Notts., v.c.56	SK79	P
River Wharfe, Ozendyke House	Mid-W.Yorks., v.c.64	SE53	P
Bishops Dyke, Caywood (stream)	Mid-W.Yorks., v.c.64	SE53	P
River Till, Ford	Cheviot, v.c.68	NT93	P
River Tweed, Union Bridge	Cheviot, v.c.68	NT95	P
Coldingham Loch	Berwicks., v.c.81	NT86	P, F
Loch Fitty	Fife, v.c.85	NT19	P, F
Tiree, Loch Riaghain	Mid Ebudes, v.c.103	NM04	F
Tiree, An Fhaodhail (stream)	Mid Ebudes, v.c.103	NM04	P, F
Tiree, Loch Earblaig	Mid Ebudes, v.c.103	NL94	P
Tiree, Loch Bhasapol	Mid Ebudes, v.c.103	NL94	P, F
Tiree, Loch an Eilein	Mid Ebudes, v.c.103	NL94	F
Tiree, Loch a'Phuill	Mid Ebudes, v.c.103	NL94	F
Loch an Aigeil	W.Sutherland, v.c.108	NC02	F
South Uist, West Loch Ollay	Outer Hebrides, v.c.110	NF73	P, F
Benbecula, Loch a'Chinn Uacraich	Outer Hebrides, v.c.110	NF75	P
Benbecula, Loch na Liana Moire	Outer Hebrides, v.c.110	NF75	P, F
Benbecula, Loch Fada	Outer Hebrides, v.c.110	NF75	P
North Uist, Loch Grogary	Outer Hebrides, v.c.110	NF77	P, F
Sweden:			
Östergötlands Län, Vadstena, Lake Vättern;	58°28'N, 14°56'E		F
Uppsala Län, Övergran, Varpsund channel;	59°38'N, 17°30'E		F
Stockholms Län, Daiarö (lake);	59°08'N, 18°26'E		P
Västernorrlands Län, near Njurundabommen, Berga (coast);	62°16'N, 17°27'E		P
Norrbottens Län, near Luleå, Bensbyn (coast);	65°38'N, 22°15'E		V
Crete:			
Georgioupoli (freshwater river);	35°20'N, 24°15'E		P
(brine spring);	35°20'N, 24°15'E		P
Argentina:			
CORFO system, Rio Colorado (irrigation channel)			S
irizo (drainage channel)			S
Pradere (drainage channel)			S

Methods

Standard horizontal starch gel electrophoresis of isozymes from leaf extracts was carried out as described by Wendel and Weeden (1989). Nine enzyme systems (AAT, ALD, G6PDH, IDH, LDH, PGD, GPI, PGM and SKD) were analysed using LiBO3 gel and electrode buffers as described by Hollingsworth *et al.* (1995a,c, - chapters 3,5).

RESULTS

Data Analysis

Based on knowledge of conserved isozyme number and compartmentalisation (Gottlieb 1981), I have tentatively interpreted the banding patterns produced by the various enzymes (Fig.13 & 14) as follows.

ALD, IDH, LDH and SKD showed patterns consistent with variation at a single locus in each case. Data for ALD for the West Loch Ollay population of *P.filiformis* is not presented because the bands were too faint to score. Owing to this ALD data are not included in the statistical analyses of either *P.pectinatus* or *P.filiformis* . G6PDH,PGD and PGM patterns could be tentatively interpreted in terms of variation at two loci, and those of AAT in terms of variation at four loci. GPI patterns were more complex and showed evidence of enzyme multiplicity. A total of at least 16 loci were surveyed. However, the fact that both *P.pectinatus* and *P.filiformis* are hexaploid (2n=6x=78) presents a problem as far as scoring allele frequencies is concerned. In particular it was not possible to infer the precise allelic constitution of heterozygotes. Furthermore, the fact that I was not able to germinate adequate progeny arrays to test for segregation ratios means that I cannot assume that any of the loci are necessarily inherited in a disomic fashion. Tests for Hardy-Weinberg equilibrium are consequently ruled out. Instead, the number and relative frequencies of single-enzyme and multi-enzyme phenotypes were used as measures of genetic diversity. In the present case data for multi-enzyme phenotypes have been analysed using the complement of Simpson's Diversity Index, D, corrected for finite samples (Pielou 1969; Peet 1974). This statistic represents the likelihood of any two individuals selected at random from a population having a different multi-

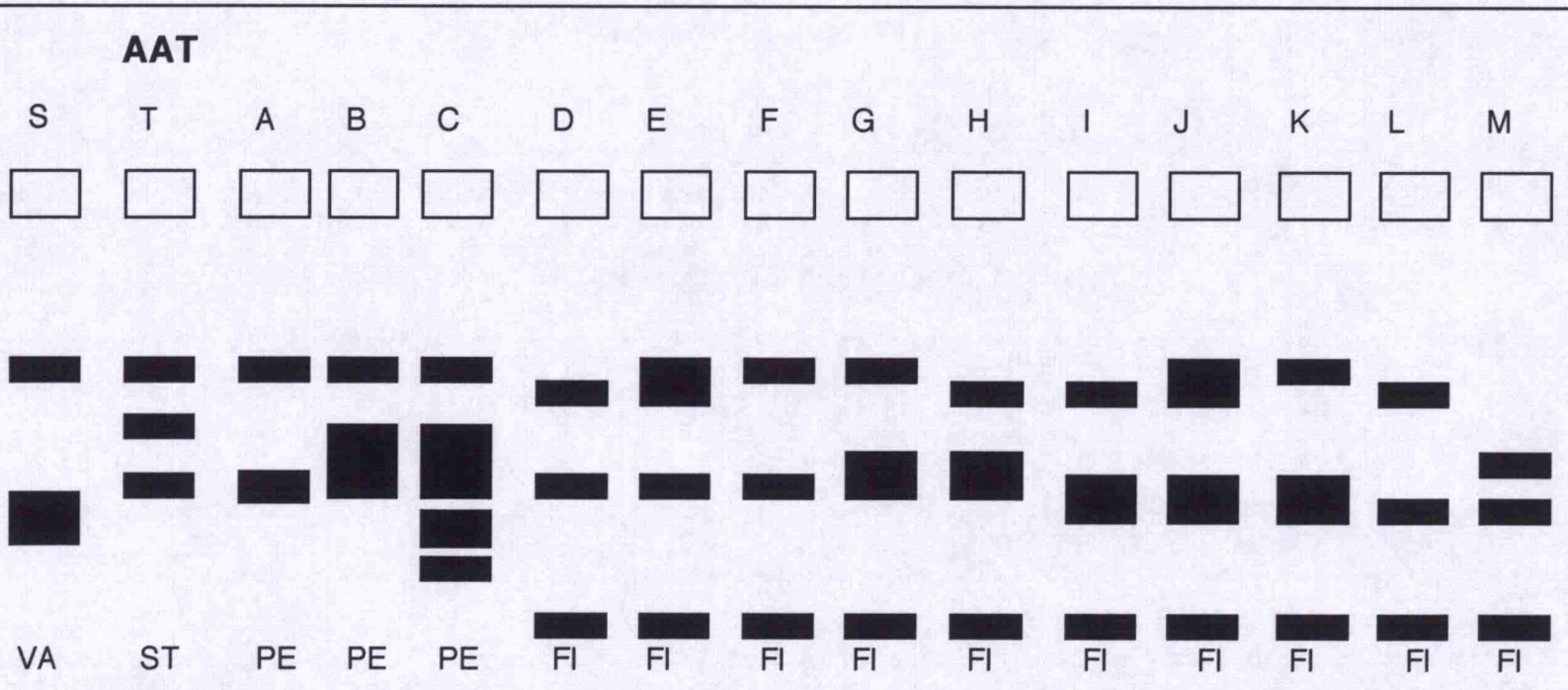
enzyme phenotype. It is calculated as: $D = 1 - \Sigma\{n_i(n_i-1)/N(N-1)\}$, where n_i is the number of individuals in the ith multi-enzyme phenotype and N is the total sample size. The evenness, E, with which multi-enzyme phenotypes are distributed within a population, was calculated using the following formula: E = (Dobs - Dmin) / (Dmax-Dmin) (Pielou 1969; Fager 1972), where Dobs is the observed value of D, and Dmax and Dmin are the theoretical maximum and minimum values obtainable given the sample size and the number of multi-enzyme phenotypes present in the population. E is thus a scaled measure ranging from 1 (complete evenness) to 0 (complete skewness, and can be used to compare populations that differ in the number of multi-enzyme phenotypes and individuals.

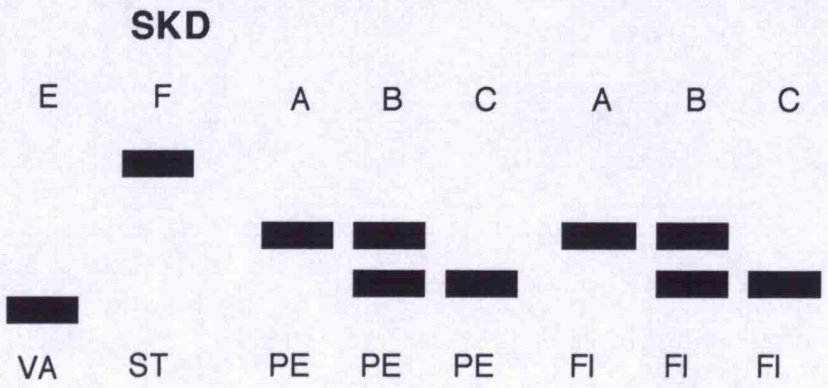
To test for similarities between populations, Euclidean distance measures (Sneath & Sokal 1973) were calculated from the frequencies of isozyme phenotypes and the resulting values were subjected to cluster analysis using UPGMA.

Fig. 13. Banding patterns of species of *Potamogeton* subgenus *Coleogeton*

VA=*P. vaginatus,* ST=*P. striatus,* PE=*P. pectinatus,* FI=*P. filiformis.* Letters above the banding patterns are codes given to the single enzyme phenotypes

Fig. 13


AAT
S T A B C D E F G H I J K L M
VA ST PE PE PE FI FI FI FI FI FI FI FI FI FI


SKD
E F A B C A B C
VA ST PE PE PE FI FI FI

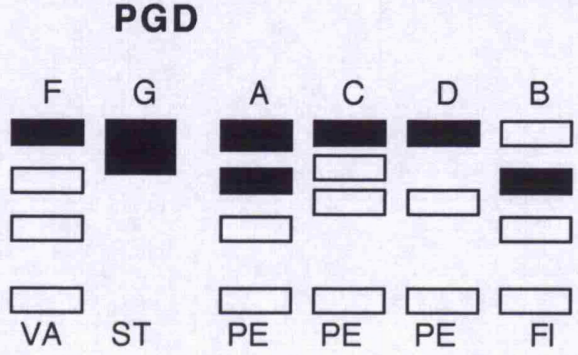

PGD
F G A C D B
VA ST PE PE PE FI

Fig. 13

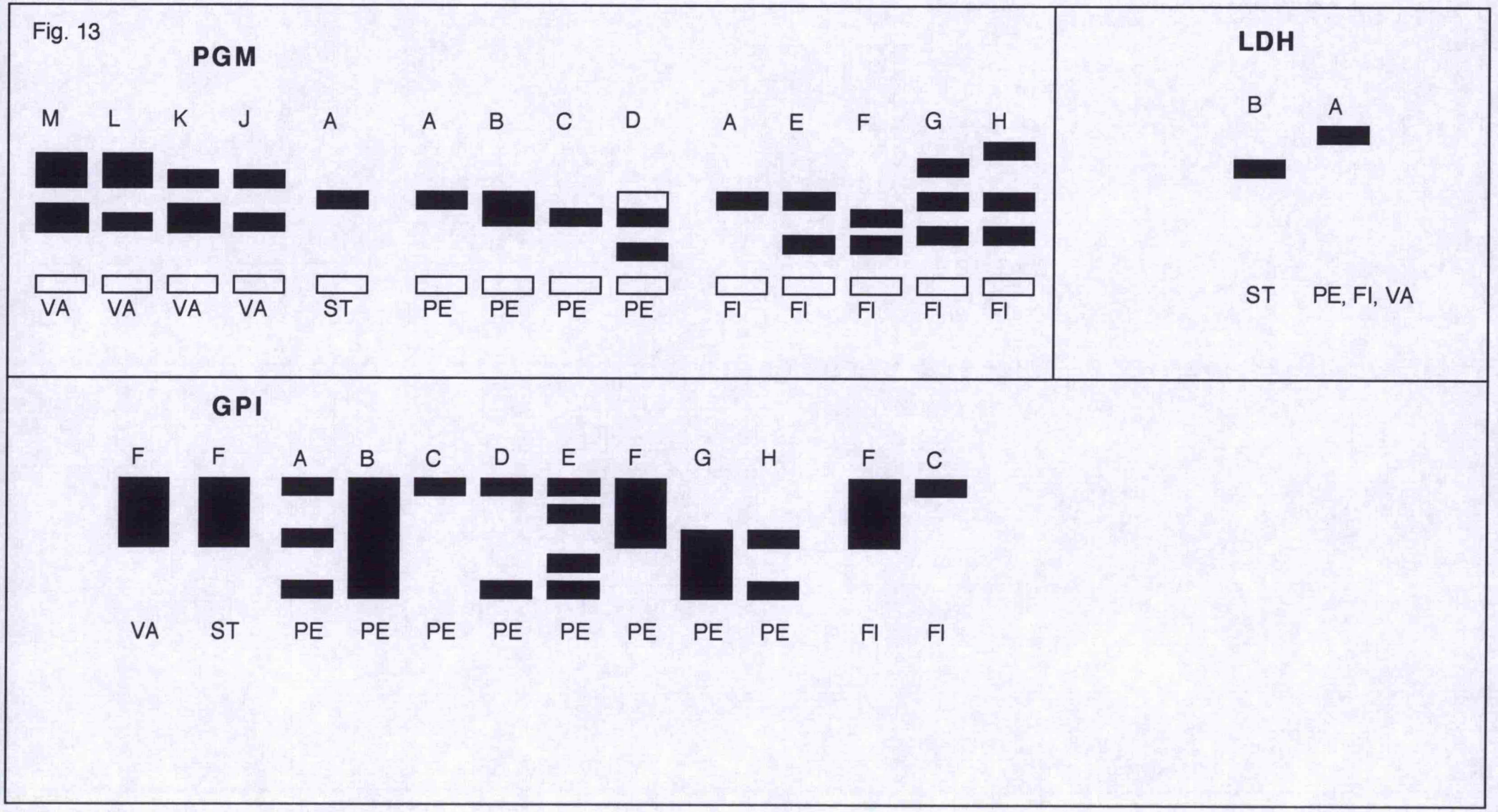

PGM
M L K J A A B C D A E F G H
VA VA VA VA ST PE PE PE PE FI FI FI FI FI
LDH
B A
ST PE, FI, VA
GPI
F F A B C D E F G H F C
VA ST PE PE PE PE PE PE PE PE FI FI

Fig. 13

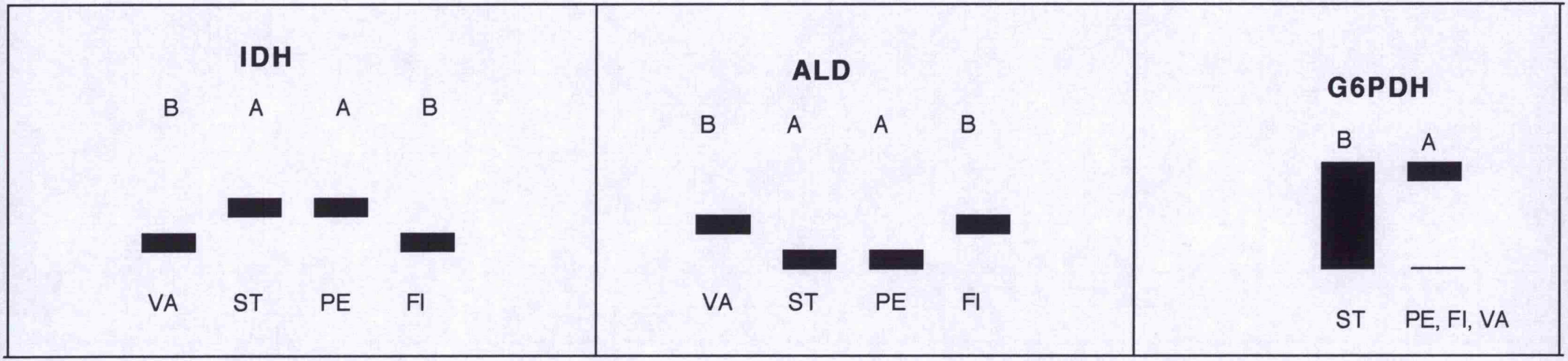


Fig.14 PGM variation in Swedish *P. filiformis.* From left to right, lanes 4 and 15 are PGM 'H' phenotype, all other lanes are PGM 'G' phenotype.

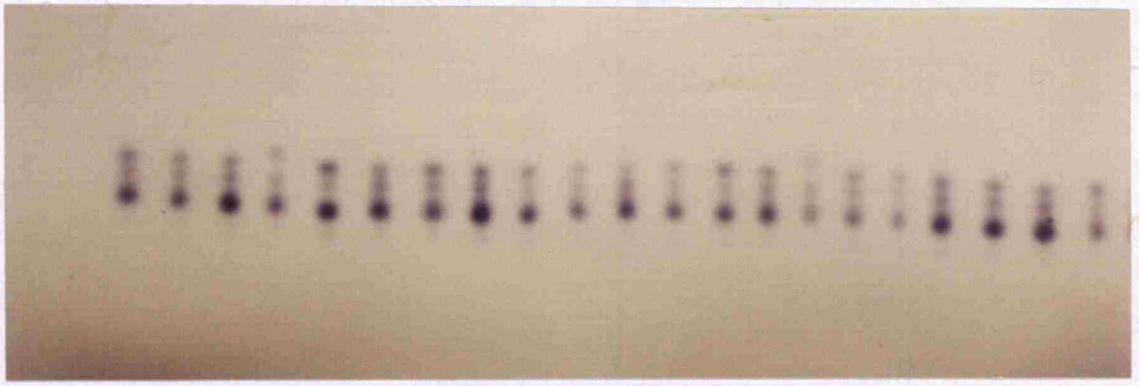
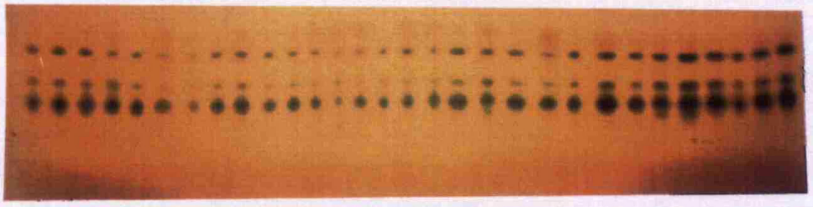


Fig.14 GOT 'A' phenotype for *P. pectinatus*



Variation between species

Markers that separate *P.pectinatus* from *P.filiformis* consistently in the material surveyed are represented by bands from the following enzymes: AAT, ALD and IDH (Fig.13). Allele dosage effects, as revealed by differential staining intensities, also separated *P.pectinatus* from *P.filiformis* with respect to their PGD phenotypes.

The Argentinian population of *P.striatus* was separable from all other populations by its G6PDH, LDH and PGD phenotypes (Fig.13), and that of *P.vaginatus* could be distinguished by its AAT, PGD, PGM and SKD phenotypes.

Variation within species

Summaries of the distribution of the different single-enzyme phenotypes within and between populations of *P.pectinatus* and *P.filiformis* are presented in Table 14. Comparative data for *P.striatus* and *P.vaginatus* are also provided.

In *P.pectinatus*, five enzyme systems (AAT, GPI, PGD, PGM and SKD) showed variation (Table 14). All non-British individuals differed from British individuals in at least one single-enzyme phenotype (Crete, freshwater river: GPI, PGD; Crete, brine springs: PGD; Sweden, Berga: GPI; Sweden, Dalarö: PGM, GPI), i.e. no multi-enzyme phenotype is shared between countries. All British and Swedish populations, however, shared a common PGD phenotype, whereas unique PGD patterns were detected in each of the two Cretan populations. Four enzymes (AAT, SKD, PGM and GPI) showed variation within and between British populations.

Table 14 SINGLE-ENZYME PHENOTYPES FOR *POTAMOGETON PECTINATUS*

Population	N	AAT	ALD	LDH	G6P	IDH	SKD	PGD	PGM	PGI	N	MEP
British Isles:												
River Stour	**17**											
Hamoon	10	A	A	A	A	A	A	A	B	E	10	P24
Mill Weir	4	A	A	A	A	A	A	A	C	A	4	P01
Marnhull	3	A	A	A	A	A	A	A	C	A	3	P01
River Loddon	**4**	A	A	A	A	A	A	A	A	F	4	P25
River Babingley	**14**	A	A	A	A	A	A	A	C	A	14	P01
Snettisham	**11**	A	A	A	A	A	A	A	A	B	10	P19
		A	A	A	A	A	A	A	A	C	1	P20
Newton Fen	**29**											
Main Drain	24	A	A	A	A	A	A	A	B	B	20	P12
		A	A	A	A	A	A	A	C	A	4	P01
		A	A	A	A	A	A	A	A	C	1	P20
N. of Bridge	2	A	A	A	A	A	A	A	A	B	2	P19
S. of Bridge	2	A	A	A	A	A	A	A	A	C	2	P20
River Torne	**30**	B	A	A	A	A	A	A	B	E	26	P22
		A	A	A	A	A	A	A	C	A	4	P01
Groby Pool	**37**	A	A	A	A	A	A	A	C	A	20	P01
		A	A	A	A	A	A	A	C	B	14	P02
		A	A	A	A	A	B	A	C	A	3	P03
Chesterfield Canal	**30**	A	A	A	A	A	A	A	C	A	28	P01
		C	A	A	A	A	A	A	C	A	2	P21
River Wharfe	**30**	A	A	A	A	A	A	A	A	E	27	P23
		A	A	A	A	A	A	A	C	A	3	P01
Bishops Dyke	**30**	A	A	A	A	A	B	A	C	A	30	P03
River Till (Ford)	**30**	A	A	A	A	A	A	A	C	A	6	P01
		A	A	A	A	A	B	A	C	A	24	P03
River Tweed	**3**	A	A	A	A	A	A	A	B	B	3	P12
Coldingham Loch	**10**	A	A	A	A	A	A	A	C	A	10	P01
Loch Fitty	**30**	A	A	A	A	A	B	A	C	A	9	P03
		A	A	A	A	A	C	A	C	A	21	P17
An Fhaodhail	**3**	A	A	A	A	A	A	A	B	B	2	P12
		A	A	A	A	A	A	A	C	A	1	P01
Loch Earblaig	**30**	A	A	A	A	A	A	A	C	D	30	P18

Table 14 SINGLE-ENZYME PHENOTYPES FOR *POTAMOGETON PECTINATUS*

Population	N	AAT	ALD	LDH	G6P	IDH	SKD	PGD	PGM	PGI	N	MEP
British Isles:												
Loch Bhasapol	**6**	A	A	A	A	A	A	A	C	A	6	P01
West Loch Ollay	**2**	A	A	A	A	A	B	A	B	A	1	P08
		A	A	A	A	A	C	A	B	A	1	P09
Loch a' Chinn Uacraigh	**15**	A	A	A	A	A	A	A	B	A	15	P04
Loch na Liana Móire	**3**	A	A	A	A	A	C	A	A	A	2	P10
		A	A	A	A	A	A	A	A	A	1	P11
Loch Fada	**8**	A	A	A	A	A	C	A	A	B	4	P05
		A	A	A	A	A	C	A	A	F	3	P06
		A	A	A	A	A	C	A	B	B	1	P07
Loch Grogary	**3**	A	A	A	A	A	A	A	C	A	2	P01
		A	A	A	A	A	A	A	B	A	1	P04
Crete:												
River	**24**	A	A	A	A	A	A	C	B	G	24	P13
Brine Spring	**30**	A	A	A	A	A	A	D	B	F	30	P14
Sweden:												
Berga Sundsvall	**10**	A	A	A	A	A	B	A	C	H	10	P15
Dalarö	**8**	A	A	A	A	A	B	A	D	H	8	P16

SINGLE-ENZYME PHENOTYPES FOR *POTAMOGETON STRIATUS*

Population	N	AAT	ALD	LDH	G6P	IDH	SKD	PGD	PGM	PGI	N	MEP
Argentina:												
CORFO System	**31**											
Colorado	20	T	A	B	B	A	F	G	A	F	20	P26
Irizo	10	T	A	B	B	A	F	G	A	F	10	P26
Pradere	1	T	A	B	B	A	F	G	A	F	1	P26

SINGLE-ENZYME PHENOTYPES FOR *POTAMOGETON FILIFORMIS*

Population	N	AAT	ALD	LDH	G6P	IDH	SKD	PGD	PGM	PGI	N	MEP
British Isles:												
Coldingham Loch	**30**	D	-	A	A	B	C	B	A	F	30	F01
Loch Fitty	**30**	D	B	A	A	B	C	B	A	F	20	F01
		L	B	A	A	B	C	B	A	F	6	F12
		L	B	A	A	B	B	B	A	F	4	F14
Loch Rhiagain	**5**	D	B	A	A	B	C	B	A	F	5	F01
An Fhaodhail	**28**	D	B	A	A	B	C	B	A	F	28	F01
Loch an Eilein	**31**	D	B	A	A	B	C	B	A	F	2	F01
		D	B	A	A	B	C	B	E	F	1	F02
		E	B	A	A	B	C	B	E	F	3	F03
		E	B	A	A	B	C	B	A	F	1	F04
		F	B	A	A	B	C	B	A	F	3	F05
		G	B	A	A	B	C	B	A	F	2	F06
		H	B	A	A	B	C	B	A	F	1	F07
		I	B	A	A	B	C	B	A	F	4	F08
		K	B	A	A	B	C	B	E	F	1	F09
		K	B	A	A	B	C	B	A	F	2	F10
		L	B	A	A	B	C	B	E	F	1	F11
		L	B	A	A	B	C	B	A	F	4	F12
		M	B	A	A	B	C	B	A	F	6	F13
Loch a' Phuill	**16**	I	B	A	A	B	C	B	A	F	5	F08
		M	B	A	A	B	C	B	E	F	1	F15
		L	B	A	A	B	C	B	A	F	3	F12
		D	B	A	A	B	C	B	A	F	6	F01
		L	B	A	A	B	C	B	E	F	1	F11
Loch Bhasapol	**7**	D	B	A	A	B	C	B	A	F	2	F01
		F	B	A	A	B	C	B	A	F	2	F05
		I	B	A	A	B	C	B	A	F	3	F08
Loch an Aigeil	**10**	D	B	A	A	B	C	B	A	F	4	F01
		D	B	A	A	B	C	B	E	F	6	F02
Loch Grogary	**30**	D	B	A	A	B	C	B	E	F	12	F02
		D	B	A	A	B	C	B	F	F	3	F17
		D	B	A	A	B	B	B	E	F	2	F18
		D	B	A	A	B	B	B	A	F	1	F19

Table 14 SINGLE-ENZYME PHENOTYPES FOR *POTAMOGETON FILIFORMIS*

Population	N	AAT	ALD	LDH	G6P	IDH	SKD	PGD	PGM	PGI	N	MEP
British Isles:												
Loch Grogary (cont.)		D	B	A	A	B	C	B	A	F	10	F01
		D	B	A	A	B	B	B	F	F	1	F20
		D	B	A	A	B	C	B	E	C	1	F21
West Loch Ollay	**24**	D	-	A	A	B	B	B	A	F	2	F19
		D	-	A	A	B	C	B	A	F	5	F01
		D	-	A	A	B	A	B	A	F	1	F22
		J	-	A	A	B	C	B	A	F	1	F23
		J	-	A	A	B	C	B	E	F	14	F24
		J	-	A	A	B	B	B	E	F	1	F25
Loch na Liana Móire	**26**	D	B	A	A	B	B	B	E	F	1	F18
		D	B	A	A	B	C	B	E	F	14	F02
		D	B	A	A	B	C	B	F	F	3	F17
		D	B	A	A	B	C	B	A	F	3	F01
		D	B	A	A	B	B	B	F	F	1	F20
		I	B	A	A	B	B	B	E	F	2	F27
		I	B	A	A	B	C	B	E	F	2	F28
Sweden:												
Lake Vättern	**29**	D	B	A	A	B	C	B	G	F	27	F29
		D	B	A	A	B	C	B	H	F	2	F30
Varpsund	**4**	D	B	A	A	B	C	B	G	F	4	F29

SINGLE-ENZYME PHENOTYPES FOR *POTAMOGETON VAGINATUS*

Population	N	AAT	ALD	LDH	G6P	IDH	SKD	PGD	PGM	PGI	N	MEP
Sweden:												
Bensbyn	**24**	S	B	A	A	B	E	F	J	F	12	V01
		S	B	A	A	B	E	F	K	F	8	V02
		S	B	A	A	B	E	F	L	F	1	V03
		S	B	A	A	B	E	F	M	F	3	V04

Letters refer to the banding phenotype codes given in Fig 13

Statistics summarising the frequency of multi-enzyme phenotypes in *P.pectinatus* are given in Fig. 15 and Table 15. Seven (26%) populations shared no multi-enzyme phenotype with any other population, but only three (11%) of these were British and of the latter only one (4%), Loch Earblaig, possesed a unique single-enzyme phenotype (GPI, phenotype D) rather than a unique combination. Chesterfield Canal and the River Torne were the only other British populations which, while sharing some of their muiti-enzyme phenotypes with other populations, had some individuals with a unique single-enzyme phenotype.

When populations with a sample size of fewer than ten plants are omitted from consideration, values for the diversity index, D, in *P.pectinatus* ranged from 0 (at eight sites) to 0.574 (at Groby Pool) and of evenness, E, from 0 (at nine sites) to 0.965 (River Stour) (Table 16).

In *P.filiformis*, four enzyme systems (AAT, SKD, PGM and GPI) were variable (Table 14). Of these, GPI variation was due to a unique phenotype in one individual at one site (Loch Grogary). The other polymorphic enzymes showed some intra- and inter-population variation.

Both of the Swedish populations had PGM phenotypes that were not found in any British population (Fig. 14). Of the British populations only Loch an Eilein, Loch Grogary, and West Loch Ollay had atleast some individuals with a unique single-enzyme phenotype that was not present in any other population.

Statistics relating to the frequency of muiti-enzyme phenotypes in *P.filiformis* are given in Fig. 15 and Table 15. All populations shared at least one muiti-enzyme phenotype with at least one other population. Values of the diversity index, D, ranged from 0 (Coldingham Loch and An Fhaodail) to 0.923 (Loch an Eilein) and those of evenness, E, from 0 (at two sites) to 0.937 (Loch an Aigeil) (Table 16).

In populations where ten or more plants were sampled, the mean number of multi-enzyme phenotypes per population, the mean diversity (D) and the mean evenness (E) within populations are significantly higher in *P.filiformis* than in *P.pectinatus* (Mann-Whitney U-test, $p < 0.05$, 0.05 and 0.1 respectively).

The single population of *P.vaginatus* that was studied yielded four multi-enzyme phenotypes (D=0.649, E=0.754), whereas the Argentinian population of *P.striatus* contained only one (D=0, E=0).

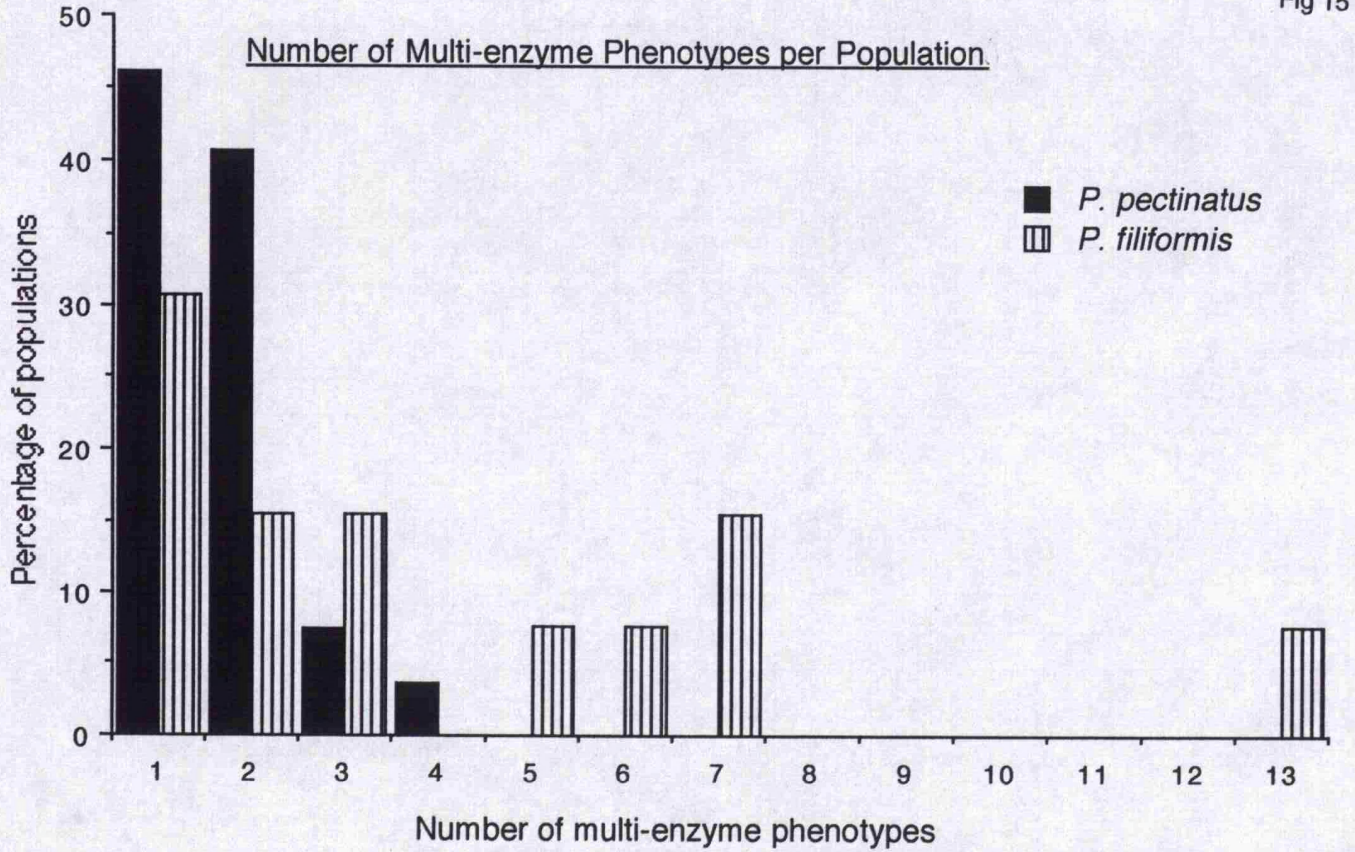
Table 15 : Statistics relating to the frequency of multi-enzyme phenotypes (MEPs) in *P.pectinatus* and *P.filiformis*.

	P.pectinatus	*P.filiformis*
No. MEPs/no. populations	25/26	28/13
Mean no. MEPs per population (SD)	1.7 (0.8) 1.7 (0.8)	4.0 (3.5) (all pops) 4.7 (3.7) (pops N>10)
No. MEPs represented by one individual	4 (all from Outer Hebrides)	8 (4 from Tiree, 4 from Outer Hebrides)
No. MEPs found in only one population	19 (76%)	17 (61%)
No. monomorphic populations	12 (48%)	4 (31%)
No. populations with commonest MEP	12 (48%)	11 (85%)

Table 16 : Simpson's Diversity Index (D) and Evenness (E) for populations of P.pectinatus and P.filiformis. G = no. multi-enzyme phenotypes, N = no. plants sampled.

Locality	*P.pectinatus*			*P.filiformis*		
	D	E	G/N	D	E	G/N
Britain:						
River Stour	0.515	0.965	2/17			
River Loddon	0.0	-	1/4			
Babingley River	0.0	-	1/14			
Snettisham (brackish pool)	0.182	0.000	2/11			
Newton Fen	0.507	0.534	4/29			
River Torne	0.239	0.382	2/30			
Groby Pool	0.574	0.809	3/37			
Chesterfield Canal	0.129	0.138	2/30			
River Wharfe	0.186	0.265	2/30			
Bishops Dyke (stream)	0.0	-	1/30			
River Till	0.331	0.587	2/30			
River Tweed	0.0	-	1/3			
Coldingham Loch	0.0	-	1/10	0.0	-	1/30
Loch Fitty	0.434	0.815	2/30	0.515	0.687	3/30
Loch Riaghain			-	1/5		
An Fhaodhail (stream)	0.667	1.000	2/3	0.0	-	1/28
Loch Earblaig	0.0	-	1/30			
Loch Bhasapol	0.0	-	1/6	0.762	1.000	3/7
Loch an Eilein				0.923	0.913	13/31
Loch a'Phuill				0.767	0.792	5/16
Loch an Aigeil				0.533	0.932	2/10
West Loch Ollay	1.000	1.000	2/2	0.630	0.510	6/24
Loch a'Chinn Uacraich	0.0	-	1/15			
Loch na Liana Moire	0.667	1.000	2/3	0.695	0.590	7/26
Loch Fada	0.679	0.751	3/8			
Loch Grogary	0.667	1.000	2/3	0.736	0.713	7/30
Sweden:						
Lake Vättern				0.133	0.143	2/29
Varpsund				0.0	-	1/4
Dalarö (Baltic coast)	0.0	-	1/8			
Berga (Baltic coast)	0.0	-	1/10			
Crete:						
Georgioupoli (freshwater)	0.0	-	1/24			
(brine spring)	0.0	-	1/30			
Mean value (SD) of populations with N>10	0.182	0.264		0.493	0.529	
	(0.213)	(0.347)		(0.333)	(0.358)	

Fig 15


Number of Multi-enzyme Phenotypes per Population
P. pectinatus
P. filiformis
Percentage of populations
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Number of multi-enzyme phenotypes

Cluster Analysis

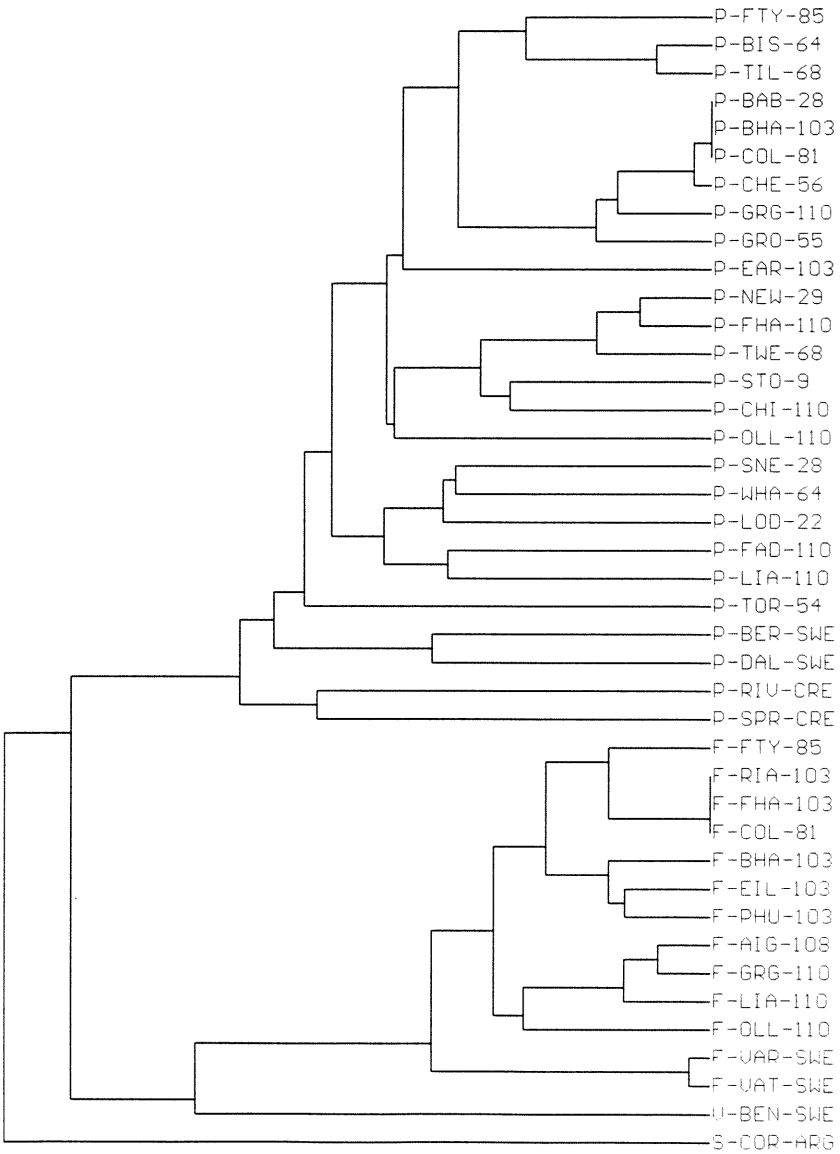
Cluster analysis of single-enzyme phenotypes showed an excellent correlation with taxonomy, inasmuch as populations of each species were more similar to each other than they were to those of a different species (Fig. 16). At the level of species, *P.filiformis* clustered with the single population of *P.vaginatus*, and this pair then grouped with *P.pectinatus*. Last to join the cluster was *P.striatus*.

Within *P.pectinatus*, cluster analysis of single-enzyme phenotypes grouped together populations from the same country (Fig. 16). Within Britain, however, the populations showed no clustering based on their geographical proximity to one another (Fig. 16).

Cluster analysis of the populations of *P.filiformis* showed that both the Swedish populations grouped together, as did all of the British populations. Within Britain, all of the populations from the Outer Hebrides clustered together, along with plants from Loch an Aigeil, and all of the populations from Tiree formed a group, together with those from south-eastern Scotland, viz. Loch Fitty and Coldingham Loch (Fig. 16).

Fig. 16 UPGMA phenogram based on single enzyme phenotypes

The first letter of the code relates to the species i.e. P= *P. pectinatus,* F= *P. filiformis,* V= *P. vaginatus,* S= *P. striatus.*
The group of three letters is a code for the popuiation, and thefollowing numbers are the vice county number for British populations. For non-British material a three letter country suffix is used. Thus P-FTY-85 is *P. pectinatus* from Loch Fitty (vice county 85).
Codes (from top to bottom) FTY= Loch Fitty, BIS= Bishops Dyke, TIL= River Till, BAB= Babingley River, BHA= Loch Bhasapol, COL=Coldingham Loch, CHE=Chesterfield Canal, GRG=Loch Grogary, EAR=Loch Earblaig, NEW=Newton Fen, FHA=An Fhaodhail, TWE=River Tweed, STO=River Stour, CHI=Loch a' Chinn Uacraich, OLL=West Loch Ollay, SNE, Snettisham, WHA=River Wharfe, LOD=River Loddon, FAD=Loch Fada, LIA=Loch na Liana Moire, TOR=River Torne, BER=Berga, DAL=Dalarö, RIV=Crete River, SPR=Crete Spring, RIA=Loch Riaghain, EIL=Loch an Eilein, PHU=Loch a' Phuill, AIG=Loch an Aigeil, VAR=Varpsund, VAT=Vättern, BEN=Bensbyn, COR=CORFO


P-FTY-85
P-BIS-64
P-TIL-68
P-BAB-28
P-BHA-103
P-COL-81
P-CHE-56
P-GRG-110
P-GRO-55
P-EAR-103
P-NEW-29
P-FHA-110
P-TWE-68
P-STO-9
P-CHI-110
P-OLL-110
P-SNE-28
P-WHA-64
P-LOD-22
P-FAD-110
P-LIA-110
P-TOR-54
P-BER-SWE
P-DAL-SWE
P-RIV-CRE
P-SPR-CRE
F-FTY-85
F-RIA-103
F-FHA-103
F-COL-81
F-BHA-103
F-EIL-103
F-PHU-103
F-AIG-108
F-GRG-110
F-LIA-110
F-OLL-110
F-VAR-SWE
F-VAT-SWE
U-BEN-SWE
S-COR-ARG

DISCUSSION

Variation between species

The isozyme data demonstrate that *P.pectinatus* and *P.filiformis* can be separated by three species-specific markers (AAT, ALD, IDH). This finding is important in the context of subsequent analyses of putative hybrid populations (Hollingsworth *et al.* 1995e - chapter 7).

Little comment may be made about the taxonomic relationships of *P.vaginatus* since only one population was examined. It is interesting, however, that cluster analysis showed it to be most similar to *P.filiformis*, a position supported by data on fruit and pollen morphology (Sorsa 1988) but at variance with the view of Hagström (1916) who believed the species to be closer to *P.pectinatus*.

Similarly I can say little about the single population of *P.striatus*, except that in the cluster analysis it was separated from all other populations of *P.pectinatus*, a species of which it was originally considered to be a variety (Hagström 1916). In the absence of material of *P.pectinatus* from South America, however, it is not clear to what extent the differences should be interpreted in geographic rather than taxonomic terms. On the one hand, the Argentinian material has the same IDH and FBA phenotypes that are characteristic of all European *P.pectinatus* plants analysed, lending support to a close taxonomic relationship. On the other hand, however, the Argentinian material shows a unique LDH phenotype, which differs from all three European species of subgenus *Coleogeton*, as well as a unique G6PDH pattern. This G6PDH phenotype is the only variation I have detected from the standard 'A' phenotype, which is monomorphic and uniform in *P.pectinatus, P.filiformis* and their hybrid, *P.x suecicus* (Hollingsworth *et al.* 1995e - chapter 7), as well as in *P.coloratus* (Hollingsworth *et al.* 1995a - chapter 3), *P.nodosus, P.natans, P.lucens* and their hybrids *P.x schreberi* and *P.x fluitans* (Hollingsworth *et al.* 1995c - chapter 5).

Partitioning of variation within & among populations

Of the factors influencing the amount and pattern of variability within and

among populations, the breeding system is the most important (Hamrick & Godt 1989). *P.pectinatus* and *P.filiformis* are self-compatible and pollinated at the water-surface (epihydrophily), and as such might be expected to apportion variation between rather than within populations. Indeed, some studies of hydrophilous angiosperms have revealed extremely low levels of variability within populations, and have led to the inference that fixation rates are high and levels of sexual recombination low in such species (Les 1988, 1991). Few cases have been adequately studied, however. Laushman (1993) reviewed four such, i.e. *Vallisneria americana, Ceratophyllum demersum, C.echinatum* and *Zostera marina*, and concluded that the percentage of polymorphic loci in hydrophilous taxa over all populations is equal to or higher than the average for all plants; at the population level, however, he concluded that hydrophilous species have a reduced frequency of polymorphic loci, fewer alleles per locus and lower levels of heterozygosity than species with other poliination syndromes. Furthermore, apart from *Zostera*, all show a much greater partitioning of variability between populations than non-hydrophiles. He agreed with Les (1988) that this genetic profile could be attributed to the isolation of populations, clonal spread and limited sexuality (infrequent flowering). The extent to which this model applies to the two hydrophilous species of *Potamogeton* studied here is assessed below.

Isolation of populations

Populations may be isolated ecologically as well as geographically. Thus, in their analysis of inter-population variation in *P.pectinatus*, Hettiarachchi & Triest (1991) reported a fundamental division between those populations from freshwater sites and those from brackish water. This dichotomy is not evident, however, in our data, where the brackish water populations of Snettisham (North Sea), Sweden (Baltic Sea) and Crete (brine spring) did not group together in the cluster analysis. Instead I have found strong evidence implicating geography, rather than ecology, as an important isolating mechanism, promoting inter-population variability. Gene flow between populations of freshwater aquatic plants is likely to be restricted by the discontinuous nature of their habitat in an otherwise terrestrial landscape (i.e. considering lakes or rivers as islands). Cluster analysis of the

populations of *P.filiformis* revealed that geographically close populations tended to group together. There was, however, no statistical correlation between overall similarity (as measured by Euclidean distance based on single enzyme phenotypes) and geographical distance within the British Isles (Spearman rank correlation n.s.). This is due to the populations from the Scottish mainland clustering together with the Hebridean groups. The reasons for the similarity of the eastern Scottish populations to those from Tiree, and the similarity of Loch an Aigeil to the populations of the Outer Hebrides, are not immediately obvious. All British populations do, however, share a single detectable multi-enzyme phenotype with every other British population, which suggests that perhaps historically, the alleles involved may have become widespread, with subsequent divergence (by mutation?) accounting for the local genotypes in each region. Thus I feel that the separate clustering of the Inner and the Outer Hebridean populations should be considered meaningful and therefore suggest that the data for *P.filiformis* are consistent with the stepping-stone model of population structure (under which increasing geographical distance is expected to lead to increasing genetical isolation), rather than the island model (in which dispersal to one population is as likely as to any other) (Hartl & Clark 1989). The dendrogram produced using multi-enzyme phenotypes (not shown), rather than single-enzyme phenotypes, did not show any geographically meaningful groups, leading to the conclusion that gene flow is by means of sexually-derived propagules (seeds or pollen) rather than by vegetative diaspores such as turions or fragments of stem or rhizome.

A similar analysis of the single-enzyme phenotypes for populations of *P.pectinatus*, gave rather different results. Although clustering occurred at an international level, no pattern between geographical distance and similarity of enzyme phenotypes was evident at all within Britain. There are at least three possible reasons for this:

1) dispersal of alleles to one population is as likely as to any other (island model of genetic structure);

2) dispersal is at a very low level such that the populations are virtually isolated from one another;

3) dispersal occurs mainly by seed, but seedling recruitment is very low, so

again the populations are isolated from one another.

I have no additional evidence to offer in support of the first explanation. I doubt the second because the fruits of *P. pectinatus* are a favourite food of waterfowl (Preston 1995a), can survive passage through the digestive system (Smits *et al.* 1989) and may therefore be dispersed at high frequency. Field observations suggest that the third explanation, which supposes that seedling recruitment is a limiting factor on population variability, is at least as likely, and probably more so, as any other. Thus, although fruits may be produced in quantity by large populations of *P. pectinatus* in standing waters, seedling recruitment in established populations appears to be rare. In exposed waters all the fruits are sometimes blown away to the margin, where conditions are unsuitable for growth of this species, and even in more sheltered sites where there is a persistent seed bank, seedlings cannot be found or are only seen very rarely (van Wijk 1983). As with *P. filiformis,* the dendrogram produced using multi-enzyme phenotypes (not shown), did not show any geographically based groupings

On an international scale, all individuals of *P. pectinatus* and *P. filiformis* sampled from outside Britain, have at least one single enzyme phenotype which distinguishes them from all of the British material examined. This international divergence of populations is clearly shown in Fig. 16. From this data, there is little evidence of direct gene flow between countries, although further sampling of non-British populations would be required to confirm this. It cannot, however, be ruled out that the *P. filiformis* genotypes characteristic of the different Hebridean Islands have arrived from (as yet unsampled) non-British populations via wildfowl migratory routes, as opposed to originating *in situ* by recombination and mutation.

Clonal spread

There is evidence to suggest that one of the most important factors determining the partitioning of variability between and within the popuiations of *Potamogeton* studied here is the extent of clonal growth, with this more prevalent in *P.pectinatus* than in *P.filiformis*. 46% of *P.pectinatus*

populations were monomorphic, with 1-4 (mean 1.7) multi-enzyme phenotypes per population and a mean D of 0.18 (Tables 15 & 16). In *P.filiformis* 31% of the populations were monomorphic, with 1-13 (mean 4.0) multi-enzyme phenotypes per population, and a mean D of 0.49 (Tables 15 & 16). These results indicate that variabiiity tends to be partitioned between rather than within populations, especially so in *P.pectinatus*. The data are closely matched by those of Les (1991), who studied nine American populations of the hydrophilous *Ceratophyllum demersum* and found that 56% of them were monomorphic, with 1-8 (mean 2.7) multi-locus genotypes per population; Simpson's D was calculated as 0.45. A GST value for this species was calcuiated as 0.580, indicating that much of the variability was held between rather than within populations.

The only relevant previous study of *P.pectinatus* was by Hettiarachchi & Triest (1991) who, in a world-wide study of leaf isozymes (different from those examined by us), found that 57 or 63% of *P.pectinatus* populations were monomorphic, a value reasonably close to that of our own of 46%.

According to Ellstrand & Roose (1987) the mean value of Simpson's diversity index, D, for multiclonal terrestrial species is 0.62 (range 0.1-1.0). This value is only a little higher than in *P.pectinatus* (0.48) and almost the same as in *P.filiformis* (0.63). This leads to the conclusion that these aquatic clonal species may be no different on average from their terrestrial counterparts - particuarly as the value calculated by Ellstrand & Roose (1987) is based on purely multiclonal populations, which makes it an artificially high estimate.

The foregoing discussion has assumed that the multi-enzyme phenotypes in our study are equivalent to single genotypes. Evidence that this may be so, at least in some cases, is indicated by data on the populations of *P.pectinatus* in the Rivers Wharfe and Torne. In the River Wharfe I collected 27 plants with the PGM 'A' and the GPI 'E' phenotypes, and three plants with the PGM 'C' and the GPI 'A' phenotypes. The River Torne sample consisted of 26 plants with the AAT 'B', the PGM 'B' and the GPI 'E' phenotypes, and a further four plants had the AAT 'A', the PGM 'C' and the GPI 'A' patterns. In

both rivers variation in the different enzymes is complementary, with apparently two sets of patterns present in different individuals in each case. Ruling out genetic linkage, which is unlikely as other populations show apparent random assortment between these enzymes, this strongly suggests the co-existence of two clones in each population.

Further support for the existence of extensive clonal growth comes from cases where putatively heterozygous genotypes exist to the exclusion of the putative homozygotes (which are found in other populations). There are good examples of this phenomenon in *P.pectinatus* at Bishops Dyke (where all individuals have a heterozygote phenotype for SKD), the River Stour at Hamoon, Loch a'Chinn Uacraigh and in Crete (where purely heterozygotes were detected at the most anodally migrating putative PGM locus).

In terms of overall levels of variability, there is little evidence in either *P.pectinatus* or *P.filiformis* of the lack of genetic variation seen in British populations of the wind-pollinated *P.coloratus*, which could perhaps be attributed to founder effects and drift following post-glacial recolonisation (Hollingsworth *et al.* 1995a - chapter 3). Indeed the population of *P.filiformis* from Loch an Eilein on Tiree, well north of the limit of the Devensian glaciation, showed as much variation as was found in populations of the aquatic, but wind-pollinated, *Myriophyllum alterniflorum* from the same general area, many of whose populations were found to be in Hardy-Weinberg equilibrium (Harris *et al.* 1992).

Sexual reproduction

Further insight into the levels and patterns of variability in *P.pectinatus* and *P.filiformis* comes from a direct comparison of the two. The average level of variability within populations of *P.filiformis* is significantly higher than it is in *P.pectinatus*, both in terms of the numbers of multi-enzyme phenotypes per population and of values of D and E (Tables 15 & 16). This seems somewhat suprising considering that *P.pectinatus* is the more widespread and more phenotypically variable species.

One explanation is that water-flow could influence the level of genetic

variability by affecting the frequency of flowering and hence of sexual reproduction. All populations of *P.filiformis* that were sampled were growing in still water where flowering is generally abundant, whereas several populations of *P.pectinatus* came from flowing water, where the frequency of flowering and therefore of sexual reproduction is reduced. However, no difference in the mean diversity index was found between populations of *P.pectinatus* from flowing and still-water sites (for sample sizes of $N > 10$, flowing mean $D = 0.3556$, still-water mean $D = 0.3298$; Mann-Whitney U-test, n.s.). This finding leads me to suspect that seedling recruitment rather than the frequency of sexual reproduction itself is an important factor in controlling the observed levels of variability. Two other observations lead to the same conclusion. Firstly, *P.filiformis* is often common in the shallow water at the edge of lochs. These marginal areas are subject to periodical drying out, and this could result in conditions where reproduction from desiccation-resistant propagules such as fruits would be favoured over the less tolerant vegetative propagules. In contrast, *P.pectinatus* grows at a range of water depths, but it is not well adapted to shallow water at the edge of lakes where the water level fluctuates and plants may be subject to desiccation (van Wijk 1988). Secondly, *P.filiformis* often grows as discrete clumps surrounded by bare areas of sand with much unshaded substrate available for seedling establishement. In contrast, *P.pectinatus* often forms denser stands covering much of the water surface thereby increasing competition for both light and nutrients, both of which might impede seedling recruitment.

CHAPTER 7

ISOZYME EVIDENCE FOR THE MULTIPLE ORIGIN OF *POTAMOGETON* X *SUECICUS* (POTAMOGETONACEAE)

ABSTRACT

Isozyme evidence is presented to support the hypothesis that plants morphologically intermediate between *Potamogeton pectinatus* and *P. filiformis* are of hybrid origin between these two species. Variation in enzyme banding patterns suggests that the hybrid has arisen on at least eleven occasions.

The identity of plants from the Rivers Tweed and Till that were previously identified as *P.* x *suecicus* is questioned, and an alternative opinion, that they are *P.* x *bottnicus* (*P. pectinatus* x *P. vaginatus*) is suggested.

INTRODUCTION

Potamogeton pectinatus L. is the most widespread of all *Potamogeton* species and occurs in all continents of the world. It grows throughout the British Isles where it can be found in eutrophic or brackish water in a wide range of lowland habitats. *P.filiformis* Pers. in contrast, is primarily a circumboreal species (of more northerly latitudes). In the British Isles it is distributed mainly in Scotland. In England it occurs at only one site, Rayburn Lake, in S.Northumberland (v.c.67), and in Wales it grew on Anglesey (v.c.52) up until the 19th century but has not been seen there since (Preston 1990); in Ireland the species has been recorded chiefly from the north and west. *P.filiformis* grows in lowland lakes, usually in shallow, eutrophic or calcareous water, often near the sea, and is a characteristic species of machair lochs.

P.pectinatus can be distinguished from *P.filiformis* mainly by characters of the stipular sheath and the fruit. In *P.pectinatus* the margins of the stipular sheath are not united, the sheath remaining open, and the fruits are longer than 3mm and have a distinct style on the ventral side; *P.filiformis*, in contrast, has stipular sheaths in which the margins are united to form a tube, and the fruits are shorter than 3mm and have a sessile stigma at the apex

(Preston 1990).

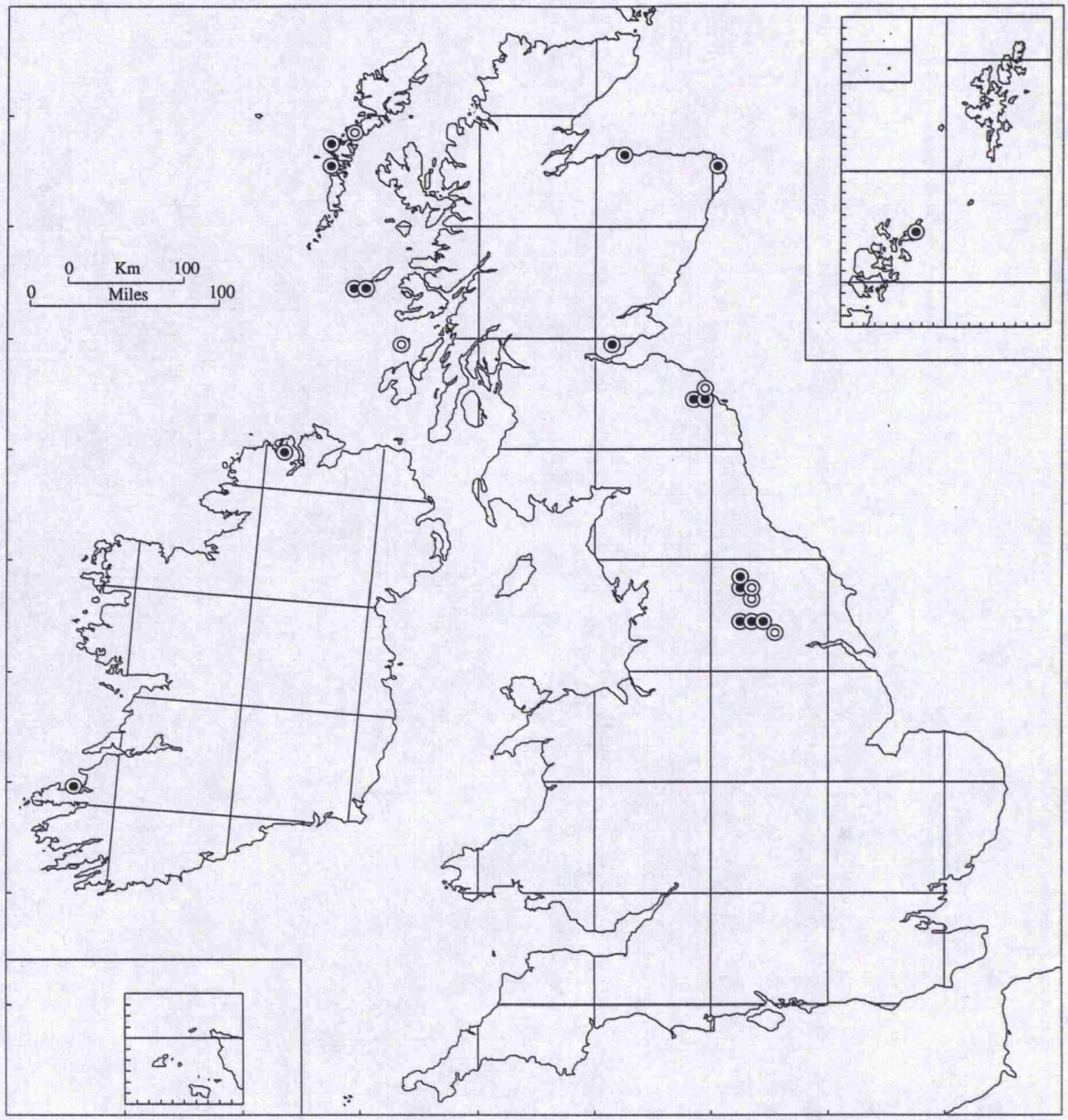
The two species are alleged to hybridise in the British Isles, and putative hybrids (*P.* x *suecicus* Richt.) have been recorded from localities in Scotland, where the parental ranges overlap, as well as from four sites in England where they do not: the Rivers Tweed and Till in Northumberland, and the Rivers Wharfe and Ure in Yorkshire (Dandy & Taylor 1946; Dandy 1975) (Fig.17). Evidence for the hybrid status of such plants comes from the intermediate nature of the stipular sheaths and the apparently complete sterility of the pollen and ovules (Dandy & Taylor 1946; Bance 1946). English populations, however, resemble *P.filiformis* in vegetative anatomy and carpel morphology (Bance 1946), whereas Scottish and Irish populations have a carpel like that of *P.pectinatus* (Preston & Stewart 1994).

The occurrence of the putative hybrid in the English localities poses some problems regarding the distribution of the parents. In the Rivers Till and Tweed, although *P.pectinatus* can be found, there are no records of *P.filiformis*, the nearest locality for that species being Coldingham Loch (Berwickshire, v.c.81), about 18km to the north. This separation clearly will reduce the chances of hybridisation, although the barrier may not be insuperable. The other two English localities, however, present a much greater degree of isolation. In the Rivers Wharfe and Ure not only is *P.pectinatus* extremeiy uncommon, but also *P.filiformis* has never been recorded, the nearest site being Rayburn Lake, some 150km to the north.

Dandy & Taylor (1946) offer two alternative explanations for the mismatch between the distribution of the putative hybrid and that of its parents. Both explanations are historical and are not mutually exclusive. The first supposes that *P.* x *suecicus* in England is a relic from per-glacial or inter-glacial times, and was formed *in situ* on at least two or three occasions, as *P.pectinatus* and *P.filiformis* came into contact during migration in response to changing climatic conditions. The exigencies of climate were presumed now to have restricted *P.filiformis* to its northern range and the vigour of the hybrid to have largely ousted *P.pectinatus* from the Rivers Ure and Wharfe.

This scenario derives some support from fossil evidence which shows that *P.filiformis* was much more widespread in Britain in the last (Weichselian) glacial period than it is today (Godwin 1975).

Fig. 17 Distribution map of *P.* x *suecicus* in the British Isles



Closed circles are post-1970 records, open circles are pre-1970 records

In the second explanation, *P.* x *suecicus* was presumed to have arisen in the north and become displaced southwards by the advancing ice sheets to England, where it now remains as a relic. Under this hypothesis the hybrid need have originated only once and could thus represent a single clone. Once formed, moreover, its distribution would be independent of its parents, being mediated solely through vegetative spread.

The purpose of this study is to use isozymes to examine plants described as *P.* x *suecicus* and compare the results with a thorough study of isozyme variation in the putative parental taxa and closely related species (Hollingsworth *et al.* 1995d - chapter 6). This investigation focuses on whether *P.* x *suecicus* actually is a hybrid and if so whether it has arisen more than once. The extent of clonal growth is also assessed.

MATERIALS AND METHODS

Materials

Plant material was collected from the localities described in Table 17 and returned to the laboratory for analysis. Identification of the plants was based on microscopic examination of leaf sheath structure and, where available, flower structure. Representative voucher specimens of each population have been placed in LTR. 275 plants from eleven populations of *P.* x *suecicus* were sampled from a representative range of sites in Britain. A further 30 plants were collected from the River Till and two plants from the River Tweed from populations that were considered by Dandy and Taylor (1946) and Dandy (1975) to be *P.* x *suecicus*. However upon microscopic examination, all individuals had open and convolute leaf sheaths. This contrasts with all of the other individuals of *P.* x *suecicus* I have examined, (which have at least some tubular sheaths), and raised a question as to the identity of these plants. These 'River Till' type plants are thus treated separately from *P.* x *suecicus* in this paper.

Methods

Standard horizontal starch gel electrophoresis was carried out on crude protein extracts of fresh green leaf tissue on all samples using the following nine anodally migrating enzyme systems AAT, ALD, G6PDH, IDH, LDH,

SKD, PGD, GPI and PGM. Electrophoretic procedures and data analysis are as described by Hollingsworth *et al.* (1995d - chapter 6)

Table 17.

Sampling Locations of putative *P.* x *suecicus*

Location	Vice county	Grid. Ref.
River Wharfe:Linton	Mid-west York vc64	SE/3.4
Netherby	Mid-west York vc64	SE/3.4
Harewood	Mid-west York vc64	SE/3.4
River Ure, West Tansfield,	North-west York vc65	SE/2.7
Loch Fitty	Fife vc85	NT/1.9
River Lossie: Calcots	Elgin vc95	NJ/2.6
Arthurs Bridge	Elgin vc95	NJ/2.6
An Fhaodhail, Tiree	Mid Ebudes vc103	NM/0.4
Loch a' Phuill, Tiree	Mid Ebudes vc103	NL/9.4
West Loch Ollay, South Uist	Outer Hebrides vc110	NF/7.3
Loch a' Chinn Uacraich, Benbecula	Outer Hebrides vc110	NF/7.5
Loch na Liana Móire, Benbecula	Outer Hebrides vc110	NF/7.5
Loch Grogary, North Uist	Outer Hebrides vc110	NF/7.7
River 'Till' type plants		
River Till, Twizel Bridge,	Cheviotland vc68	NT/8.4
River Tweed, Union Bridge	Cheviotland vc68	NT/9.5

The banding patterns obtained were compared to those obtained from 447 individuals of *P. pectinatus* from 26 populations; 270 individuals of *P. filiformis* from 13 populations; and 24 individuals of *P. vaginatus* Turcz. from a single population (Hollingsworth *et al.* 1995d - chapter 6).

RESULTS

Isozyme banding patterns in two enzyme systems (G6PDH and LDH) were identical over all individuals surveyed of *P.* x *suecicus* ; these were the same patterns as were discovered in all individuals of *P. pectinatus, P.filiformis* and *P. vaginatus* (Hollingsworth *et al.* 1995d - chapter 6). More informative enzyme systems were AAT, ALD[1] , IDH, PGD, GPI, PGM and SKD. The electrophoretic phenotypes for these polymorphic enzymes (Fig. 18) and their distribution among populations (Table 18) are described in turn below.

[1] Faint bands for ALD for the River Lossie populations meant that they were not scorable for this population, and because of this, coupled with their uniformity in all other Individuals, they were excluded from any of the formal analysis.

Fig. 18

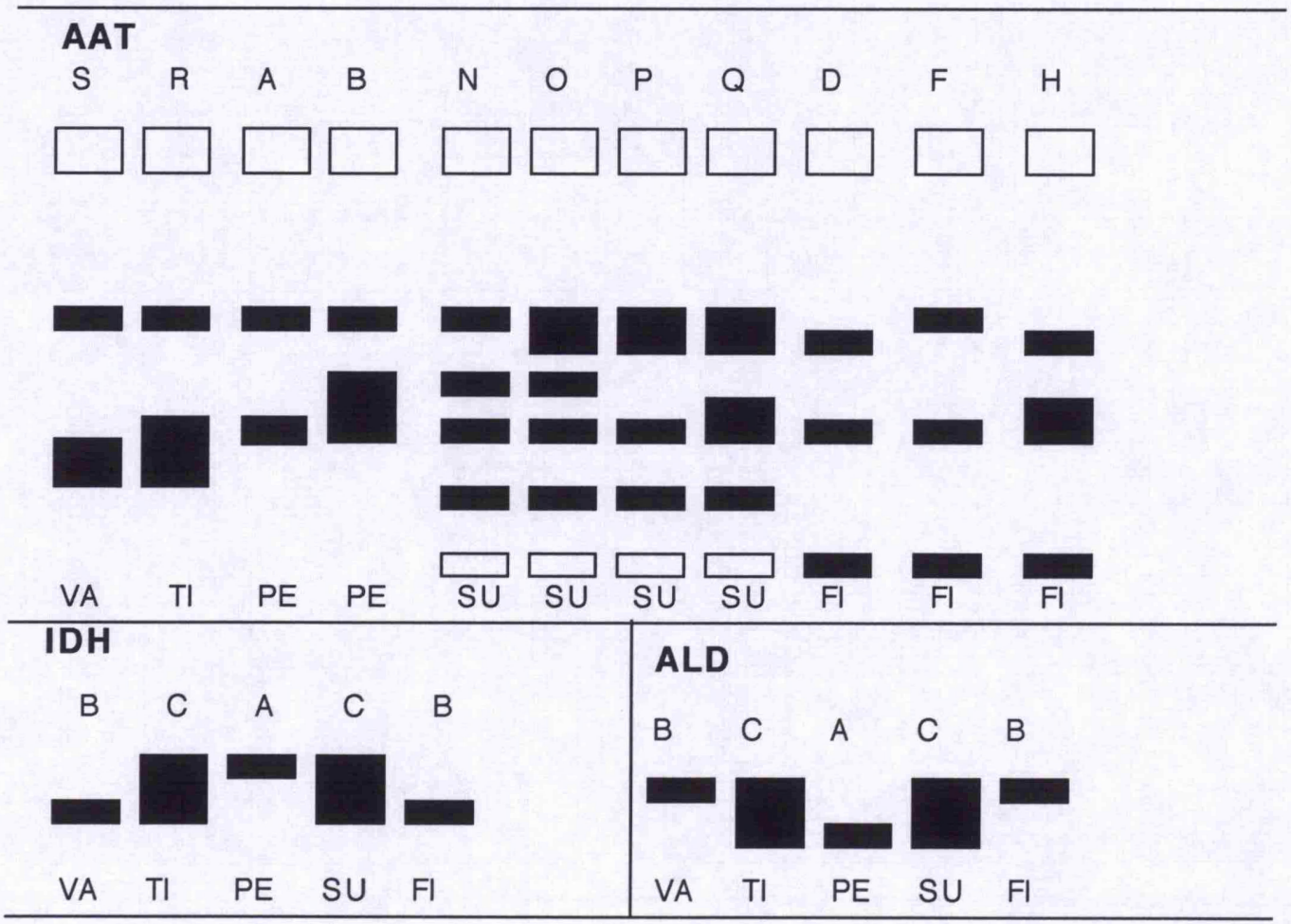


Fig.18 Isozyme phenotypes.
VA= *P. vaginatus,* TI= River Till type plants, PE=*P . pectinatus,* SU= *P.* x *suecicus,* FI= *P. filiformis.* Letters above the banding patterns are the codes given to the different single-enzyme phenotypes

Fig. 18

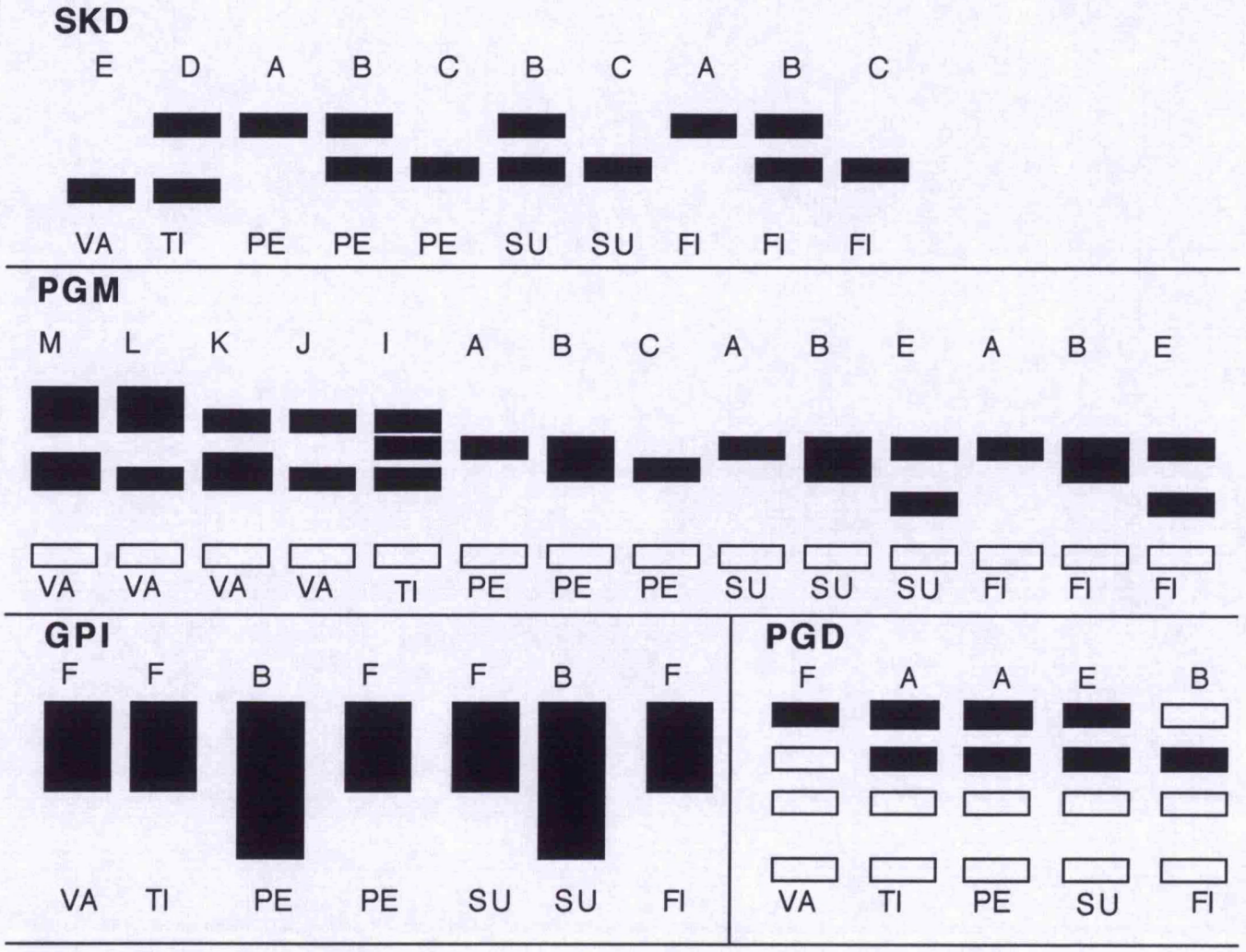


Fig. 18 Isozyme phenotypes.
VA= *P. vaginatus*, TI= River Till type plants, PE=*P . pectinatus*, SU= *P.* x *suecicus*,
FI= *P. filiformis.* Letters above the banding patterns are the codes given to the different single-enzyme phenotypes

Fig.19 Additive inheritance of IDH banding patterns in *P.* x *suecicus*

From left to right, lanes 1 and 2 = *P. pectinatus* 'A' phenotype, lanes 3, 4 and 5 = *P.* x *suecicus* 'C' phenotype, lanes 5 and 6 = *P. filiformis* 'B' phenotype. The faint band beneath the main band for *P. pectinatus* and *P. filiformis* was highly variable in its expression and was considered to be a non-genetic ghost band.

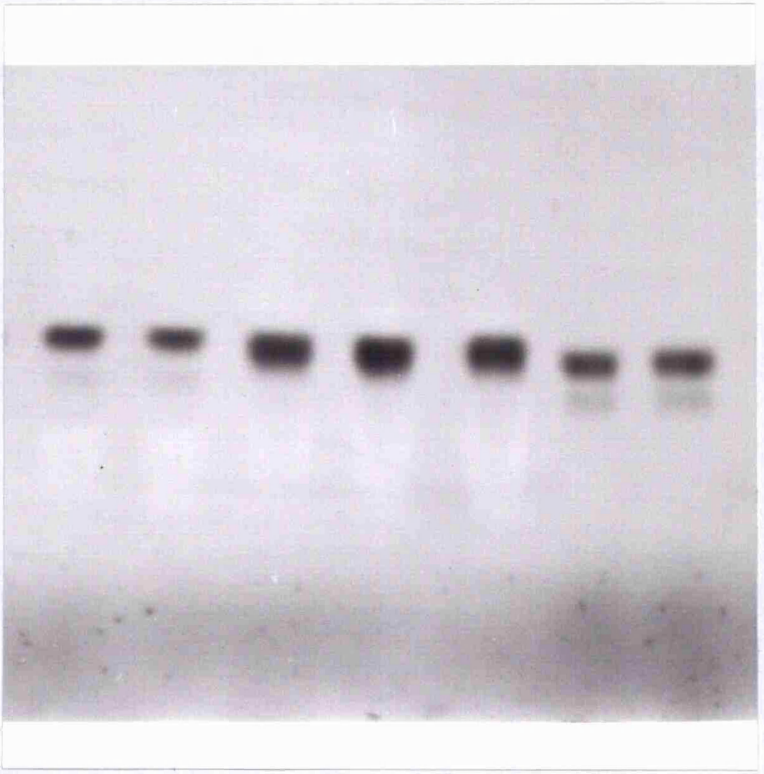


Fig.19 Additive inheritance of SKD banding patterns in *P.* x *suecicus* from Tiree

From left to right, lane 1 = *P. pectinatus* 'A' phenotype, lanes 2 and 3 = *P.* x *suecicus* 'B' phenotype, lanes 5, 6, 7 and 8 = *P. filiformis* 'C' phenotype.

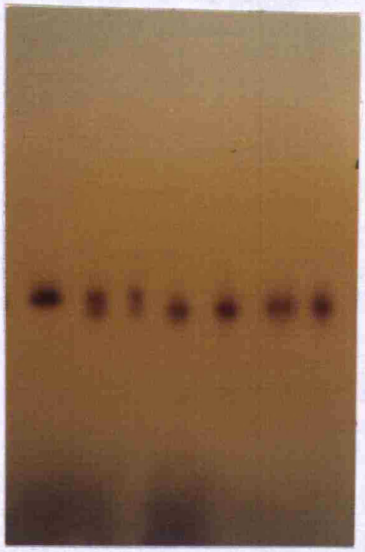


Table 18. SINGLE-ENZYME PHENOTYPES FOR *POTAMOGETON* X *SUECICUS*

Population	N	AAT	ALD	LDH	G6P	IDH	SKD	PGD	PGM	PGI	Freq	MEP
British Isles:												
River Wharfe	**44**											
Harewood	17	N	C	A	A	C	B	E	E	F	17	S01
Netherby	7	N	C	A	A	C	B	E	E	F	7	S01
Linton	20	N	C	A	A	C	B	E	E	F	20	S01
River Ure	**8**	O	C	A	A	C	B	E	E	F	8	S02
Loch Fitty	**37**	P	C	A	A	C	C	E	B	B	25	S03
		P	C	A	A	C	B	E	B	B	12	S04
An Fhaodhail	**66**	Q	C	A	A	C	B	E	A	F	9	S06
		Q	C	A	A	C	B	E	B	F	1	S07
		Q	C	A	A	C	B	E	B	B	3	S08
		P	C	A	A	C	B	E	B	B	25	S04
		P	C	A	A	C	B	E	B	F	15	S09
		P	C	A	A	C	B	E	A	F	13	S05
Loch a' Phuill	**33**	P	C	A	A	C	B	E	A	F	20	S05
		P	C	A	A	C	B	E	B	B	13	S04
Loch a' Chinn Uacraigh	**15**	P	C	A	A	C	B	E	A	B	15	S10
Loch na Liana Móire	**34**	P	C	A	A	C	B	E	E	I	34	S11
Loch Grogary	**2**	P	C	A	A	C	B	E	A	I	2	S05
Loch Fada	**3**	P	C	A	A	C	B	E	E	F	2	S11
		P	C	A	A	C	B	E	A	F	1	S05
West Loch Ollay	**3**	P	C	A	A	C	B	E	B	B	2	S04
		P	C	A	A	C	B	E	B	I	1	S09
River Lossie	**30**											
Calcots	25	Q	-	A	A	C	B	E	A	F	25	S06
Arthurs Bridge	5	Q	-	A	A	C	B	E	A	F	5	S06

SINGLE-ENZYME PHENOTYPES FOR *POTAMOGETON* 'TILL'

Population	N	AAT	ALD	LDH	G6P	IDH	SKD	PGD	PGM	PGI	N	MEP
British Isles:												
River Till, Twizel Bridge	**30**	R	C	A	A	C	D	A	I	F	30	T01
River Tweed, Union Bridge	**2**	R	C	A	A	C	D	A	I	F	2	T01

Letters refer to the banding phenotype codes given in Fig. 18

Genetic variation within taxa

P. x *suecicus.* Variation was detected in the same four enzymes (44%: AAT, SKD, PGM and GPI) that were variable in British populations of the putative parental species (Hollingsworth *et al.* 1995d - chapter 6). Six (55%) populations were monomorphic. A total of eleven different multi-enzyme phenotypes were detected from eleven populations, with the mean number of multi-enzyme phenotypes per population being 1.6 (range 1-6). Six (55%) of the multi-enzyme phenotypes were restricted to one population, although only three (23%) of these were due to unique single enzyme phenotypes as opposed to unique combinations. Conversely the most widespread multi-enzyme phenotypes were present in four (37%) of the populations. Only one multi-enzyme phenotype was found in only one individual. For populations where N>10, the mean value of Simpson's D=0.243 and mean evenness E=0.383 (Table 19)

Table 19 : Simpson's Diversity Index (D) and Evenness (E) for populations of *P.* x *suecicus* . G = no. multi-enzyme phenotypes, N = no. plants sampled.

Locality	D	E	G/N
River Wharfe	0	0	1/44
River Ure	0	0	1/8
Loch Fitty	0.45	0.86	2/37
An Fhaodhail	0.76	0.87	6/66
Loch a' Phuill	0.49	0.95	2/33
Loch a' Chinn Uacraigh	0	0	1/15
Loch na Liana Móire	0	0	1/34
Loch Grogary	0	0	1/2
Loch Fada	0.67	1	2/3
West Loch Ollay	0.67	1	2/3
River Lossie	0	0	1/30
Mean (SD) of populations with N=>10	0.243 (0.304)	0.383 (0.431)	

The high levels of variation found at An Fhaodhail, where six multi-enzyme phenotypes were identified, is not an artifact of its higher sample size. Five of the six multi-enzyme phenotypes were detected in an initial sample of 30 plants. A further 36 collected on a second visit revealed only one more multi-enzyme phenotype.

River Till and Tweed plants. No variation was detected between any of the individuals from these sites, with only a single multi-enzyme phenotype noted.

Is *P.* x *suecicus* actually a hybrid and if so how often has it arisen?
IDH and ALD phenotypes: one putative isozyme locus was seen for each enzyme. In *P.filiformis* and *P.pectinatus* the phenotypes consisted of a single band, the electrophoretic mobility of which was different in the two species. Putative hybrids exhibited an apparently additive pattern (Figs. 18 & 19). The inference is that *P.pectinatus* and *P.filiformis* were monomorphic and homozygous for different alleles at these enzyme loci, with the putative hybrid behaving as a heterozygote with associated heteromeric products in each case - the resulting pattern being an unresolvable block of activity occurring in the zone between the parental alleles.

PGD phenotypes: two putative isozyme loci were seen, with one being monomorphic and uniform in all three taxa. The other (most anodally migrating locus) showed the following subtle variation in British material. *P.filiformis* showed a three-banded pattern, with the centre band darker than the two outer bands. A similar three-banded pattern was observed in *P.pectinatus*, except that the upper band was much darker than the other two. The putative hybrids exhibit the same three-banded pattern but with the relative intensities of the three bands intermediate between those of *P.filiformis* and *P.pectinatus*, consistent with additive inheritance (Fig. 18).

AAT phenotypes: AAT was the only one of the variable enzymes which had patterns that were always diagnostic for a given species. Although some bands were variable, the slowest *P. filiformis* band (Fig. 18) was present in

all individuals of that species, and never present in any individuals of *P. pectinatus*. The putative hybrid always had a band that corresponded to this slow *P. filiformis* band (albeit with a lesser staining intensity), as well as a band that corresponded to the slowest band in the *P. pectinatus* 'A' genotype, (this band being present in all *P. pectinatus* individuals sampled). Using a four locus model, variation in *P.* x *suecicus* for AAT appears to be explainable in terms of inheritance of the above mentioned invariant bands, along with different numbers of the variant bands as well as associated heteromeric products. This suggestion works well for the most common *P.* x *suecicus* patterns ('P' and 'Q'), if the slowest band in the *P. pectinatus* 'A' pattern is considered to be the product of two co-migrating loci. However for the *P.* x *suecicus* patterns found in the Yorkshire rivers (patterns 'N' and 'O') the situation is less clear. If the four locus model that is evident for patterns 'P' and 'Q' is applied, then the fourth slowest band for patterns 'N' and 'O' could have been inherited from a plant similar to that of the *P. pectinatus* 'B' genotype. As no heteromer is present in the *P.* x *suecicus* plants, one must assume that an allele with the same mobility has been inherited from *P. filiformis.* Although a band with this mobility has not been recovered in *P. filiformis,* the putative locus in question is variable in this species, and this anomaly may merely represent a sampling artifact.

SKD phenotypes: both putative parents appeared to show the same diallelic variation at a single locus, although the fast moving allele was more common in *P. pectinatus* and the slow moving allele more common in *P. filiformis*. The putative hybrid typically displayed a two banded pattern consistent with inheritance of the fast band from *P. pectinatus* and the slow band from *P. filiformis* (Fig. 19). Interestingly the only site where some individuals of the hybrid showed only a single band (corresponding to the slow moving band) was at Loch Fitty where both alleles were present in both *P. pectinatus* and *P. filiformis.*

PGM and GPI phenotypes: both showed variation with some banding patterns that were shared between *P. pectinatus* and *P. filiformis* (GPI 'F', GPI 'C'; PGM 'A', PGM 'B', PGM 'C') and some banding patterns that were not shared between these species (*P. pectinatus* : GPI 'A', GPI 'B', GPI 'D',

GPI 'E'; P. filiformis: PGM 'E', PGM 'F'.). Banding patterns for both of these enzyme systems are not readily interpretable in terms of loci and alleles. The putative hybrid did, however, show banding patterns that are consistent with crosses between various combinations of the above mentioned phenotypes.

From the limited data on *P. vaginatus* (Hollingsworth *et al.* 1995d - chapter 6), isozyme evidence adds support to morphological evidence (Dandy & Taylor 1946) eliminating this species from the parentage of the plants identified in this chapter as *P.* x *suecicus*. Although *P. vaginatus* and *P. filiformis* possess identical IDH and ALD phenotypes (which eliminates a *P. vaginatus* x *P. filiformis* cross (see Fig. 18)), they are clearly distinguishable by their AAT, PGM and SKD phenotypes. *P. vaginatus* lacks the slow moving *P. filiformis* AAT band that is present in all *P.* x *suecicus* individuals. Furthermore all *P. vaginatus* individuals have an SKD band that is not found in either *P. filiformis* or *P.* x *suecicus*. For PGM none of the bands that was detected in *P. vaginatus* was detected in *P.* x *suecicus*.

The above evidence is strongly consistent with the putative hybrid plants actually being *P. pectinatus* x *P. filiformis*. From the variation detected in *P.* x *suecicus*, which is explainable on the basis of crosses between different parental genotypes, there is evidence that the hybrid has arisen on at least eleven occasions.

The identity of the plants from the Rivers Till and Tweed, however, is not so clear. These plants have a single multi-enzyme phenotype with unique AAT, SKD and PGM patterns. They display the heterozygous IDH and ALD profiles which are consistent with their being a hybrid (*P. pectinatus*, *P. filiformis* and *P. vaginatus* all show patterns that are homozygous for these enzymes), with the data suggesting a cross between *P. pectinatus* and either *P. filiformis* or *P. vaginatus*. The 'Till' type plants consistently differ from *P.* x *suecicus* in three enzyme systems, AAT, SKD and PGM, and these unique phenotypes are entirely consistent with those that would be predicted in a *P. pectinatus* x *P. vaginatus* hybrid

DISCUSSION

Status of putative hybrids between *P. pectinatus* and *P. filiformis*

There can be little doubt that the plants with a morphology intermediate between *P.pectinatus* and *P.filiformis* are in fact hybrids between these species. The evidence of additive inheritance in seven enzyme systems (AAT, ALD, IDH, PGD, GPI, PGM and SKD), coupled with the morphological and anatomical evidence of Dandy & Taylor (1946) and Bance (1946) is virtually conclusive, and as such these plants are correctly identified as *P.* x *suecicus* Richt. The only case where a pattern occurs that is not directly explainable on the basis of inheritance from observed parental genotypes is for AAT in two populations of *P.* x *suecicus*. This apparent discrepancy relates to the fact that a band in *P.* x *suecicus* has not been found (where expected) in *P. filiformis*. The *P.* x *suecicus* plants possessing this band are from the Yorkshire rivers, and the *P. pectinatus* plants with this band are from N. Lincoln, relatively close by. If the *P.* x *suecicus* plants originated in this area a long time ago, it may well be that the *P. filiformis* genotypes that were involved in the hybridisation event have died out - they have certainly not survived nearby. In Scotland, where the putative parents and the hybrid occur together, the variation in *P.* x *suecicus* in these populations is always explicable in terms of the variation in the local putative parental genotypes. Furthermore, considering the additive inheritance of species specific markers in all *P.* x *suecicus* individuals, coupled with the fact that the anomalous band is from the most variable AAT locus, I feel that this does not in any way challenge the evidence for the hybrid status of *P.* x *suecicus*.

Frequency of hybridisation

According to reports (Dandy & Taylor 1946; Bance 1946) and my own observations, *P.* x *suecicus* is completely sterile and thus unable to recombine its genotype. The finding of eleven genotypes in the populations studied therefore indicates that the hybrid has arisen on at least eleven occasions. Observations of flowering behaviour show that the flowering periods of the two species overlap, and if they grow together in the same water body the inflorescences may often occur in close proximity (inflorescences virtually touching each other have been observed at Loch Fitty). Under these circumstances hybridisation is presumably a far from rare

event - although establishment of hybrid seeds must face similar problems to the seeds of the parental species as described by Hollingsworth *et al.* (1995d - chapter 6).

I support a multiple origin theory to explain the variation in the hybrid, rather than the occurrence of rare sexual events as was suggested for the largely sterile but variable triploid grass,*Puccinellia* x *phryganodes* (Jefferies & Gottlieb 1983). This is because variation in *P.* x *suecicus* is mirrored by variation in the parental species. Furthermore, despite searching, no trace of an embryo or an intact pollen grain has ever been found in a *P.* x *suecicus* inflorescence in a British population (Dandy & Taylor 1946, Bance 1946, Hollingsworth, Gornall and Preston, 1994 unpublished observations). The pollen of *P. pectinatus* and *P. filiformis* are typically rounded to oval, whereas those of *P.* x *suecicus* are more irregular in shape - with all of the grains having a crumpled and collapsed appearance (Fig. 20). Three hundred pollen grains from each of three individuals of *P. pectinatus,* two individuals of *P. filiformis,* and two individuals of *P.* x *suecicus* were examined to compare relative pollen viability. Using a rather crude estimate of viability based on the regularity of the pollen grain shape, the values in Table 20 were obtained, with no apparently viable *P.* x *suecicus* pollen found.

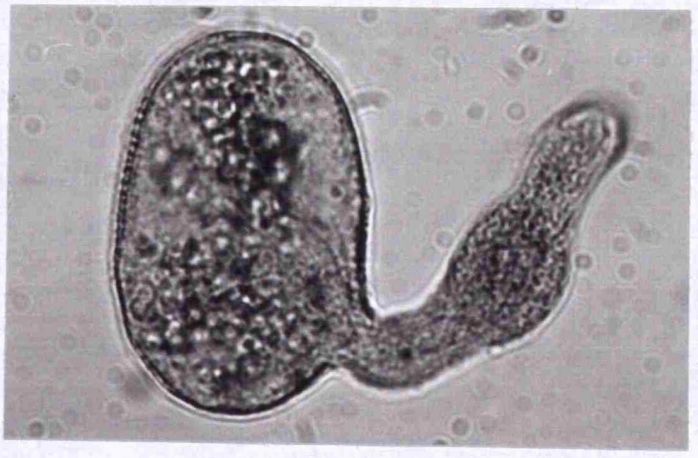
Table 20. Pollen viability estimates for *P. filiformis, P. pectinatus* and *P.* x *suecicus* .

		Pollen grain shape	
Species	Location	Regular	Irregular
P. pectinatus	Loch Fitty	285	15
		289	11
		293	7
P. filiformis	Loch Fitty	291	9
		267	33
P. x *suecicus*	River Lossie	0	300
		0	300

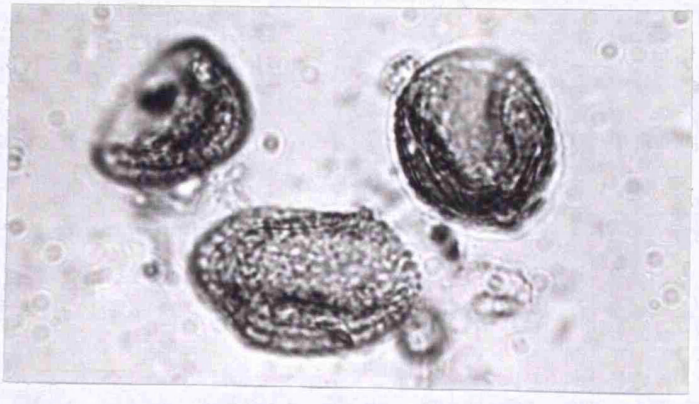
FIG. 20

Pollen grains of *Potamogeton pectinatus, P. filiformis,* and *P.* x *suecicus* (stained with safranin in glycerol)

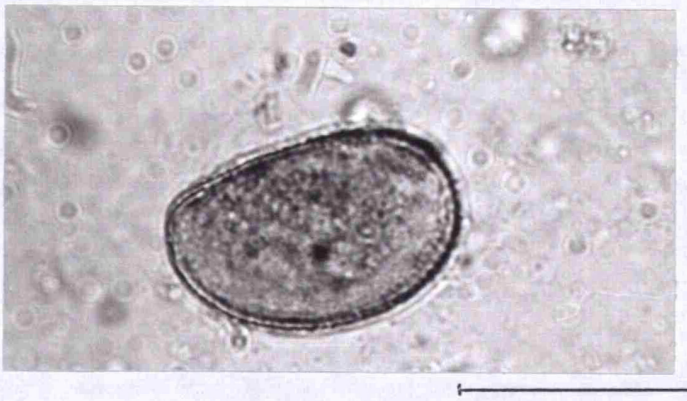
P. filiformis



P. x *suecicus*



P. pectinatus



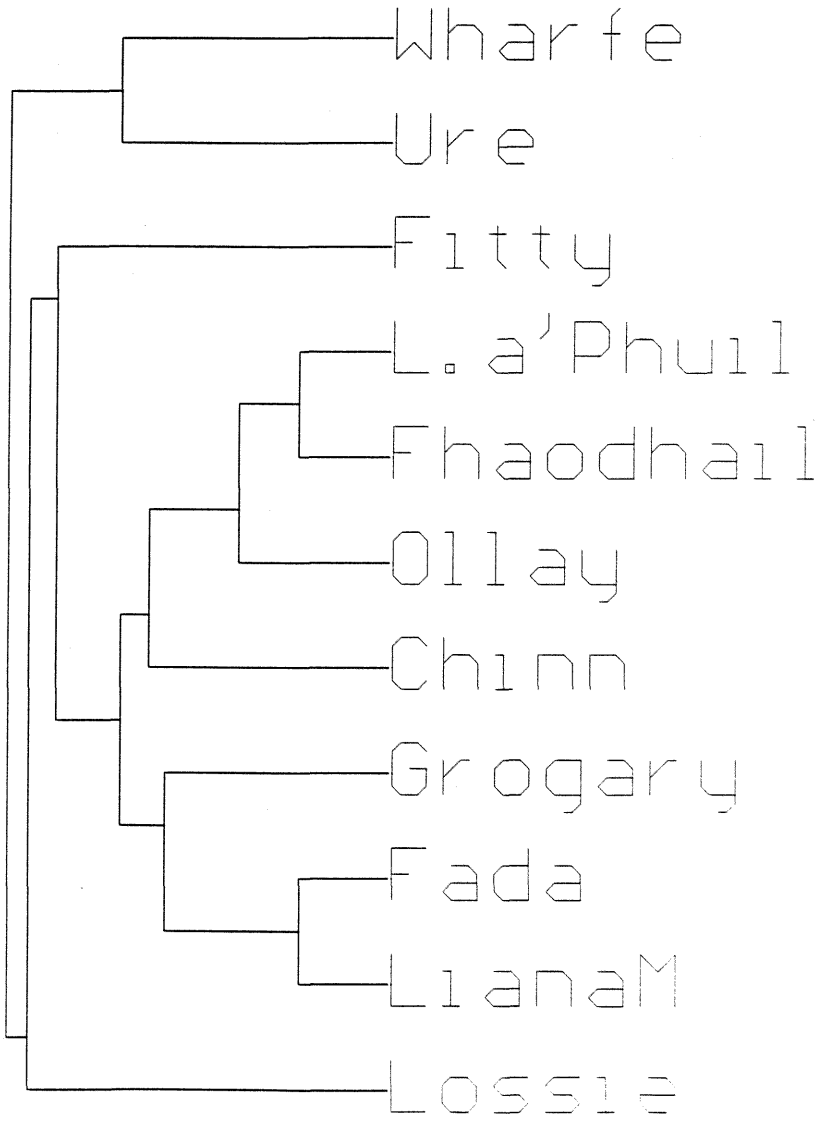
=30μm

This data suggests that multiple origin accounts for the genetic variation in *P.* x *suecicus* rather than recombination. The estimate of eleven separate origins of the hybrid represents a minimum. Where a multi-enzyme phenotype is shared between two geographically separated populations , I cannot distinguish between multiple origin from the same parental multi-enzyme phenotypes at different sites, and one multi-enzyme phenotype being dispersed to other sites as vegetative material by a vector such as birds. At sites where all three taxa grow together, or are found within close proximity, any variation in *P.* x *suecicus* is usually explicable on the basis of the variation in the local parental genotypes, which implies a polytopic origin. The presence of broadly geographically based clustering for the single-enzyme phenotypes (Fig. 21) but its absence in the multi-enzyme phenotypes (not shown) is informative. Presumably the correlation for the single-enzyme phenogram is due to the multiple origins of the hybrid in an area reflecting the local parental gene pool. The fact that nearby sites do not all contain the same clones of the hybrid implies that vegetative dispersal is not common and that the same multi-enzyme phenotypes detected in widely separated populations have arisen *in situ* from coincidentally similar parental genotypes.

At sites where one or both parents are not found, one could suggest that the parent(s) have been ousted by vigorous growth of the hybrid, or that they have died out due to other processes such as habitat change. At An Fhaodhail, a narrow river (<10m wide) on Tiree, the main channel is almost completely covered by *P.* x *suecicus*, with *P. filiformis* only growing in still back waters and side pools. *P. pectinatus* appeared to be represented by only a very few individuals sporadically scattered in among the *P.* x *suecicus* stands and looking in considerable danger of being smothered. At sites where habitat heterogeneity is sufficient such as at Loch Fitty, all three taxa occur on slightly different substrates, and appear to be co-existing, with *P.filiformis* usually growing on gravel, *P.pectinatus* on mud, and *P.* x *suecicus* on muddy gravel. Another possible (although perhaps less likely explanation) is that the hybrid could have arrived as seed, transported by wildfowl. It is worth noting that seeds of hybrid origin are presumably as

capable of being transported by birds as non-hybrid seed and as such, range extension of the hybrid to non-parental sites cannot be excluded.

Fig. 21 Single-enzyme phenogram (UPGMA) for *P.* x *suecicus*



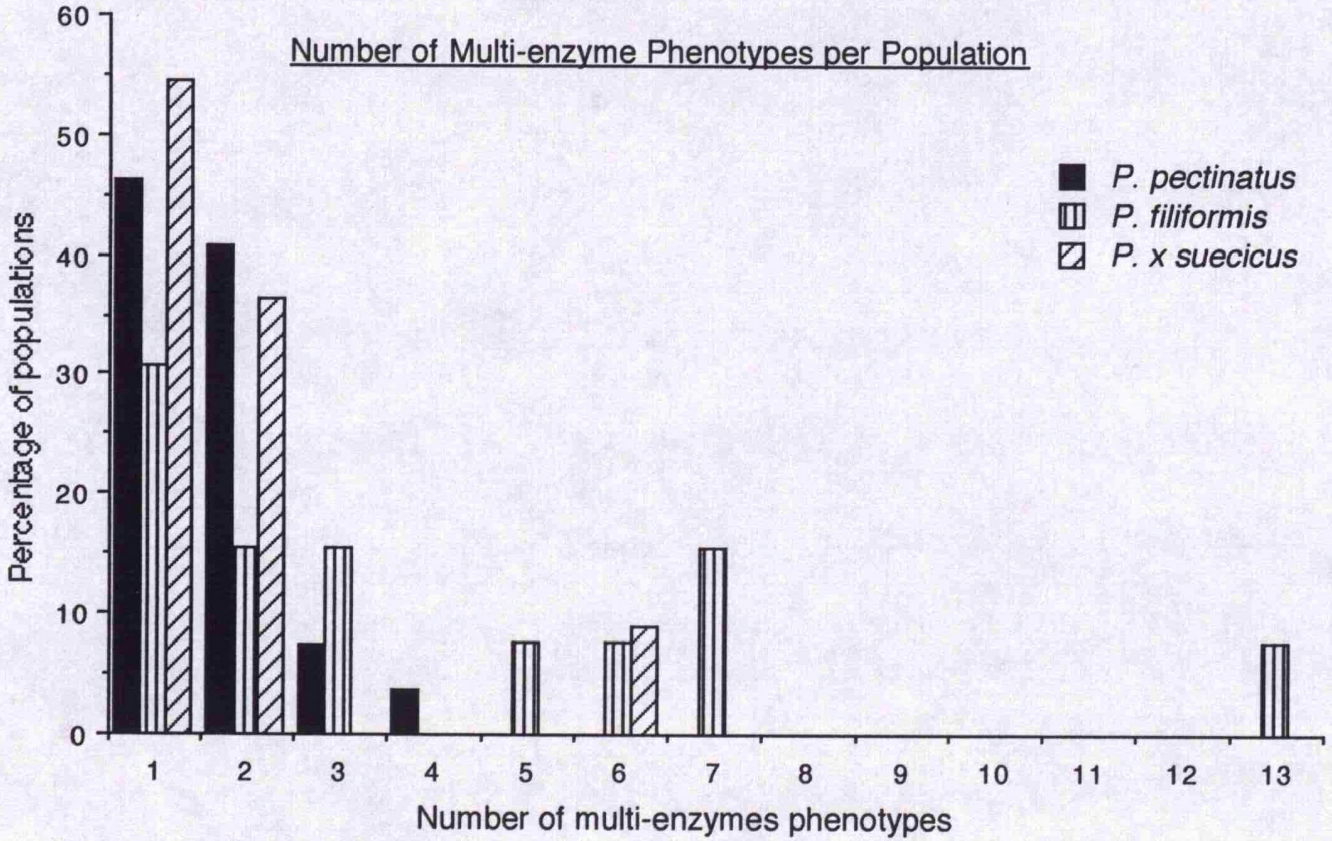
Extent of clonal growth

Assuming the absence of sexual reproduction in *P.* x *suecicus,* growth must be entirely clonal, by means of vegetative spread either from non-specialised structures such as rhizomes or from specialised structures such as tubers. Electrophoretic data from the two English populations is consistent with the presence of a single clone in each of the Rivers Wharfe and Ure. In Scotland, the data suggest the presence of one clone in the River Lossie, Loch a' Chinn Uacraigh, Loch Grogary and Loch na Liana Moire; two clones in Loch Fitty, Loch Fada, West Loch Ollay and Loch a'Phuill; and six at An Fhaodhail.

That the more genetically diverse populations are all Scottish is interesting, and is best explained by the fact that at the Scottish sites the two parents are in the same water body (or are nearby) and thus can cross repeatedly to produce different hybrid genotypes. It is worthy of note that the relatively rare *P.* x *suecicus* has a slightly higher mean D value (0.24) than *P. pectinatus* (D=0.18), one of the most cosmopolitan of all aquatic macrophytes. This is presumably due to the diversity *P.* x *suecicus* inherits from the more variable *P. filiformis* (D=0.49) (Fig. 22).

In English populations, in contrast, either one or both parents are absent, with the result that hybridisations are presently unlikely or impossible. The single remaining genotype in each of the Yorkshire rivers may thus represent genetically depauperate, relict populations, quite possibly vigorous clones that have survived from the Weichselian as envisaged by Dandy & Taylor (1946) and outcompeted others, including the parents.

Fig. 22


Number of Multi-enzyme Phenotypes per Population
P. pectinatus
P. filiformis
P. x suecicus
Percentage of populations
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Number of multi-enzymes phenotypes

As far as the 'Till ' type plants are concerned, the isozyme data implies that they are not *P.* x *suecicus*, and from comparisons with *P. vaginatus* (Hollingsworth *et al.* 1995d - chapter 6) the data is consistent with a *P. pectinatus* x *P. vaginatus* parentage. If this is correct these plants should be called *P.* x *bottnicus* Hagstr. If this identity is confirmed by morphological studies planned for the coming field season, then this will certainly represent an interesting distributional anomaly, as the nearest known *P. vaginatus* locality is in Norway (Elven & Johansen 1984). To the best of our knowledge *P. vaginatus* has never been recorded in Britain, but subfossil fruits are abundant in lake sediments in Holland (Cappers 1993). A similar situation also occurs in Russia where robust plants of *P.* x *bottnicus* , apparently very morphologically similar to the plants in the Rivers Till and Tweed, survive in rivers in Lithuania and near St. Petersburg in European Russia, south of the southern limit of *P. vaginatus* (Juzepczuk 1955, Galinis 1977).

CHAPTER 8

A REVIEW OF MOLECULAR POPULATION GENETIC STUDIES OF AQUATIC ANGIOSPERMS

INTRODUCTION

Clonal growth via vegetative spread is considered to be an important part of the life history of many aquatic plants. Extensive rhizomatous growth coupled with the dispersal of fragments such as stems, roots, rhizomes, and specialised vegetative propagules, either singularly or in combination, enable various species of aquatic plants to colonise and exist over large areas by asexual means (Sculthorpe 1967). The well documented spread of the mainly female *Elodea canadensis* Michx. in Britain is a classic example (Richards 1986). The efficiency of vegetative reproduction and dispersal in many aquatic plants, coupled with the perceived stability of aquatic environments (Les 1988), has led to predictions that genetic diversity in populations of waterplants may be lower than in their terrestrial counterparts (Hutchinson 1975).

Since the advent of protein electrophoresis (Harris 1966, Lewontin & Hubby 1966) and with recent advances in DNA-based techniques (Burke *et al.* 1991), tools are available for accurately assessing the levels of genetic diversity at the population level. Several reviews have appeared in recent years, such as those by Wain *et al.* (1985), Les (1988) and Triest (1990, 1991b,c), that have summarised studies of genetic variation in aquatic plants. However, as Harris et *al.* (1992) pointed out, many of the summarised studies have had taxonomic (eg: comparing species within a genus) rather than population genetic aims (comparing genetic diversity within and between populations), and therefore the possibility exists that variation within populations may have been underestimated. To the best of my knowledge this is the first review which has attempted to assess critically the levels of variation within and between populations of aquatic plants from a population genetic perspective. This study reviews molecular data on 96 aquatic angiosperm species from 35 genera. I have used Cook's '*Aquatic Plant Book*' (1990) as my guideline as to what taxa are considered aquatic.

In this review I make a clear distinction between the genetic diversity within and between populations (measured by numbers of detectable genotypes), and the levels of genetic variation within individuals. Thus, when considering levels of clonal growth, measures such as P (the proportion of loci that are polymorphic), A (the mean number of alleles per locus) and H (mean heterozygosity) (although reported in this review where available) are not directly relevant. It may well be that having high levels of genetic diversity within an individual is advantageous for a clonal plant, but here I concentrate primarily on assessing which species show evidence of clonal growth, of which measures such as PP (percentage of populations that are polymorphic) and the number of detectable genotypes per population are more relevant .

In the following review, taxa are arranged alphabetically by genus, with the appropriate family (taken from Mabberley 1987) given in brackets. The data are also presented as a table (Table 21), to enable the reader to access the relevant information more easily.

Original papers have been used as the source for all the data except for work by Triest and co-authors, McMillan, and Verkleij & Pieterse and co-authors. These authors all publish some data in a cumulative fashion, with some studies including data in the results sections from previous publications. As it would be repetitive and unnecessary to cite the same data numerous times, I have cited the most recent summary paper for each author, although the original papers were consulted and information from these, such as sampling strategy, has been included and cited where required.

MOLECULAR POPULATION GENETIC STUDIES OF AQUATIC ANGIOSPERMS

Alisma (Alismataceae)

Nine populations of the predominantly entomophilous *Alisma lanceolatum* With. were sampled from Belgium and the Netherlands (Triest & Roelandt 1991). 17 enzymes and total protein were analysed on seed (only single embryos were used) and leaf material. No variation was detected within or between populations, with A=1.2, P=22% and H=0.18 although the authors reported fixed heterozygosity for an ADH gene. Triest (1991e) when summarising the data from this paper gave the following values: A=1.1, P=12.5% and H=0.13 In this study and that of *A. plantago-aquatica* and *A. gramineum* reported below, values of A, P and H were based on 16 loci (no indication is given as to which enzymes / loci were excluded from those originally described in the materials and methods).

Triest & Roelandt (1991) sampled 29 populations of the predominantly entomophilous *A. plantago-aquatica* L. from Finland, Denmark, Belgium and France. 17 enzymes and total protein were analysed in seed (only single embryos were used) and leaf material. No variation was detected within or between populations, with A=1 P=0% and H=0. When summarising the data from this paper Triest (1991e) gave the same values for P, A and H, but cited the number of populations examined as 24.

Four populations of the predominantly entomophilous *A. gramineum* Lej. were sampled from Belgium, France and Italy (Triest & Roelandt 1991). 17 enzymes and total protein were analysed in seed (only single embryos were used) and leaf material. One population was variable (PP=25%) with two allelic forms of ADH, with the four populations giving the following values: A=1.1, P=11% and H=0.02. In a summary of the data from this paper Triest (1991e) quoted the following values:, P=6.3% and H=0.06.

Alternathera (Amaranthaceae)

Wain *et al*. (1984) studied 225 individuals from two populations (100 and

125 plants per population) of the introduced entomophilous Alligator weed - *Alternathera philoxeroides* (Mart.) Griseb. - from Louisiana, using nine different protein / enzyme systems encoded by 16 putative loci. Three loci were polymorphic (three enzymes), with both populations being variable (PP=100%). The two populations were morphologically different, one with slender stems and short, rounded leaves, and one with broad stems and long, narrow leaves. A one-way nested analysis of variance on measures of this morphological difference, between plants from both populations, indicated that it was statistically significant. The mean genetic identity across all loci was 0.886 and the authors suggested that the biotypes could represent separate introductions of previously differentiated populations. However, no comparison was made between populations with the same morphological type, which must thus cast some doubt on the validity of the comparison between forms.

Amphibolis (Cymodoceaceae)

A single population each of the hydrophilous *Amphibolis antarctica* (Labill.) Sonder et Aschers. and *A. griffithii* (J. M. Black) Den Hartog, both from Australia, were sampled. Ten enzyme systems were examined in each case, and no isozyme variation detected (McMillan 1991).

Baldellia (Alismataceae)

Triest & Vuille (1991) presented isozyme results on three predominantly entomophilous taxa of *Baldellia* Parl., namely a Spanish population of *B. alpestris* (Cosson) Lainz, *B. ranunculoides* (L.) Parl. subsp. *ranunculoides* from the UK and France (five populations), and the self-incompatible *B. ranunculoides* subsp. *repens* (Lamark) Löve & Löve, from the Netherlands, Belgium and Portugal (three populations). Results for two further populations whose identity was uncertain were presented, as was data of controlled crossings with one of these unknown populations and different subspecies of *B. ranunculoides*. Nine loci from five enzyme systems and total protein were analysed in fruits.

B. alpestris showed no variation within or between populations, with PP=0% (Triest & Vuille 1991). The following values were given: A=1.1, P=11% and H=0.11. Triest (1991e) in his summary table of genetic variability in aquatic plants gave the following values: A=1, P=0% and H=0, although here he gave the number of loci examined as eight, thus data from one locus has presumably been omitted.

B. ranunculoides subsp. *ranunculoides* showed no within or between population variation, but showed fixed heterozygosity for an ADH locus (Triest & Vuille 1991). The following values were given: A=1.1, P=11% and H=0.06. However somewhat confusingly, these figures include values on the controlled cross between an unknown plant and *B. ranunculoides* subsp. *ranunculoides* and as such are difficult to interpret. In a summary paper, Triest (1991e) cited the following values: A=1.1, P=12.5% and H=0.06. It is not clear whether these values include data from the controlled cross. Zymograms were presented of all banding patterns and it may well be possible to calculate values from these although, without further information on sample sizes, staining intensities, and what was actually considered a locus, it would be unsafe to do so.

B. ranunculoides subsp. *repens* showed some variation between populations, although this variation appears to be in the intensity of ADH and total protein bands rather than mobility (Triest & Vuille 1991).The following values were given: A=1.2, P=22% and H=0.11-0.17. Allele frequencies of 0.25 / 0.75 and 0.42 / 0.58 and 0.50 / 0.50 were given for the three populations. Coupled with the statement that "no allelic variation was noted within a population" (page 40) one must assume that these allele frequencies relate to staining intensity in a fixed heterozygote, thus some question must surely be raised as to the 0.42 / 0.58 value. In a uniform population this means that each individual has 21 copies of one allele and 29 copies of another - this being something which would be virtually impossible to quantify from a gel. In Triest (1991e) the following values were given: A= 1.1, P=12.5 and H=0-0.06, based on eight loci with no indication of which locus has been omitted from the original paper. Furthermore, five populations are mentioned, which is two more than are described in the

paper that is cited. It is interesting to note that more variation was detected in the self-incompatible *B. ranunculoides* subsp. *repens* , than in the self-compatible *B. ranunculoides* subsp. *ranunculoides* . The difficulties in interpreting the data mentioned above, however, make this difference difficult to assess.

Additional data are presented on populations whose identity could not be determined. Since their identity is unknown, I have made no attempt to summarise this here.

Cabomba (Cabombaceae)
Wain *et al.* (1983) studied genetic relationships of the predominantly entomophilous *Cabomba caroliniana* Gray *var. caroliniana* (two populations), *C. caroliniana* var. *multipartita* (three populations) and *C. pulcherrima* (Harper) Fass. (three populations), all from the U.S.A. Each individual was assayed for six different enzyme / protein systems encoded by twelve putative loci with the average population sample size being 43 (range 40-45). There were no monomorphic populations (inferred from allele frequencies). Four out of the twelve loci (four out of six enzyme / protein systems) were polymorphic, although allele frequency was similar between populations and consequently Nei's genetic identity values, 0.992-1.0 are high. The authors reported a high overall genetic identity between all three taxa and suggested that they are actually part of a common gene pool and that morphological differences are best explained by environmentally induced variation.

Callitriche (Callitrichaceae)
Philbrick (1993) used RAPDs to demonstrate underwater outcrossing in *Callitriche hermaphroditica* L. A total of three maternal plants from two sites in California and six progeny were analysed using twelve 10-mer primers that were shown to be polymorphic after a preliminary survey. The presence of non-maternal bands in the offspring was considered to be indicative of outcrossing, with seven primers showing novel bands in the progeny.

Ceratophyllum (Ceratophyllaceae)
Les (1986) studied isozyme variation in a single population of the hydrophilous *Ceratophyllum demersum* L. 20 individuals were analysed for variation in eleven enzymes encoded by 14 putative loci. No variation was detected.

A further study of hydrophilous *Ceratophyllum* species was reported by Les (1991), in which 262 plants of *C. demersum* from nine populations (19-33 plants per population), and 79 individuals from three populations of the rarer *C. echinatum* (22-32 plants per population), were analysed for variation in ten enzyme systems representing 17 putative loci. All plants were collected from Wisconsin.

For *C. demersum*, populations ranged from monomorphic (five out of nine populations) to having eight multi-enzyme genotypes (Les 1991). 44.4% of the populations were multi-clonal with a mean of 2.7 genotypes per popuation. Simpson's D=0.45, P=0.20 (0-0.35), A=1.22 (1-1.41), H=0.076 (0-0.294). Over all loci H_T=0.1559, H_S=0.0655, D_{ST}=0.0904, G_{ST}= O.5797.

C. echinatum was uniclonal in all three populations examined, but differences existed between all three populations (Les 1991). P=0.07 (0-0.14), A=1.07 (1-1.14), H=0.071 (0-0.143). Over all loci H_T=0.2343, H_S=0.0364, D_{ST}=0.1979, G_{ST}= O.8446. Les suggested that the low genetic identity among populations may reflect a history of repeated bottlenecks following the Pleistocene glaciations.

Cymodocea (Cymodoceaceae)
A single population of the hydrophilous *Cymodocea angustata* Ostenfeld from Australia was sampled. Ten enzyme systems were examined, and no isozyme variation was detected (McMillan 1991).

Four populations of the hydrophilous *C. rotundata* Ehrenb. & Hempr. and three of *C. serrulata* (R. Br.) Aschers. & Magnus were sampled from Kenya, Micronesia and Australia and ten enzyme systems were examined; no isozyme variation was detected (McMillan 1991). 6-15 plants per population were sampled (McMillan 1980).

Eichhornia (Pontederiaceae)

Wain & Martin (1980) studied three different biotypes of the entomophilous *Eichhornia crassipes* (Mart.) Solms., the Water Hyacinth, from Florida. A total of 150 individuals from three populations (40-60 plants per population) was studied using seven enzyme systems encoded by 14 putative loci. No genetic variation was found either within or between biotypes, with only two out of 150 individuals differing from the other individuals by a single anodally migrating peroxidase band. This difference was concluded to be an experimental artifact.

A technical communication on *E. crassipes* by Watson & Cook (1982) showed that different extraction buffers could result in different electromorph bands, although the differences were only noted in certain enzyme systems with certain bands and certain clones. From the nine enzymes that were analysed, two showed variation with respect to extraction buffer, these being PGM and APH with the discussion being limited to PGM.

Glover & Barrett (1986) studied six populations of the tristylous *Eichhornia paniculata* (Spreng.) Sloms. from N.E. Brazil and five populations from Jamaica. The main aim of the paper was to compare outcrossing rates of populations with different floral traits. Populations were either trimorphic with long, medium and short styled plants, dimorphic with long and medium styled plants, or monomorphic consisting of a range of self-pollinating, semi-homostylous, mid-styled variants. Eight enzyme systems were used, and progeny tests were carried out to demonstrate Mendelian inheritance of variation. 10-65 seed families were collected from each population and the number of progeny analysed was 80-1,480 per population. Only polymorphic loci were used for the estimation of the mating system, with a

maximum of six loci being polymorphic. In polymorphic populations (nine out of 11) the multi-locus outcrossing rate varied from 0.98-0.29 (text gives this as being 0.96-0.29) with trimorphic populations having uniformly high outcrossing rates of 0.98-0.84 (text says 0.96-0.84). Two of the Jamaican populations, which consisted completely of self-pollinating plants, were invariant at 21 isozyme loci precluding estimates of outcrossing frequency. It was suggested that founder events may be responsible for the lower levels of variation detected in the Jamaican populations as compared to the Brazilian populations. They suggested that self-pollination would be favoured in establishment of the populations in Jamaica, particularly if such dispersal events are rare. Self-pollination following founder events would be expected to lead to low levels of variation within populations.

Glover & Barrett (1987) presented comparative data to test the above hypothesis. Using the samples from the same eleven populations described by Glover & Barrett (1986), seed families were analysed for 21 loci from eleven enzyme systems. Of the 21 loci, 13 were polymorphic in at least one population. Out of the eleven populations nine were polymorphic. In Brazil 36 alleles were detected among the 21 loci, compared with 24 alleles in the Jamaican populations. The mean percentage of loci that were polymorphic was 23.8% (range 4.7-35.0) for Brazil and 7.6% (0-14.3) for Jamaica. The mean number of alleles per locus was 1.27 (SD 0.37) for Brazil, and 1.07 (SD 0.27) for Jamaica. The mean observed heterozygosity was 7.8 (SD 1.98) for Brazil and 2.0 (SD 1.4) for Jamaica. In the study it was shown that the Jamaican populations were depauperate with respect to allozyme variability at both regional and population levels. Island isolation and a restricted number of dispersal events along with high levels of self-fertilisation were suggested as important contributory factors. F_{IT} was 0.574, F_{ST} was 0.528 and F_{IS} was 0.096 for all populations. For Brazil F_{IT} was 0.384, F_{ST} was 0.324 and F_{IS} was 0.088. For Jamaica F_{IT} 0.734, F_{ST} was 0.633 and F_{IS} was 0.275. In Brazil the strongest contribution to F_{IT} comes from F_{ST} - indicating that population differentiation is pronounced. The Jamaican F_{IT} value receives a strong contribution from F_{ST} but also a significant contribution from within population differentiation (F_{IS}). The following measures were also given: G_{ST} = Brazil 0.4, Jamaica, 0.57; R_{ST} =

Brazil 0.81, Jamaica 1.70; mean H_T = Brazil 0.15, Jamaica 0.06; and mean H_S= Brazil 0.09, Jamaica 0.03.

Barrett & Husband (1990), carried out a more intensive survey from the same regions using 21 populations from N.E. Brazil and eleven from Jamaica. Seed families were collected from each population, and 7-25 progeny raised from each family. The number and size of each family varied according to population size and germination levels. Populations were screened for between 19 and 27 loci. Results were as follows:
Brazil P=0.235 Na=0.477, H=0.507 f=-0.486
Jamaica P=0.562 Na=-0.196 H=0.811 f=-0.876
Overall P=0.702 Na=0.648 H=0.818 f=-0.741
- where Na is the average number of alleles per polymorphic locus and f is the inbreeding coefficient.

Populations varied from predominantly outcrossing in Brazil to those that are highly autogamous in Jamaica (outcrossing rate ranged from 0.002-0.960), This was reflected in the associated variation in floral morphology. All populations had outcrossing rates calculated and are thus assumed to be polymorphic. Partial correlations and multiple regression indicated that population size, plant density and style morph diversity each show a significant association with outcrossing rate. Small populations of *Eichhornia paniculata* are common with approximately 57% of populations (N=110) containing less than 100 individuals. This study includes all populations from Glover & Barrett (1986, 1987) except for the two Jamaican populations that were uniform for 21 loci.

Enhalus (Hydrocharitaceae)
McMillan (1991) sampled a single population of the dry-epihydrophilous *Enhalus acoroides* (L. f.) Royle from Australia. Ten enzyme systems were examined and no isozyme variation detected.

Groenlandia (Potamogetonaceae)
Hettiarachchi & Triest (1991) sampled seed and leaf tissue from three

populations of *Groenlandia densa* (L.) Fourr. from Belgium and France. Variation at 17 loci was studied in five enzyme systems (no indication was given of which loci were visualised in which tissue). Two out of the three populations were variable, with PP=67%, A=1.2-1.5, P=24-47%, and H=0.027-0.118.

Halodule (Cymodoceaceae)
McMillan (1991) studied a single population of the hydrophilous *Halodule pinifolia* (Miki) Den Hartog from Australia. Ten enzyme systems were examined, and no variation was detected .

Similarly McMillan (1991) investigated four populations of the hydrophilous *H. uninervis* (Forsk.) Aschers. from Kenya, Micronesia, Australia and Fiji, and seven populations of the hydrophilous *H. wrightii* Aschers. from the USA, Bermuda and Mexico. Ten enzyme systems were examined in both species, and no isozyme variation detected. Between 6 and 15 plants per population were sampled (McMillan 1980)

Halophila (Hydrocharitaceae)
McMillan (1991) summarised the results of an investigation of seven species of the hydrophilous seagrass genus *Halophila* : *Halophila decipiens* Ostenfeld (one population from St Croix), *H. engelmannii* Aschers. (one population from Texas), *H. hawaiiana* Doty & Stone (one population from Hawaii), *H. johnsonii* Eiseman (one population from Florida), *H. minor* (Zoll.) Den Hartog (six populations from Guam, Western Samoa, Fiji and Australia), *H. ovalis* (R. Br.) Hook. f. (6-15 plants per population from each of six populations from Kenya, Micronesia and Australia), *H. stipulacea* (Forsk.) Aschers. (one population from Kenya). Ten enzyme systems were studied. Variation was detected only in *H. minor* . Five enzymes were polymorphic with slightly different patterns noted between the four geographical regions. No isozyme variation was detected within populations although the plants from Fiji and Western Samoa showed some intra-population morphological variation in the degree of bullation of the leaves.

McMillan and Bridges (1982) noted that at least some variation in bullation on the same rhizome occurs in experimental cultures, suggesting some kind of environmental interaction. McMillan and Bridges (1982) also commented that *H. minor* could perhaps be treated as a group of endemic taxa, each confined to a single island, as opposed to a single polymorphic species.

Heterozostera (Zosteraceae)

A single Australian population of the hydrophilous *Heterozostera tasmanica* (Martens ex Aschers.) Den Hartog was sampled, with ten enzyme systems examined and no isozyme variation detected (McMillan 1991).

Howellia (Campanulaceae)

Lesica *et al.* (1988) studied four (of a total of ten known) North American populations (approx. 60 plants per site) of *Howellia aquatilis* Gray, an annual member of the Campanulaceae. *H. aquatilis* produces flowers that are cleistogamous early in the season but chasmogamous later in the season. The authors repeatedly observed embryo development before flowers opened. No variation within or between populations was detected from eight enzyme systems representing 18 putative loci, with the estimated genetic diversity (H_T) being zero.

Hydrilla (Hydrocharitaceae)

Verkleij & Pieterse (1991) presented a review of a number of papers (Pieterse *et al.* 1984, 1985, Verkleij *et al.* 1983a, 1983b, Verkleij & Pieterse 1986, Ryan 1988, 1989) studying isozyme variation in the epihydrophilous *Hydrilla verticillata* (L.f) Royle. Verkleij & Pieterse (1986) studied a total of 48 plants from 36 populations from around the world. 23 multi-enzyme phenotypes were detected, with six out of ten enzymes being polymorphic. Of the four populations where more than one individual was sampled, (2, 2, 5, and 7 plants respectively) two were polymorphic (although a third population - Kota (India) - has two plants that have apparently identical banding phenotypes yet they are given different multi-enzyme phenotype

codes by the authors). No correlations between morphological variation, chromosome number and isozyme variation were detected.

Ryan (1989) studied isozyme variation in *Hydrilla verticillata* in the U.S.A. 36-38 plants per population were sampled from four sites in N. Carolina. 36 plants were also sampled from each of three sites in the Washington D.C. area (the text actually says two replicates of 18 samples although it is not clear whether this means repeated sampling or repeated analysis of the same samples). Variation was detected in three out of the four enzyme systems analysed in three of the N. Carolina populations, with all other populations being invariant. Later, however, Ryan *et al.* (1991) reported that this variation was non-genetic.

Hydrocharis (Hydrocharitaceae)
Scribailo *et al.* (1984) studied isozyme variation in the dioecious entomophilous *Hydrocharis morsus-ranae* L. This species was deliberately introduced into Canada in 1932. Variation was examined in two widely separated populations in Canada. One was from Ottawa and was thought to be the site of the original introduction, the second was from Lake Erie. The Lake Erie population actually consists of a number of sub-populations. 20 adult plants were collected from Ottawa, with isozymes in turions from ten of these plants also analysed. 32 adult plants were collected from a number of locations from the Lake Erie site, along with 76 progeny from 14 fruits, as well as two turions. Four turions were also sampled from the botanic garden in Berlin and seven from that in Zurich. Of the 29 loci (17 enzyme systems) examined in the North American plants 28 were always monomorphic, with the same allele present in all individuals, whether adults or progeny, indicating that they are all probably homozygotes. No variation was detected among adults. MDH showed a heterozygous pattern (although asymmetric in position) in adults; whereas the heterozygote and the slow band were detected in the progeny. Compared with turion samples from Europe, all isozymes were identical, apart from EST in which three multi-locus phenotypes were detected. North American populations were represented by one of these. ADH showed considerable variation in expression,

although not mobility, in seedlings.The following values were given: A=1.04 and P=0%.

Lagarosiphon (Hydrocharitaceae)
Triest (1991c) studied isozyme variation in three dioecious epihydrophilous *Lagarosiphon* species from South Africa, *L. major* (Ridley) Moss, *L. muscoides* Harvey, *L. verticillifolius* Oberm. No details of sample size were given although "about five plants per population" is mentioned in the discussion (page 81).

Three populations of *L. major* were analysed (one male, one female and one mixed) for variation at 21 putative loci from 13 enzyme systems (Triest 1991c). No variation within populations was detected (PP=0%) but three out of the 21 loci showed some variation between populations (three out of 13 enzymes). Triest (1991e) gave the following values: PP=0%, A=1.0-1.1, P=0-9.5% and H=0.095

Four populations of *L. muscoides* (two male and two female, or three populations: one male, one female and one mixed) were analysed for variation at 21 putative loci in 13 enzyme systems (Triest 1991c). The percentage of polymorphic populations was 0. Variation in two out of the 21 loci (two out of 13 enzymes) was detected between populations. A=1-1.1 and P=9.52%, calculated by PMH. Triest (1991e) however gave the following values: PP=0%, A=1.0, P=0 and H=0.

One population of *L. verticillifolius* (femaie) was analysed for variation at 21 putative loci in 13 enzyme systems and no variation was detected (Triest 1991c). Triest (1991e) gave the following values: PP=0%, A=1.0, P=0 and H=0.

Lemna (Lemnaceae)
Vasseur *et al.* (1993) studied allozymic variation in local apomictic populations of *Lemna minor* L. Eight sites along a twelve kilometre transect

in Ontario, Canada, were sampled. An average of 24 plants (1-30) per population was tested for different enzyme systems. All eight populations were multi-cionai, with the average number of genotypes detected per population being 19.6 (SD 2.8), and the total number of genotypes detected being 157. Simpson's D=0.973 (SD 0.014) with E=0.882 (SD 0.084). 60% of the genotypes were restricted to a single population, with the most widespread genotype being present in six populations. No genotypes were found in all eight populations. 13 of the 18 putative loci were polymorphic (13 out of 13 enzyme systems). Large deviations from Hardy Weinberg equilibrium were frequent in all populations, although the nature of these deviations was not consistent within a site, with excesses of homozygotes and heterozygotes being present in different enzymes. The mean genetic distance between populations was D_H=0.801 (0.232-0.980). No flowering individuals were observed throughout the study. Multiple introductions and somatic mutation, as well as the possibility of rare sexual events, were suggested as explanations for these relatively high levels of variability in an aquatic plant.

Myriophyllum (Haloragidaceae)

Harris *et al.* (1992) studied genetic varation within and between populations of the anemophilous *Myriophyllum alterniflorum* DC. 273 individuals from 13 populations (ten from NW Scotland, one from central Scotland, one from England and one from Norway) were analysed for variation in twelve enzyme systems. Eight of the twelve (67%) enzyme systems were polymorphic across the populations, and a total of 58 multi-enzyme phenotypes was detected. No monomorphic populations were found (PP=100%) with Simpson's D = 0.536-0.978 (mean=0.792). Most of the populations were in Hardy-Weinberg equilibrium for the two loci from which such calcuiations were possible. Wright's F_{IT} was 0.498, with the deviation from panmixia being attributed to subdivisions between populations (F_{ST}=0.496) rather than inbreeding within populations (F_{IS}=0.054).

Furnier & Mustaphi (1992) studied isozyme variation in Minnesota populations (and a single plant from Wisconsin) of the anemophilous

Myriophyllum spicatum L. A total of 80 plants was collected from seven populations with the sample sizes ranging from 1-54 (mean =11). Eleven enzyme systems encoded by 19 putative loci were examined with only three loci found to be polymorphic. PP=0% and only two multi-locus genotypes were detected, with no evidence for sexual reproduction in any of the sites.

Najas (Najadaceae)

Triest (1989, 1991d) presented data on 54 populations of the hydrophilous *Najas marina* L. Three subspecies were examined: subsp. *marina*, subsp. *intermedia* (Gorski) Casper and subsp. *armata* (Lindb. f.) Horn af Rantz. Seeds and leaves were examined for twelve enzyme systems and total denatured protein, giving a total of 24 loci. The mean sample size per locus per population varied from 2.3 - 99 (taken from Table 16 of Triest 1989). As the results presented by Triest (1991d) are unintelligible, I have taken the values of PP, P, A, and H from Triest's (1991e) summary.

N. marina subsp. *marina* was sampled from 23 populations in Europe and the Far East (Triest 1991d). 13% of the populations were polymorphic with A=1.0-1.1, P=0-4.2% and H=0-0.012 (Triest 1991e).

N. marina subsp. *intermedia* was sampled from 21 populations from Europe, Turkey and the USA (Triest 1991d). 62% of the populations were polymorphic, with A=1.0-1.3, P=0-8.3%, H=0-0.030 (Triest 1991e). Some populations contained "B" chromosomes, with these populations also possesing some unique alleles.

N. marina subsp. *armata* was sampled from ten populations in Israel, Egypt, Burundi and South Africa (Triest 1991d). 30% of the populations were polymorphic, with A=1-1.1, P=0-8.3%, H=0-0.083 (Triest 1991e).

Two populations of *N. horrida* Magn. from South Africa are listed in Triest (1991e), although I could find no trace of this species in the source references cited (Triest 1989 , 1991d). Neither population was polymorphic at the 19 loci examined, with A=1.1, P=5.3% and H=0.053.

Phragmites (Gramineae)
Neuhaus *et al.* (1993) studied genetic diversity in 53 samples of *Phragmites australis* (Cav.) Trin. ex Steud. using DNA fingerprintlng. Five samples from each of four populations in Germany were collected, along with an additional 18 samples from one of these sites. Differences were noted between all populations, and two of the four populations were polymorphic (PP=50%). A further sample of 15 plants from a large area at one of the monomorphic sites failed to detect any variation.

Phyllospadix (Zosteraceae)
McMillan & Phillips (1981) examined isozyme variation in three species of *Phyllospadix*: *P. serrulatus* Rupr. ex Aschers. (two populations), *P. scouleri* Hook. (five populations) and *P. torreyi* S. Watson (five populations), all from North America. Ten enzyme systems were analysed in 5-15 plants per population. Only *P. scouleri* showed some (between population) variation in the mobility of an ADH isozyme. No other consistent variation was noted within or between populations.

Posidonia (Posidoniaceae)
Single Australian populations of *Posidonia australis* Hook. f. and *P. sinuosa* Cambridge & Kuo. were sampled. Ten enzyme systems were examined, and no isozyme variation was detected within either species (McMillan 1991).

Potamogeton (Potamogetonaceae)
Following a preliminary survey of genetic variation in *P. pectinatus* L. (Hettiarachchi & Triest 1986), Hettiarachchi & Triest (1991) presented results from isozyme analysis of 188 populations (only 187 populations actually listed) of 18 species of *Potamogeton* L. One of these species was *P. densus* L. which in my review is treated as *Groenlandia densa* (L.) Fourr., and the results for this species are presented under that heading. While no results

are presented for a nineteenth species, *P. gramineus* L. , Triest (1991e) included values of genetic variability for *P. gramineus* in his general summary of variation in aquatic plants.

A single population of *P. acutifolius* Link was sampled from Belgium (Hettiarachchi & Triest 1991). 17 loci from five enzyme systems were examined in leaf tissue. The population was polymorphic with A=1.2, P=18% and H=0.059.

P. alpinus Balb. was sampled from two Belgian populations. Variation was found at 13 loci from five enzyme systems (Hettiarachchi & Triest 1991). Seed and leaf tissue was examined although it is not clear which loci were visualised in which tissue. Both populations were polymorphic (PP=100%) with A=1.4-1.6, P=29-50% and H=0.1-0.129.

P. berchtoldii Fieber was sampled from nine populations from Belgium and France (Hettiarachchi & Triest 1991). 17 loci from five enzyme systems were examined in leaf tissue, with the following results: 78% of the populations were polymorphic, with A=1.1-1.5, P=6-35% and H=0.019-0.118.

P. coloratus Hornem. was sampled from a single Belgian population. 17 loci from five enzyme systems were analysed in leaf tissue (Hettiarachchi & Triest 1991). The population was polymorphic (PP=100%) with A=1.2, P=18% and H=0.059.

Hollingsworth *et al* . (1995a - chapter 3) studied genetic variation in British populations of *P. coloratus* . 390 plants were collected from twelve populations (8-69 plants per population) including man-made and natural / semi-natural sites. All individuals were studied for variation in nine enzyme systems in leaf tissue. One of these enzyme systems (ADH) was variable in its expression and was not considered in the results, thus a total of 14 loci from eight enzyme systems was analysed. Ten out of the twelve populations were monomorphic (PP=17%) with nine of these possessing the same multi-locus genotype. Of the eight enzyme systems only one, PGM, showed variation, with diallelic variation at two loci. The two polymorphic populations

were both at sites with a long post-glacial (and possibly pre-glacial) history as wetlands, both south of the Devensian glacial boundary. Over all populations, P=0-14.3% (mean=1.8%), A=1-1.14 (mean=1.02), and H=0-0.013 (mean=0.001). A total of seven multi-locus genotypes was detected (1-6 per population, mean=1.6). The overall inbreeding coefficient F_{IT} was 0.939, which received a substantial contribution both from genetic sub-division among populations (F_{ST}=0.702) and a high level of inbreeding within populations (F_{IS}= 0.796).

Holiingsworth *et al.* (1995b - chapter 4) examined the partitioning of genetic variation within the polymorphic population of *P. coloratus* from the Gordano Valley described by Hollingsworth *et al.* (1995a - chapter 3). Leaves from 647 individuals were sampled from this population and examined for variation at two polymorphic loci. Wright's overall inbreeding coefficient F_{IT} was 0.749 which received contributions from genetic sub-division among populations (F_{ST}=0.575) and a high level of inbreeding within populations (F_{IS}= 0.408). Strong evidence for clonal spread within ditches was observed, as were high ievels of partioning of variation within the site.

20 populations of *P. crispus* L. were sampled from Belgium, Luxemburg, France and Switzerland (Hettiarachchi & Triest 1991). 16 loci from five enzyme systems were examined in leaves and seeds. It is not clear from the results which of the loci were scored in which tissue. 90% of the populations were polymorphic with A=1-1.5, P=0-50%, and H=0-0.073. No clear division between fresh and brackish water populations was found.

Hollingsworth *et al.* (1995d - chapter 6) studied isozyme variation in *P. filiformis* Pers. by sampling 270 individuals from 13 populations (4-31 per population mean=21). Eleven of the populations were British and two were Swedish. Nine enzyme systems from leaf tissue were analysed, and variation was detected in four of these. 69% of the populations were polymorphic, with a total of 28 multi-enzyme phenotypes detected. The mean number of multi-enzyme phenotypes detected per population was 4.0 (range=1-13). When populations with ten plants or more are considered, the mean Simpson's D=0.493 (S.D. 0.333).

A single population of *P. friesii* Rupr. was sampled from Finland. 15 loci in five enzyme systems were examined in leaf tissue (Hettiarachchi & Triest 1991). The population was polymorphic with A=1.3, P=27% and H=0.017.

Seeds from three Finnish populations of *P. gramineus* L. were analysed for variation at seven loci. No mention of the enzymes studied or the levels of genetic variation in *P. gramineus* is given by Hettiarachchi & Triest (1991). However results for this species are presented in Triest (1991e) and these are presumably from the populations described by Hettiarachchi & Triest (1991). One out of the three populations was variable (PP=33%) with A=1.3-1.7, P=29-43% and H=0.286-0.429.

Five populations of *P. lucens* L. from Belgium and France were analysed for variation at 16 loci in five enzyme systems (Hettiarachchi & Triest 1991). Seeds, leaves and tubers were used for the analysis, but it is not clear which loci were examined in which tissue. All populations were polymorphic (PP=100%) with A=1.3-1.4, P=19-38% and H=0.021-0.125.

Leaves from 36 plants from two British populations (6-30 plants per population) of *P. lucens* were analysed for variation in eight enzyme systems (Hollingsworth *et al.* 1995c - chapter 5). Only one enzyme was polymorphic. Variation between but not within the two populations was detected. The total number of multi-enzyme phenotypes detected was two, one at each locality.

Ten populations of *P. natans* L. were sampled from Finland, Denmark and Belgium (Hettiarachchi & Triest 1991). However, data on only seven of these is presented in the results; it is not clear which populations are omitted. 13 loci in five enzyme systems were analysed in seeds and leaves, although it is not clear which loci were examined in which tissue. All populations were polymorphic (PP=100%) with A=1.4-1.6, P=14-50%, and H=0.071-0.214.

Leaves from a total of 98 individuals of *P. natans* from one Austrian and three British populations (2-34 plants per population, mean=24.5) were

sampled. All plants were examined for variation in eight enzyme systems (Hollingsworth *et al.* 1995c - chapter 5). Four out of the eight enzyme systems were variable, with two out of the four populations being polymorphic (PP=50%). Eleven multi-enzyme phenotypes were detected with 1-8 per population. Especially strong evidence for clonal spread was noted at one site.

Five populations of *P. nodosus* Poiret were sampled from Luxemborg, France and Egypt (Hettiarachchi & Triest 1991). Results were only presented for four populations although it is not clear which population was omitted. 13 loci in five enzyme systems were analysed. Seed and leaf tissue was examined although it is not clear which loci were visualised in which tissue. All four populations were polymorphic (PP=100%) with A=1.2-1.4, P=14-29% and H=0.071-0.143. The authors commented that *P. nodosus* showed divergences at the geographical level, populations from W. Europe being different from those of S. France and Egypt.

Leaves of 49 plants of *P. nodosus* from two British populations (8-41 plants per population) were analysed for variation in eight enzyme systems (Hollingsworth *et al.* 1995c - chapter 5). Only one enzyme system was variable, with one of the two populations being polymorphic (PP=50%). The total number of multi-enzyme phenotypes detected was three, with the number detected per site being 1-3. Strong evidence for clonal spread of *P. nodosus* was noted at one site.

P. obtusifolius Mert. & Koch was sampled from three populations from Belgium and Luxemburg (Hettiarachchi & Triest 1991). 17 loci from five enzyme systems were examined in seed and leaf tissue, with the following results: 67% of the populations were polymorphic with A=1.2-1.4, P=6-24% and H=0.059.

Van Wijk *et al.* (1988) examined isozyme variation in ten populations (several plants per site) of *Potamogeton pectinatus* L. in the Netherlands and France. In the two enzyme systems that were found to be informative (PER and SOD), considerable variation was noted between populations with

variation within populations being very low or absent. Further isozyme data on populations from Hungary, South Africa and Egypt, although not fully resolvable, showed further differences. The authors could detect no distinct population groups on the basis of the isozyme results, and suggested that more populations from a wider geographical range using more polymorphic enzymes would be required to understand the patterns of genetic variation in *P. pectinatus*.

Hettiarachchi & Triest (1991) sampled a total of 72 populations of *P. pectinatus* (68 populations from Europe, one from the USA and three from Egypt). The results, however, summarise variation in a total of 70 populations, with no indication which populations are excluded. Population six has a '-' mark for all localities, which the key describes as meaning idem. If the locality is exactly the same as the locality above it in the Table, it is not clear if this represents the same or another population. Of the 70 populations, 37% were polymorphic (although the text says about 57% of the populations were monomorphic (page 104)). The results summary is based on 16 loci from seeds, leaves and tubers. The results text describes 16 loci from leaves, 16 loci from tubers (eight of these were not examined in leaves or seeds) and six loci from seeds (two of these were not examined in leaves or tubers). Thus the results text describes a total of 26 loci, but there is no indication which of these constitute the 16 loci in the results table. The values in the results table, however, are consistent with 'leaf results' only, with A=1-1.5, P=13-38%, H=0.063-0.125. An association of *P. pectinatus* genotypes with fresh and brackish water habitats was noted as was a geographical trend in genotypes among the freshwater populations across major geographical areas.

Hollingsworth*et al.* (1995d - chapter 6) studied isozyme variation in 447 (2-37 plants per population, mean = 17) individuals of *P. pectinatus* from 26 populations; 22 of these were from Britain, and a further two populations were from each of Sweden and Crete. Nine enzyme systems in leaf tissue were analysed and variation was detected in five of these. 54% of the populations were polymorphic and 25 multi-enzyme phenotypes were detected, the mean number per population being 1.7 (range=1-4). When

populations with ten or more plants sampled were considered, the mean Simpson's D=0.182 (S.D. 0.213).

Seven populations of *P. perfoliatus* L. were sampled from The Netherlands, Belgium, France and Hungary (Hettiarachchi & Triest 1991). 16 loci in five enzyme systems were examined in leaf tissue, with 71% of populations being polymorphic. The following values were given: A=1.1-1.4, P=6-44% and H=0.063-0.115.

P. polygonifolius Pourr. was sampled from a total of five populations from Belgium and Luxemburg (Hettiarachchi & Triest 1991). 17 loci in five enzyme systems were analysed in leaf tissue. All populations were polymorphic (100%) with A=1.2-1.5, P=12-35% and H=0.029-0.088.

Hettiarachchi & Triest (1991) studied genetic variation in *P. pusilus* L. (=P. *panormitanus* Biv.) from Belgium. 27 populations are listed in the Materials and Methods although two of these (88 and 92) have an idem mark indicating that they are replicates. A total of 28 populations are listed in the results table (one more than in the Materials and Methods) with the following values given based on 15 putative loci (five enzyme systems) studied in leaf tissue: 96% of populations were polymorphic, with A=1.1-1.5, P=7-40%, H=0-0.111. No genetic differentiation between fresh and brackish water forms was noted.

Leaves from a single population of *P. schweinfurthii* A. Bennett from South Africa were analysed for variation at 13 loci in five enzyme systems (Hettiarachchi & Triest 1991). This population was monomorphic with A=1.1, P=14% and H=0.143.

Hollingsworth *et al.* (1995d - chapter 6) studied isozyme variation in *P. striatus* Ruiz et Pavon in 31 plants sampled from a single Argentinian population. Nine enzyme systems from leaf tissue were analysed, but no variation was detected.

14 populations of *P. trichoides* Cham. & Schl. were sampled from Belgium

and The Netherlands (Hettiarachchi & Triest 1991). 17 loci in five enzyme systems were examined in leaf tissue, with the following results: 85% of the populations were polymorphic, with A=1-1.4, P=0-24% and H=0-0.059.

Hollingsworth *et al.* (1995d - chapter 6) studied isozyme variation in leaves of 24 plants of *P. vaginatus* Turcz. sampled from a single Swedish population. Nine enzyme systems were analysed. Variation was detected in one enzyme system, with four multi-enzyme phenotypes found and Simpson's D=0.649

35 plants from a single population of *P.* x *fluitans* Roth (*P. lucens* x *P. natans*) were analysed for variation in eight enzyme systems in leaf tissue (Hollingsworth *et al.* 1995c - chapter 5). Two of the eight enzymes were variable in this population with a total of nine multi-enzyme phenotypes detected. *P.* x *fluitans* is one of the few *Potamogeton* hybrids to set fruit (albeit infrequently), and the intra-population variation detected may be a reflection of this.

36 individuals from the only known British population of the sterile hybrid *P.* x *schreberi* G.Fisch. (*P. natans* L. x *P. nodosus* Poir.) were analysed for variation in eight enzyme systems in leaf tissue (Hollingsworth *et al.* 1995c - chapter 5). No variation was detected.

275 individuals of the sterile hybrid *P.* x *suecicus* Richt. (*P. pectinatus* x *P. filiformis*) were sampled from eleven populations in Britain (2-66 plants per population mean=25) and analysed for variation in nine enzyme systems in leaf tissue (Hollingsworth *et al.* 1995e - chapter 7).Four of the nine enzyme systems showed some variation within and between populations. Eleven different multi-enzyme phenotypes were found, with the mean number per population being 1.6 (range 1-6). 45% of the populations were polymorphic, the variation being attributed to multiple origins of the hybrid. All variation in the hybrid could be attributed to variation in the parental species. When populations with ten or more plants sampled were considered, the mean Simpson's D=0.24.

Leaf tissue from a single population from Belgium of *P.* x *zizii* Koch (*P. lucens* x *P. perfoliatus* - one of the few Potamogeton hybrids that often produces fruit (Preston 1995a)) was examined for variation at 16 loci in five enzyme systems (Hettiarachchi & Triest 1991). The population was polymorphic, with A=1.3, P=19% and H=0.113.

Podostemum (Podostemaceae)

Philbrick & Crow (1992) studied isozyme variation in the aerial-flowering *Podostemum ceratophyllum* Michx in North America. 233 individuals from eleven populations (10-28 plants per population, mean=21) were analysed for variation in ten enzyme systems encoded by 19 putative loci. 58% of the loci were polymorphic. Six populations had only a single detectable multi-locus genotype, whereas the most variable population had eleven multi-locus genotypes (mean=2.9). A partitioning of genetic variation between sites north and south of the glacial boundary was noted, with northern sites being more genetically depauperate. Over all populations, total amount of genetic diversity H_T=0.162, H_S=0.025, diversity among populations D_{ST}=0.137 and diversity between populations G_{ST}=0.846. For northern populations H_T=0.024, H_S=0.001, D_{ST}=0.023 and G_{ST}=0.961. For southern populations H_T=0.273, H_S=0.089, D_{ST}=0.185 and G_{ST}=0.676. Genetic identity among populations ranged from 0.544-1. At the population level, P ranged from 0-32% and A=1-1.42.

Ruppia (Ruppiaceae)

Triest & Symoens (1991) studied isozyme variation in four hydrophilous taxa of *Ruppia* L. Ten populations of *R. maritima* L. var. *maritima* from Europe, Egypt and S. Africa; six populations of *R. maritima* var. *brevirostris* (Agardh) Aschers. & Graebn. from Egypt, Finland, The Netherlands and France; 19 populations of *R. cirrhosa* (Petagna) Grande var. *cirrhosa* from Europe and Egypt and Israel; and a single population of *R. cirrhosa* var. *drepanensis* (Tineo) Symoens from Spain, were studied for variation at 17 putative loci (although 18 loci are presented in the results table) in eleven enzyme systems in seeds and leaves.

In *R. cirrhosa* var. *cirrhosa,* between population variation was seen in three out of 18 loci (three out of eleven enzyme systems), with an additional two loci showing fixed heterozygosity (Triest & Symoens 1991). No populations were polymorphic. The following values were given: A=1-1.2 P=11-22% and H=0.11-0.17.

For the single population of*R. cirrhosa* var *drepanensis* the following values were given: A=1.2, P=17% and H=0.17 (Triest & Symoens 1991).

For *R. maritima* var. *brevirostris* variation was seen between populations in three out of 18 loci (three out of eleven enzyme systems). No populations were polymorphic (Triest & Symoens 1991). The following values are given A=1-1.1, P=0-11% and H=0-0.11.

For *R. maritima* var. *maritima* variation was seen between populations in four out of 18 loci (three out of eleven enzyme systems) (Triest & Symoens 1991). No populations were poiymorphic. The following values are given A=1-1.2, P=0-17% and H=0-0.17.

Spartina (Gramineae)

Guenegou*et al.* (1988) studied isozyme variation in two populations of *Spartina alterniflora* Loisel., three populations of *S. maritima* (Curt) Fernald, and three populations of *S. anglica* C.E. Hubbard from France. The study examined the origin of the allopolyploid *S. anglica.* No details of sample sizes were given. All individuals were analysed for variation in three enzyme systems. No variation was mentioned within or between populations of any of the species.

In a study of the evolution of the allopolyploid *Spartina anglica,* Raybould *et al.* (1991a & 1991b) studied genetic variation in British populations of that species, *S. alterniflora, S. maritima* and *S.* x *townsendii*:

13 plants from a single population of *Spartina alterniflora,* were examined

for variation in six enzyme systems, with a subset of three plants examined for variation in a further five enzyme systems. No variation was detected.

261 plants from 14 populations of *S. anglica* were examined for variation in five enzyme systems, with a subset of these analysed for variation in a further six enzyme systems. *S. anglica* was uniform apart from some inter- and intra-population variation for a GOT variant that was detected in a total of 31 individuals from five sites This variation was correlated with chromosome ioss. A further sample of 49 seeds was examined for a single enzyme with no variation detected. 225 seeds from five sites were examined for major soluble seed proteins, but no variation was detected.

253 plants from ten populations of *S. maritima* were examined for variation in six enzyme systems, with a subset of 19 piants examined for variation in a further five enzyme systems. The species showed some intra-population variation in two enzyme systems. One particular multi-enzyme phenotype was detected in all populations, with three out of the ten populations possessing this together with either a GOT or an SKD variant. A total of three multi-enzyme phenotypes was detected with a maximum of two in any population.

Tweive plants from a single population of the sterile hybrid *S.* x *townsendii* Groves were examined for variation in five enzyme systems, with a subset of these analysed for variation in a further six. No variation was detected.

Silander (1979) studied microevolution and cione structure in *Spartina patens* (Aiton) Mulh. 402 plants from a single population in North America were sampled for variation in six enzymes. Among the 346 plants that survived transplantation, 101 genotypes were detected. Population subdivision relative to different local habitats, e.g. dune, swaie and salt marsh, was noted.

Syringodium (Cymodoceaceae)
McMillan (1980, 1991) sampled 6-15 plants per population from nine

populations of *Syringodium filiforme* Kütz (from the USA, Bermuda, Mexico, Bahamas, Belize and Panama), and four popuiations of *S. isoetifolium* (Aschers.) Dandy (from Kenya, Micronesia and Australia). Ten enzyme systems were examined in each case and no isozyme variation was detected (McMillan 1991).

Thalassia (Hydrocharitaceae)
McMillan (1980, 1991) sampled 6-15 plants per population from seven populations of *Thalassia testudinum* Banks ex König. (from the USA, Belize, Bermuda, Bahamas and Mexico) and three populations of *T. hemprichii* (Ehrenb.) Aschers. (from Kenya, Micronesia and Australia). Ten enzyme systems were examined, and no isozyme variation was detected. Additionally six seedlings of *T. testudinum* from populations from Texas, the Florida Keys, the N. Florida coast and St Croix were assayed with "little variation" detected, despite some populations showing morphological differences. Six seedlings of *T. hemprichii* from Kenya were examined and these also showed the parental isozyme phenotype.

Thalassodendron (Cymodoceaceae)
6-15 plants per population from two populations of *Thalassodendron ciliatum* (Forsk.) Den Hartog from Kenya and Micronesia were sampled by McMillan 1980, 1991) Ten enzyme systems were examined, and no isozyme variation was detected.

Typha (Typhaceae)
Mashburn *et al.* (1978) studied 74 populations of *Typha latifolia* L. and 52 populations of *T. domingensis* Pers. Seven ramets were taken from each stand and ten enzyme systems studied. All populations were from the U.S.A. No evidence of genetic variation was detected in either species. No evidence of hybridisation between these two species was detected. *Typha* grows in large rhizomatous clones and is primarily self pollinating (Krattinger 1975, Lee 1975).

Sharitz *et al.* (1980) studied isozyme variation in ten enzyme systems in *Typha latifolia* (seven populations), *T. angustifolia* L. (five populations), *T. glauca* Godron (two populations) and *T. domingensis* (four populations) from the eastern USA. 3 -7 ramets were taken from each stand and 1-20 stands were sampled per population as follows: *Typha latifolia* 1-20 stands, mean = 4; *T. angustifolia* 1-5 stands, mean=3; *T. glauca* two stands and *T. domingensis* 1-20 stands, mean=7.5. No variation was detected in *T. angustifolia* or *T. domingensis.* In *T. latifolia* five out of the seven populations were monomorphic and identical, and only one enzyme in one population was variable. *T. glauca* showed variation in three out of the ten enzyme systems, with both populations showing some intra-population variation.

Vallisneria (Hydrocharitaceae)

Leaves from a total of 1104 plants of the hydrophilous *Vallisneria americana* Michx were analysed, with 48 plants collected from each of twelve populations in North America (Laushman 1993). One population was examined in greater detail with 192 plants sampled together with 336 seedlings from nine fruits. Eight loci were used to study variation between populations and five polymorphic loci were used to study the variation in the population that was examined in detail. The following overall values were given: P=38.5%, A=1.4, Hexp=0.085 and GST=0.457. In the population that was examined in detail, 21 distinct multi-locus genotypes were detected among 192 adult plants. 116 discrete, physical clones with a mean clone size of 1.66m (SD, 1.42) were identified. 49 multi-locus genotypes were detected among the 336 seedlings. In adult plants Hobs=0.349 was slightly higher than expected relative to Hardy-Weinberg expectations (Hexp=0.268). When all seedlings were pooled Hobs=0.270 was slightly lower than Hexp=0.277. However within individual fruits Hobs (mean=0.255) was greater than Hexp (mean=0.220).

Zannichellia (Zannichelliaceae)
Seeds from four populations of *Zannichellia contorta* (Desf.) Chamisso & Schlechtendal from Spain were examined for isozyme variation at 20 loci (Triest & Vanhecke 1991). No variation was detected with A=1.0. P=0 and H=0.

Seeds and leaves from six populations of *Z. major* Reichenb. from Denmark, Sweden and Finland were examined for isozyme variation at 20 loci (Triest & Vanhecke 1991). No variation was detected within or between populations (PP=0%), with A=1.2, P=15% and H=0.13.

Seeds and leaves from eleven populations of *Z. palustris* L. subsp. *palustris* , from Belgium, The Netherlands and Switzerland, were analysed for variation at 20 loci (Triest & Vanhecke 1991). Variation was detected between but not within populations (PP=0%), with A=1.2-1.3, P=15-25% and H=0.15-0.23.

Seeds and leaves from one population of *Z. palustris* subsp. *repens* Schübler & Marten from Denmark was analysed for isozyme variation at 20 loci (Triest & Vanhecke 1991). The following results were obtained: PP=0%, A=1.2, P=15% and H=0.15.

Seeds and leaves from 13 populations of *Z. palustris s.l.*, from Sweden, Finland, Denmark Turkey and Eygpt were analysed for isozyme variation at 20 loci (Triest & Vanhecke 1991). The following results were obtained: A=1.2, P=15% and H=0.13-0.15. Differences were detected between but not within populations (PP=0%). In Triest's (1991e) summary, data on only twelve populations are given but the values of P, A and H remain the same.

Seeds and leaves from 21 populations of *Z. pedunculata* Reichenb. from The Netherlands, Belgium, France and Spain were examined for isozyme variation at 20 loci (Triest & Vanhecke 1991). No variation was detected within or between populations (PP=0%), with A=1.2, P=20% and H=0.15 .

Seeds from a single population of *Z. peltata* Bertol and six populations of *Z.*

obtusifolia Talavera, Garcia, Murillo & Smit were examined for variation at 20 loci from sites in Spain (Triest & Vanhecke 1991). All populations were the same and uniform (PP=0%), with A=1, P=0% and H=0. There were presumably no electrophoretic differences between *Z. peltata* and *Z. obtusifolia*.

In their study of *Zannichelia* species Triest and Vanhecke (1991) mentioned that some of the ADH patterns they observed showed environmental variation although they were confident that variation in other ADH bands was genetic.

Zostera (Zosteraceae)

Three populations of *Zostera capricornia* Aschers. and one population of *Z. muelleri* Irmisch ex Aschers. from Australia were examined along with a single population of *Z. novazelandica* Setchell from New Zealand and a single population of *Z. capensis* Setchell from Kenya (McMillan 1991). The plants were examined for variation in ten enzyme systems although only seven of these were fully resolvable (McMillan 1982). I could find no indication of sample size although 3-6 plants per population is mentioned for *Z. marina* in the same publication (McMillan 1982). No isozyme variation within or between populations of these species was found, although consistent morphological differences were noted between populations of *Z. capricornia*.

Gagnon *et al.* (1980) studied variation in annual and perennial forms of *Zostera marina* L. A total of 200 individuals was collected from two populations in Maine U.S.A and analysed using 16 enzyme systems and a general protein stain. 14 of the enzymes and the general protein were invarient. PGI and AAT were variable in both populations, although no difference was detected between annual and perennial forms. Another enzyme, MDH, which was uniform in this study, showed polymorphism over a wider geographicai range (Gagnon 1977 unpublished material).

McMillan (1991) sampled five populations of *Zostera marina* from the USA

and Mexico. 3-6 plants per population were examined for variation in ten enzyme systems although only six of these were fuily resolvable (McMillan 1982). Some variation between populations was detected for PGM; and MDH showed variation within and between popuiations with three being polymorphic (PP=60%).

Fain *et al.* (1992) studied genetic variation within and between four Californian populations of *Zostera marina*. A total of 50 individuals was sampled, with a minimum of ten plants per population. Genetic diversity was anaiysed using restriction fragment length polymorphisms of 17S and 28S ribosomal DNA. Only one population was polymorphic (PP=25%) and this variation consisted of a single individual plant exhibiting a rDNA repeat length variant. Two of the populations were indistinguishable (if the above mentioned individual is ignored), with the other two populations identifiable by rDNA sequence variation and length mutations. The two genetically similar populations were also morphologically similar, whereas the genetically distinct populations were aiso morphologically distinct.

Heij & Nienhuis (1992) studied infra-specific isozyme variation in *Zostera marina* in The Netherlands and France. A total of 256 plants from five populations was collected (31-39 plants per population), and anaiysed for variation using twelve enzyme systems. Only one enzyme system appeared to be clearly polymorphic, this being peroxidase. A total of 27 multi-enzyme genotypes (based on PER) was detected, with all populations being polymorphic. A mean of 11.8 multi-locus genotypes per population (8-16) were detected. Using Nei's genetic identity, UPGMA analysis gave the following results: three non-tidal populations from Holiand clustered together, and were slightly separated from a Dutch tidal population; this group was in turn separated from the French population, which was collected some 600km south of the Dutch populations.

Laushman (1993) analysed a total of 1056 plants of *Zostera marina* with 72 plants collected from each of seven populations in North America. A more detailed analysis of a single population was carried, out with 480 plants and 72 seedlings (24 from each of three seedling beds) analysed. From 20 loci

the following values were obtained: P=18.3%, A=1.4, Hexp= 0.063 and GST=0.107. In the single population that was sampled in detail, no single intertidal pool consisted of just one clone, with the 26 pools examined having a mean of six multi-locus genotypes (SD 1.8; Range 3-9) and a total of 38 multi-locus genotypes. A high level of population substructuring was noted with GST=0.295. The seedling populations had excess heterozygosity relative to Hardy-Weinberg expectations and the Wahlund effect was suggested as a possible explanation for this.

DISCUSSION

Experimental Design

Before comparisons can be made and conclusions drawn on the biological significance of the results from the above studies, there is a number of factors relating to sampling strategy, and presentation of results, that need to be taken into account.

Amounts of genetic variation in plants are assessed for at different levels in different studies. Some of the studies summarised here have investigated variation within a species, and range from intensive intra-population studies (e.g. Silander 1979, Hollingsworth *et al.* 1995b - chapter 4, and Laushman 1993), to those looking at variation within and between populations within a country (e.g. Philbrick & Crow 1992, Les 1991), or variation between populations from different countries and continents (Hettiarachchi & Triest 1991, Verkleij & Pieterse 1991). Other studies are of a more biosystematic nature, and have focused on screening taxa for the presence, absence or relative frequency of molecular markers to enable taxonomic or evolutionary affinities to be examined (Triest & Roelandt 1991, Triest and Vuille 1991). The disciplines of population genetics and biosystematics are by no means mutually exclusive, and data obtained for one purpose often generates information that can be used for another. Indeed, if molecular markers are to be used for biosystematic purposes, an understanding of the population genetics of the species in question may greatly enhance the interpretation of the results. It does, however, remain the case that sample sizes often vary

according to study type, with many biosystematic studies concentrating on the number of populations, and population studies concentrating on the number of individuals within populations. It is thus imperative that sampling strategy is considered when comparing levels of genetic variation. A classic example of this relates to the study on *Hydrilla verticillata* by Verkleij and Pieterse (1991). Triest (1991c) quoted the study as showing low levels of intra-population variation. However, of the 36 populations analysed, only four of these had more than one individual sampled (2-7 plants per population, mean=4) and of those four, two were multi-clonal. Recommendations exist as to how many individuals it is necessary to sample from populations to gain representative measures of population diversity (Hartl 1988), but these almost always relate to diploid, outbreeding individuals, in panmictic populations. In practice, particularly when the taxa under question are polyploid and/or inbreeding and/or showing clonal growth, such calculations become highly complex and criteria such as time and money availability for studies, become the most significant factors in determining sampling strategies.

The material used for analysis can also affect the results. In perennial species, analysis of genetic variation in seeds reveals only the potential variation of the population as opposed to the actual variation. Further problems exist in that some seeds may be of hybrid origin, but not be identifiable as such at the seed stage, and also that maternal as well as offspring isozymes may well be expressed.

Another important consideration when comparing studies relates to the number and types of markers chosen for the study, and how the information obtained from them is presented. The vast majority of studies reviewed here have used isozymes and as such the following comments deal mainly with this category of marker.

The number of enzyme systems studied can affect the levels of variation detected, with the more markers analysed, the greater the chance of detecting variation. The type of isozymes studied can also affect the levels of variation detected, with substrate-specific isozymes tending to be less

variable than those with broad substrate affinities. Great caution is required here, however, as the isozymes revealed by non-specific stains such as esterases and peroxidases, appear to be particularly susceptible to environmentally induced modification (Weeden & Wendel 1989).

Using published data on quaternary structure and sub-cellular compartmentalisation of isozymes (Gottlieb 1982) and/or progeny tests it is often possible to interpret isozyme banding patterns in terms of loci and alleles as was done for Eichhornia *paniculata* (Glover & Barrett 1986, 1987, Barrett & Husband 1990). However in many cases, where progeny tests are not practical, or when dealing with polyploids, it is not possible to make such interpretations and comparisons are made on the basis of enzyme phenotypes as opposed to alleles. Allelic interpretations are to be favoured as they allow the data to be analysed using a formidable battery of population genetic statistics (Hartl 1988). However even comparisons of the relatively simple measures of A (number of alleles per locus), P (proportion of loci that are polymorphic), and H (average heterozygosity) can be confounded if the taxa under comparison are of different ploidy levels. A population of an allopolyploid may be expected to show fixed heterozygosity, giving high values for all of these parameters, and yet consist of a single multi-enzyme phenotype. Thus while maximum use of available statistics (for alielic or banding phenotype data) is recommended, care should be taken to ensure that the organism in question fits any necessary assumptions for a given parameter or, where this is not the case, data interpretation accounts for any such violations. It is worth noting that of the eight assumptions upon which the Hardy-Weinberg principle is based, the three from which the model is most sensitive to deviations are: 1, the organism in question is diploid; 2, reproduction is sexual, and 3, generations are non-overlapping (Hartl 1988). All of these are certainly not true of many aquatic plants.

In using molecular data to investigate the extent of clonal growth within a population, paradoxically, some levels of variation between individuals can often help to provide stronger evidence for clonal growth than complete monomorphism. It can, in some cases, be difficult to distinguish between

clonal growth and high levels of inbreeding, which aithough the genetic consequences for the piant may be similar, still represents a significant difference in the plant's life strategy. The fact is that in a population of plants that are homozygous and identical at all examined ioci, it is not possible to distinguish between sexual and asexual reproduction. However the presence of populations consisting solely of heterozygotes for genes that have been shown to segregate elsewhere (as was noted for *Potamogeton natans* and *P. nodosus* (Hollingsworth *et al.* 1995c - chapter 5)), or the co-occurence of plants which show consistent differences at a number of genetically unlinked markers (as was noted for *P. pectinatus* (Hollingsworth *et al.* 1995d - chapter 6)), are strong indicators of clonal growth.

Data Summary and Analysis

A summary of the review is presented in Tabie 21. It is clear from this that some taxa have some monomorphic, and some polymorphic populations, i.e. 0%<PP<100%. This range in the amount of variation within populations of the same species has obvious implications for sampling strategy. Clearly PP values for a single population wili either be 0 or 100% and as such potentially misleading.

For the main question addressed by this review, i.e. what is the extent of clonal growth within and between populations of aquatic angiosperms?, the number of polymorphic populations, and the number of genotypes detected per population are the most useful measures. The following summaries concentrate mainly on those studies that present comparative data of this sort, together with information on the number of individuals studied per population. In the following analysis, studies where at least 100 individuals, from five or more populations were sampled, are dealt with more thoroughly. I accept that this excludes some studies of rare plants where two or three populations represent a significant proportion of known populations and would thus be a good sample, e.g. *Ceratophyllum echinatum* (Les 1991), *Howellia aquatilis* (Lesica et al 1988) and *Potamogeton nodosus* (Hollingsworth *et al.* 1995c - chapter 5). However in the cases of 'rare' plants, a comparative assessment of genetic variation could be confounded by their rarity, as such species are often genetically depauperate (Avise

1994). Studies that have not given any data on sample size are not treated thoroughly, as it is not possible to ascertain what the data mean. Again, I accept that this is probably ignoring some valuable data, and that many of these studies could well have had suitable sample sizes for population analysis, but where this information is not presented it is impossible to tell.

Table 21 summarises data on 127 investigations into the genetic variation in aquatic plants. Results from a total of 108 taxa consisting of 96 species and five hybrids, as well as some infra-specific taxa, are presented.

Of the 108 taxa reviewed here, no genetic variation was detected in 51 of these (47%): all individuals were represented by a single multi-enzyme phenotype. However of these 51 taxa, 26 (51%) were sampled from only a single population.

Of the 108 taxa, 62 consist entirely of monomorphic populations (57%), with eleven of these showing some variation between populations. However for the 15 taxa where >100 individuals were sampled from five or more populations, only two (13%) consist solely of monomorphic populations.

Table 22 summarises genetic diversity statistics for taxa where a totai of more than 100 individuals were sampled from five or more popuiations.

The mean D value of 0.52 for aquatic plants is slightly higher than that calculated from Ellstrand and Roose (1987) for terrestrial clonal plant species, where D=0.45 (range=0-1) (based on 11 studies of 11 species where more than 100 plants from five or more populations were sampied and monomorphic populations were not excluded).

The mean number of genotypes per population reported by Ellstrand and Roose (1987) for terrestrial clonal plant species is 16.1 (1-167) aithough if the same criteria for inclusion used here, are used for the data from Ellstrand and Roose (1987), the mean number of genotypes per population is 10.4 (1-74). This value is higher than that for aquatic piants where the mean value is 3.8. However, when statistical comparisons were made of the D values and

the number of genotypes per population, between aquatic and terrestrial species no significant difference was found (Mann-Whitney U-statistic n.s) (n= individuals >100, populations >5). Thls is almost certainly due to the large range in variation found within each category.

Table 21. Summary of genetic variation within and between populations of aquatic plants.

No. Pops. = number of populations. Location = sampling region. Σ Indivd = total number of individuals. N/Pop = number of individuals per population. BP = between population variation. %PP = percentage of polymorphic populations (multi-clonal populations). G/Pop = number of genotypes per population. Figures in brackets represent alternative values where more than one value is given. * = See text for explanation.

References in the species column are only given where more than study on a species has been carried out. For all other taxa, see text for reference. The study by Ryan (1988) is not included as the problems of environmental variation in banding patterns make the results difficult to quantify.

Species	**No. Pops.**	**Location**	**Σ Indivd**	**N/Pop**	**B P**	**%PP**	**G/Pop**
Alisma gramineum	4	Europe	-	-	N	25	1-2 mean=1.25
Alisma lanceolatum	9	Europe	-	-	N	0	1
Alisma plantago-aquatica	29 (24)	Europe	-	-	N	0	1
Alternathera philoxeroides	2	U.S.A	225	100-125	Y	100	-
Amphibolis antarctica	1	Australia	-	-		0	1
Amphibolis griffithii	1	Australia	-	-		0	1
Baldellia alpestris	1	Spain	-	-		0	1
Baldellia ranunculoides subsp.*ranunculoides*	5	Europe	-	-	N	0	1
Baldellia ranunculoides subsp. *repens*	3 (5)	Europe	-	-	Y	0	1
Cabomba caroliniana var. caroliniana	2	U.S.A.	82	40-42	Y	100	-

Species	No. Pops.	Location	∑ Indivd	N/Pop	B P	%PP	G/Pop
Cabomba caroliniana var. *multipartita*	3	U.S.A.	133	43-45	Y	100	-
Cabomba pulcherrima	3	U.S.A.	129	42-45	Y	100	-
Callitriche hermaphroditica	2	U.S.A.	9	3-6	Y	100	2-4 mean=3
Ceratophyllum demersum (Les 1986)	1	U.S.A.	20	20		0	1
Ceratophyllum demersum (Les 1991)	9	U.S.A.	262	19-33	Y	44	1-8 mean=2.7
Ceratophyllum echinatum	3	U.S.A.	79	22-32	Y	0	1
Cymodocea angustata	1	Australia	-	-		0	1
Cymodocea rotundata	4	Worldwide	-	6-15	N	0	1
Cymodocea serrulata	3	Worldwide	-	6-15	N	0	1
Eichhornia crassipes	3	U.S.A.	150	40-60	N	0	1
Eichhornia paniculata (Glover & Barrett1986, 1987)	11	Americas	-	80-1480	Y	82	-
Eichhornia paniculata (Barrett& Husband1990)	32	Americas	-	-	Y	100	-
Enhalus acoroides	1	Australia	-	-		0	1
Groenlandia densa	3	Europe	-	-	Y	67	-
Halodule pinifolia	1	Australia	-	-		0	1
Halodule uninervis	4	Worldwide	-	6-15	N	0	1
Halodule wrightii	7	Americas	-	6-15	N	0	1
Halophila deciphiens	1	Americas	-	-		0	1
Halophila engelmannii	1	U.S.A.	-	-		0	1
Halophila hawaiiana	1	Hawaii	-	-		0	1

Species	No. Pops.	Location	Σ Indivd	N/Pop	B P	%PP	G/Pop
Halophila johnsonii	1	U.S.A	-	-		0	1
Halophila minor	6	Oceania	-	-	Y	0	1
Halophila ovalis	6	Worldwide	-	6-15	N	0	1
Halophila stipulacea	1	Kenya	-	-		0	1
Heterozostera tasmanica	1	Australia	-	-		0	1
Howellia aquatilis	4	U.S.A.	-	c. 60	N	0	1
Hydrilla verticillata	36	Worldwide	48	1-7	Y	7.5	1-6 mean=(N>1) 2.5
Hydrocharis morsus-ranae	4	Worldwide	141	4-110	Y	25	1-2 mean=1.25
Lagarosiphon major	3	South Africa	-	c. 5?	Y	0	1
Lagarosiphon muscoides	4	South Africa	-	c. 5?	Y	0	1
Lagarosiphon verticillifolius	1	South Africa	-	c. 5?		0	1
Lemna minor	8	Canada	192	1-30	Y	100	17-25 mean 19.6
Myriophyllum alterniflorum	13	Europe	273	8-25	Y	100	2-19 mean=7.8
Myriophyllum spicatum	7	U.S.A.	80	1-54	Y	0	1
Najas horrida	2	S. Africa	-	-		0	1
Najas marina subsp. *armata*	10	Worldwide	-	2.3-99	Y	30	-
Najas marina subsp. *intermedia*	21	Worldwide	-	2.3-99	Y	62	-
Najas marina subsp. *marina*	23	Worldwide	-	2.3-99	Y	13	-
Phragmites australis	4	Germany	53	5-38	Y	50	-
Phyllospadix scouleri	5	U.S.A.	-	5-15	Y	0	1

Species	No. Pops.	Location	Σ Indivd	N/Pop	B P	%PP	G/Pop
Phyllospadix serrulatus	2	U.S.A.	-	5-15	N	0	1
Phyllospadix torreyi	5	U.S.A.	-	5-15	N	0	1
Podostemum ceratophyllum	11	U.S.A	233	10-28	Y	45	1-11 mean=1.9
Posidonia australis	1	Australia	-	-		0	1
Posidonia sinuosa	1	Australia	-	-		0	1
Potamogeton acutifolius	1	Belgium	-	-		100	-
Potamogeton alpinus	2	Belgium	-	-	Y	100	-
Potamogeton berchtoldii	9	Europe	-	-	Y	78	-
Potamogeton coloratus (Hettiarachchi & Triest 1991)	1	Belgium	-	-		100	1
Potamogeton coloratus (Hollingsworth *et al.* 1995a)	12	Britain	390	8-69	Y	17	1-7 mean=1.6
Potamogeton coloratus (Hollingsworth *et al.* 1995b)	1	England	647	647		100	8
Potamogeton crispus	20	Europe	-	-	Y	90	-
Potamogeton filiformis	13	Europe	270	4-31	Y	69	1-13 mean=4
Potamogeton friesii	1	Finland	-	-		100	-
Potamogeton gramineus	3	Finland	-	-	Y	33	-
Potamogeton lucens (Hettiarachchi & Triest 1991)	5	Europe	-	-	Y	100	-
Potamogeton lucens (Hollingsworth *et al.* 1995c)	2	England	36	6-30	Y	0	1
Potamogeton natans (Hettiarachchi & Triest 1991)	10 (7)	Europe	-	-	Y	100	-
Potamogeton natans (Hollingsworth *et al.* 1995c)	4	Europe	98	2-34	Y	50	1-8 mean=3
Potamogeton nodosus (Hettiarachchi & Triest 1991)	5 (4)	Europe/Eygpt	-	-	Y	100	-

Species	No. Pops.	Location	Σ Indivd	N/Pop	B P	%PP	G/Pop
Potamogeton nodosus (Hollingsworth *et al.* 1995c)	2	England	49	8-41	Y	50	1-3 mean=2
Potamogeton obtusifolius	3	Europe	-	-	Y	67	-
Potamogeton pectinatus (Van Wijk 1988)	10	Worldwide	-	'several'	Y	0?	1?
Potamogeton pectinatus (Hettiarachchi & Triest '91)	70 (72)	Worldwide	-	-	Y	37(43)	-
Potamogeton pectinatus (Hollingsworth *et al.* 1995d)	26	Europe	447	2-37	Y	54	1-4 mean=1.7
Potamogeton perfoliatus	7	Europe	-	-	Y	71	-
Potamogeton polygonifolius	5	Europe	-	-	Y	100	-
Potamogeton pusillus	27 (28)	Belgium	-	-	Y	96	-
Potamogeton schweinfurthii	1	S. Africa	-	-		0	1
Potamogeton striatus	1	Argentina	31	31		0	1
Potamogeton trichoides	14	Europe	-	-	Y	85	-
Potamogeton vaginatus	1	Sweden	24	24		100	4
Potamogeton x *fluitans*	1	England	35	35		9	9
Potamogeton x *schreberi*	1	England	36	36		0	1
Potamogeton x *suecicus*	11	Britain	275	2-66	Y	45	1-6 mean=1.6
Potamogeton x *zizii*	1	Belgium	-	-		100	-
Ruppia cirrhosa var. *cirrhosa*	19	Europe/Med.	-	-	Y	0	1
Ruppia cirrhosa var. *drepanensis*	1	Spain	-	-		0	1
Ruppia maritima var. *brevirostris*	6	Europe/Eygpt	-	-	Y	0	1
Ruppia maritima var. *maritima*	10	Worldwide	-	-	Y	0	1

Species	No. Pops.	Location	Σ Indivd	N/Pop	B P	%PP	G/Pop
Spartina alterniflora (Guenego *et al.* 1988)	2	France	-	-	N	0	1
Spartina alterniflora (Raybould *et al.* 1991b)	1	Britain	13	13		0	1
Spartina anglica (Guenego *et al.* 1988)	3	France	-	-	N	0	1
Spartina anglica (Raybould *et al.* 1991a)	14	Britain	261	2-107	Y	36	1-2 mean=1.4
Spartina maritima (Guenego *et al.* 1988)	3	France	-	-	N	0	1
Spartina maritima (Raybould *et al.* 1991b)	10	Britain	253	2-58	Y	30	1-2 mean= 1.3
Spartina patens	1	U.S.A	346	346		100	101
Spartina x *townsendii*	1	Britain	12	12		0	1
Syringodium filiforme	9	Americas	-	6-15	N	0	1
Syringodium isoetifolium	4	Worldwide	-	6-15	N	0	1
Thalassia hemprichii	3	Worldwide	-	6-15	N	0	1
Thalassia testudinum	7	Americas	-	6-15	N	0	1
Thalassodendron ciliatum	2	Worldwide	-	6-15	N	0	1
Typha angustifolia	5	U.S.A	*	*	N	0	1
Typha domingensis (Mashburn *et al.* 1978)	52	U.S.A	364	7	N	0	1
Typha domingensis (Sharitz *et al.* 1980)	4	U.S.A	*	*	N	0	1
Typha glauca	2	U.S.A.	*	*	Y	100	-
Typha latifolia (Mashburn *et al.* 1978)	74	U.S.A	518	7	N	0	1
Typha latifolia (Sharitz *et al.* 1980)	7	U.S.A	*	*	Y	14	1-2 mean=1.1
Vallisneria americana	12	U.S.A	1104	48-528	Y	-	-

Species	No. Pops.	Location	Σ Indivd	N/Pop	B P	%PP	G/Pop
Zannichellia contorta	4	Spain	-	-	N	0	1
Zannichellia major	6	Europe	-	-	N	0	1
Zannichellia obtusifolia	6	Spain	-	-	N	0	1
Zannichellia palustris subsp. *palustris*	11	Europe	-	-	Y	0	1
Zannichellia palustris subsp. *repens*	1	Denmark	-	-		0	1
Zannichellia palustris s.l.	13 (12)	Europe/Med.	-	-	Y	0	1
Zannichellia pedunculata	21	Europe	-	-	N	0	1
Zannichellia peltata	1	Spain	-	-		0	1
Zostera capensis	1	Kenya	-	-		0	1
Zostera capricornia	3	Australia	-	-	N	0	1
Zostera novazelandica	1	New Zealand	-	-		0	1
Zostera marina (Gagnon*et al.* 1980)	2	U.S.A	200	80-120	N	100	-
Zostera marina (McMillan 1991)	5	Americas	-	3-6	Y	60	1-2 mean=1.6
Zostera marina (Heij & Nienhuis 1992)	5	Europe	256	31-39	Y	100	8-16 mean=11.8
Zostera marina (Fain *et al.* 1992)	4	U.S.A.	50	>10	Y	25	1-2 mean=1.25
Zostera marina (Laushman 1993)	7	U.S.A	1056	72-552	Y	-	-
Zostera muelleri	1	Australia	-	-		0	1

Table 22 Genetic diversity in taxa where the number of individuals sampled is >100, and the number of populations studied is > 5.

Species	D	E	FIS	FST	FIT	HT	HS	GST	G/P	Reference
Ceratophyllum demersum	0.45	-	-	-	-	0.156	0.065	0.580	2.7	Les 1991
Eichhornia paniculata	-	-	0.096	0.528	0.574	0.105*	0.060*	-	-	Glover & Barrett 1987
Lemna minor	0.97	0.882	-	-	-	-	-	-	19.6	Vasseur *et al.* 1993
Myriophyllum alterniflorum	0.79	0.899	0.054	0.496	0.498	-	-	-	7.8	Harris *et al.* 1992
Podostemum ceratophyllum	-	-	-	-	-	0.162	0.025	0.846	1.9	Phibrick & Crow 1992
Potamogeton coloratus	-	-	0.796	0.702	0.939	-	-	-	1.6	Hollingsworth *et al.* 1995a
Potamogeton filiformis	0.49	0.529	-	-	-	-	-	-	4.0	Hollingsworth *et al.* 1995d
Potamogeton pectinatus	0.18	0.264	-	-	-	-	-	-	1.7	Hollingsworth *et al.* 1995d
Potamogeton x suecicus	0.24	0.383	-	-	-	-	-	-	1.6	Hollingsworth *et al.* 1995e
Spartina anglica	-	-	-	-	-	-	-	-	1.4	Raybould *et al.* 1991a
Spartina maritima	-	-	-	-	-	-	-	-	1.3	Raybould *et al.* 1991b
Typha domingensis	-	-	-	-	-	-	-	-	1.0	Mashburn *et al.* 1978
Typha latifolia	-	-	-	-	-	-	-	-	1.0	Mashburn *et al.* 1978
Vallisneria americana	-	-	-	-	-	-	-	0.457	-	Laushman 1993
Zostera marina	-	-	-	-	-	-	-	0.107	-	Laushman 1993
Mean	0.52	0.591	0.315	0.575	0.670	0.141	0.050	0.498	3.80	
Mean value for terrestrial species	0.45	0.587	-	-	-	0.310	0.230	0.224	10.4	see legend

D =Simpsons diversity index, E = Evenness, FIS = Inbreeding within populations, FST = subdivision between populations, FIT = total inbreeding coefficient, HT = total genetic diversity, HS = genetic diversity within populations, GST = proportion of genetic diversity among populations (equivilent to FST), G/P= number of genotypes per population. - = No value available.

* Mean values for HT and HS were only presented for separate Brazilian and Jamaican populations of *Eihhornia paniculata*. The value given here is a mean of these two values, and has not been calculated from averaged raw data.

Mean values for terrestrial clonal species for D, E and G/P where calculated from Ellstrand & Roose (1987) from studies where the number of individuals > 100, and the number of populations > 5.

Mean values for all terrestrial species where obtained for HT, HS and GST from Hamrick & Godt (1989). It was not possible to establish the exact criteria for inclusion in the review by Hamrick & Godt (1989) or to obtain values for clonal species.

No general review of F-statistics was found

Several studies have stressed the importance of life history and ecological characteristics in the determination of electrophoretically detectable variation in plant species (Hamrick 1987, 1989, Hamrick & Godt 1989, Hamrick *et al.* 1979, Loveless & Hamrick 1984). These studies particularly stress the importance of the breeding system and the pollination mechanism on the levels of variation. Considering the studies on aquatic species in which >100 individuals were sampled from five or more populations, the following life history characteristics occur.

Ceratophyllum demersum is a submersed, perennial, self-compatible, monoecious species and is a member of the only hydrophilous genus of dicots (Les 1991). *Eichhornia paniculata* is a tristylous, self-compatible annual or short lived perennial (Glover & Barrett 1986, Glover & Barrett 1987, Barrett & Husband 1990). *Lemna minor* is a highly reduced floating aquatic plant, which flowers infrequently, but is well suited to vegetative dispersal by biotic vectors, with the fronds of the plants being able to survive desiccation for long periods of time (Vasseur *et al.* 1993). *Myriophyllum alterniflorum* is an anemophilous, submersed, hermaphrodite perennial (Harris *et al.* 1992). *Podostemum ceratophyllum* is a predominantly autogamous, hermaphrodite perennial that grows in waterfalls and rapids, with its flowering restricted to the dry season (Philbrick & Crow 1992). *Potamogeton coloratus* is an anemophilous perennial, *P. pectinatus* and *P. filiformis* are both perennial species, and their pollination appears to be at the water surface or below, by bubble borne self-pollination. *P.* x *suecicus* is a sterile perennial hybrid. All of these species of *Potamogeton* have bisexual hermaphroditic flowers and are thought to be self-compatible (Hollingsworth *et al.* 1995a,d,e - chapters 3,6,7). *Spartina anglica* and *S. maritima* are both perennial, hermaphrodite, anemophilous salt marsh species (Raybould *et al.* 1991a , 1991b). *Typha domingensis* and *T. latifolia* are both self-compatible, monoecious, emergent, anemophilous perennials. *Vallisneria americana* is a submerged, dioecious, epihydrophilous perennial (Laushman 1993). *Zostera marina* is a submerged , monoecious, epihydrophilous, perennial or annual species (Laushman 1993, Heij & Nienhuis 1992).

Correlations between life history characteristics and genetic diversity are not really evident at this level, with a great range of variability observed within categories. Thus *Potamogeton coloratus* and *Myriophyllum alterniflorum* are both perennial, anemophilous, submerged, bisexual, hermaphrodite diploids, yet the number of clones per population varies considerably from a mean of 1.6 (*P. coloratus*) to a mean of 7.8 (*M. alterniflorum*). Conversely the hydrophilous *Ceratophyllum demersum* has a very similar mean number of genotypes per population to the aerial flowering *Podostemum ceratophyllum* (2.7 & 2.9 respectively). There are simply not enough studies of species in each life history category for a representative mean to be generated for comparative purposes. Table 22 shows that clonal growth (as measured by the number of genotypes detected per population) is so extensive that it affects the levels of genetic variation to such an extent, that predictions of genetic diversity based on other life history characteristics may not be safe.

Of the 15 taxa where sampling allows comparisons to be made, *Lemna minor* is the most variable in terms of genotypes per population. It is interesting that a high number of different genotypes per population was found despite a lack of flowering individuals. Vasseur *et al*. (1993) attributed this to multiple introductions (which considering the small size of *Lemna* represents a plausible hypothesis), rare sexual events and somatic mutation. As full results were not presented for *Vallisneria americana* (part of an ongoing study) and *Zostera marina* (results in preparation) (Laushman 1993) it is difficult to compare these species. The studies on *Eichhornia paniculata* (Glover & Barrett 1986, Glover & Barrett 1987, Barrett & Husband 1990) do not present data on numbers of genotypes detected per population, but data on the percentage of polymorphic populations is presented (although for the study by Husband & Barrett 1990, it appears that only polymorphic populations were selected). The results that are given for these taxa do, however, suggest that high levels of variation are present compared to other aquatic species.

Myriophyllum alterniflorum also shows relatively high levels of variation for an aquatic species, with Harris *et al.* (1992) attributing this to its frequent

flowering, anemophilous pollination syndrome and its abundance and long post-glacial history in the study sites. However genetic differences between species with similar life history characteristics caution against using the data presented here to make predictions based on such factors.

Where low levels of genetic variation are found, there are a number of factors that may be important. In some *Potamogeton* species, although fruit set is often abundant, seedling recruitment is thought to be low to non-existent (Hollingsworth *et al.* 1995a,d - chapters 3,6). Furthermore if extensive clonal growth occurs, pollen transfer between adjacent ramets may well be equivalent to a genetic selfing, even if it represents a physical outcrossing, and thus any seed that does germinate may well be of a very similar genotype to that of its parent(s). It is probable that extensive clonal growth, rare seedling establishment and inbreeding all contribute to the generally low values of genetic variation obtained. An exception to this is the only predominantly annual aquatic species for which comparative data are available, namely *Eichhornia paniculata* . The relatively high levels of genetic variation found within some *Eichhornia paniculata* populations could be attributed to its tristylous annual life cycle. Clonal growth is limited and regeneration is chiefly from seed, which as a result of its floral morphology is often the product of cross-pollination.

In the case of taxa of hybrid origin, such as *Potamogeton* x *suecicus* (a sterile hybrid) and *Spartina anglica* (an allopolyploid of recent origin), the levels of variation are inextricably linked to those shown in the parental species (barring somatic mutations). In dioecious species with efficient vegetative spread, there is the possibility of single sex populations, which have no means of generating variation by recombination.

Strong evidence was obtained for multi-clonal populations (as opposed to sexual populations) in all cases where data on the number of genotypes per population was presented. In all cases, the number of detectable genotypes was low, except for *Lemna minor* which is discussed above. In taxa where allelic segregation was apparent, no evidence was found to suggest that individuals showing high levels of heterozygosity were at any competitive

advantage compared to those individuals that were predominantly homozygous at the loci examined.

Of the three studies that examined populations in detail (Hollingsworth 1995b -chapter 4, Laushman 1993, and Silander 1979), all showed some evidence of partitioning of genetic variation within a population. This clearly has sampling implications, showing the need for sampling strategy to be determined on a site by site basis.

Although only a limited number of studies on aquatic plants have reported values for Nei's gene diversity statistics, the mean value of total genetic diversity (H_T) is lower for aquatic (0.141) than for terrestrial species (0.310) (Table 22). Likewise, for the amount of genetic diversity within populations (H_S), the mean value obtained for aquatic taxa (0.050) is lower than the mean value for terrestrial species (0.230). Conversely, the mean amount of genetic diversity held between populations, is higher in aquatic (G_{ST}=0.498, F_{ST}=0.575), than in terrestrial species where the mean G_{ST} = 0.224. This indicates that in aquatic plants genetic diversity is held between rather than within populations. The reverse is true for terrestrial species where the majority of genetic variation is held within populations (Hamrick & Godt 1989). If, however, a comparison is made with mean values for terrestrial selfing species, where H_T=0.334, H_S= 0.149 and G_{ST}=0.510, a slightly different picture emerges. The total gene diversity (H_T) in aquatic plants is still lower than in terrestrial selfing species, but the values for H_S and G_{ST} are more similar. As Hamrick & Godt (1989) did not present raw data for the individual species in their study, it is not possible to test the statistical significance of the above comparisons.

Isolation of populations in water bodies such as lakes, surrounded by unsuitable terrestrial habitat, can lead to low levels of gene flow between populations (Laushman 1993). Wright's multilocus F_{ST} is considered to be equivalent to Nei's G_{ST} (Swofford & Selander 1981), and Wright (1978) considered a F_{ST} value of >0.25 as indicating very great subdivision. In this review, reported F_{ST} values ranged from 0.496-0.702 and G_{ST} values ranged from 0.107-0.846. Interestingly the only value below the >0.25 level was

GST=0.107 for *Zostera marina* , the only marine species for which such values were presented. Laushman (1993) suggested that the sea may be a less fragmented habitat and comparable to the situation in many terrestrial habitats, in being large and relatively uniform, and thus not as restrictive to gene flow between populations as inland water bodies surrounded by land.

As many of the aquatic taxa used here for comparisons are self-compatible it is not clear to what extent the high GST and FST values are due to habitat fragmentation (e.g. lakes as islands in a terrestrial sea) or due to the effects of self-pollination, which appears to generate similarly high values in terrestrial selfing species. The high GST value for the obligately outcrossing dioecious *Vallisneria americana* , coupled with the low GST value for *Zostera marina,* which occurs in a relatively unfragmented habitat, does, however, suggests that self-pollination is not entirely responsible for the levels of subdivision between populations, and that habitat fragmentation may well be a significant factor. All of these generalisations using F-statistics and gene diversity statistics should, however, be treated with some caution as clonal growth will almost certainly have affected some of these values.

SUMMARY

That clonal growth occurs in aquatic plants is a certainty - extensive rhizomatous spread can be observed in many species in many populations. Of the 127 reports of genetic variation in aquatic plants, surprisingly few, however, offer enough conclusive evidence to gain an accurate picture. From those that do, the most common factors affecting the levels of genetic variation appear to be clonal growth, rare seedling recruitment, inbreeding and high levels of population subdivision. Although no significant difference was noted between the levels of genetic diversity within populations of clonal aquatic and clonal terrestrial species, there does appear (albeit from a very small sample) to be a difference between aquatic and terrestrial species *sensu lato* as measured by gene diversity statistics. Thus the high levels of clonal growth, rare seedling recruitment, inbreeding, and population subdivision that seem to occur in many aquatic species results in them having lower levels of genetic variation than terrestrial species *per se*,

and what variation there is tends to be distributed between rather than within populations

It does, however, remain, that no firm conclusions can be drawn at present due to the lack of comparative studies, although a strong recommendation has emerged that a mention of sample size and the number of clones detected per population should be included in all future studies, even if this is only in the form of a total, a range, and a mean. This enables studies that were clearly not investigating intra-population variation to be easily identified as such. It would also prevent suitable studies from being excluded from comparisons.

APPENDIX 1 ISOZYME LAB. GUIDE

CONTENTS

Subject	Section
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Protocol	2
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SECTION 1: INTRODUCTION

This Isozyme Lab Guide is based on a compilation of information from *Isozymes in Plant Biology* (Soltis & Soltis 1989), *Molecular Systematics* (Hillis & Moritz 1990), *Handbook of Enzyme Electrophoresis in Human Genetics* (Harris & Hopkinson 1976), and *The Use of Isozyme Analysis In Fungal Taxonomy and Genetics* (Micales 1986), personal communications from Richard Abbott, (St Andrews University) and Richard Gornall (Leicester University) along with my own modifications.

Suggestions for improvements and alterations welcome!

SECTION 2 : ISOZYME PROTOCOL

A. GEL PREPARATION

EQUIPMENT NEEDED: 1L Buchner flask & solid rubber bung
Gel former & clean glass plate
Bunsen burner
Heat resistant gloves
Vacuum pump
Safety cage
Glass pipette & teat

CHEMICALS NEEDED: Starch
Gel Buffer

PROCEDURE:

1. Place the gel former on a level surface (check), preferably on top of a paper towel. The glass plate should be placed next to the gel former.

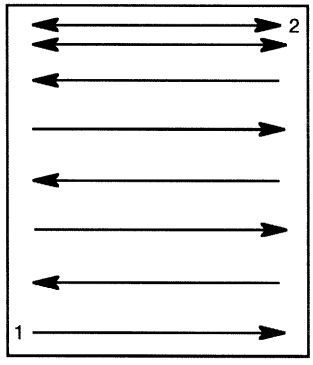
2. Weigh out the required amount of starch. 36g per 300ml gel buffer (15-25 samples) 42g per 350ml gel buffer (20-30 samples) for a 12% gel. Place the starch in the Buchner flask. For information on gel buffers see section 3.

3. Measure out the required volume of gel buffer, and pour into the flask with the starch. Agitate the solution by swirling for 30 seconds. Make sure that all of the starch has gone into suspension.

4. Heat the solution over a Bunsen burner, and continue to agitate. After a few minutes the solution will become viscous, (this represents the halfway stage). With continued swirling the solution will become clearer and eventually will start to boil. Remove from the heat source as soon as the solution begins to boil. From this stage onwards it is important to work quickly as the starch will be cooling and hence setting all the time, increasing the difficulty of pouring the gel successfully.

5. Attach the vacuum hose to the flask's side arm and place a rubber bung on top of the flask. Ensure a vapour trap is in place between the flask and the vacuum pump to prevent damage to the pump. Place the flask inside the safety cage and degas until the small bubbles have disappeared and large bubbles are distributed evenly throughout the solution. Switch off the vacuum pump, slide the rubber bung slowly off the top of the flask and then disconnect the vacuum hose.

6. Pour the gel into the gel former. Start at the edge of the gel former closest to, and pour the starch evenly in a horizontal zig zag pattern working gradually away from the worker to cover the base of the gel former. Once the far end of the gel former has been reached, any remaining starch in the flask should be poured to form a ridge across the far end of the gel. See diagram.



7. Use the glass pipette to remove any air bubbles greater than 3mm in diameter or foreign bodies.

8. Place one edge of the glass plate at the far end of the gel former and lower it back towards the worker, which will subsequently push with it the ridge of excess starch. Continue to lower gently until the glass plate is resting fully on the gel former. Then gently press the glass plate down allowing a slight overlap of starch around the top of the gel former to make a seal.

9. Allow to cool for 30 minutes, then move to another part of the bench for further cooling down to room temperature.

10. When room temperature is reached, place the gel in the fridge and allow to cool to 4^{o}C.

B. SAMPLE PREPARATION

EQUIPMENT NEEDED: Washing up bowl filled with ice
Ceramic welled paint trays (grinding plates)
Glass rod with flared end
Fine forceps
Filter paper wicks 7mm x 3mm (Whatman 3mm)
100ml Beaker
Food dye

CHEMICALS NEEDED: Extraction buffer

PROCEDURE:

Note: It is usual to prepare the samples while the gel is cooling. It is important to remember to put the gel in the fridge when it reaches room temperature so that it is ready for loading when the samples are prepared.

1. Fill the bowl with ice. Fill the beaker with water and place in the ice bucket. Place the ceramic grinding plates on ice (if the ice supply is limited the grinding plates can be taped to a blue ice pack).

2. After approximately 5 minutes, when the plates are cold, place a 5mm square portion of fresh, healthy leaf tissue in one of the wells. Add 2 drops of extraction buffer, and using the flared glass rod, homogenise the tissue. Initially it may be necessary to experiment with different tissue to buffer volumes to get the ideal ratio.

3. Once the sample is homogenised, place a filter paper wick in the homogenate and leave it to absorb the extract. Repeat the above for however many samples are required. Note: It is important to decide on a system to identify which samples are in which wells.

4. Place a drop of food dye in a empty well and add a filter paper wick.

C. ELECTRODE TANK PREPARATION

EQUIPMENT NEEDED: Electrode tank
Cellulose sponges
CHEMICALS NEEDED: Electrode buffer (approx. 700ml per gel)

1. Make sure the electrode tank and sponges have been thoroughly washed and dried. If the sponges are still damp with distilled water from being washed, rinse them with electrode buffer prior to use. Even when sponges are new they need washing thoroughly before the first use.

2. Place a sponge in each electrode tank. The sponges need to be at least the same width as the gel to be run. Fill the tanks to within 1cm of the top, with electrode buffer. For information on electrode buffers and running multiple gels off the same power pack see section 3.

D. GEL LOADING

EQUIPMENT NEEDED: Fine forceps
2 Blue ice packs per gel
2 A4 Plastic bags per gel
Clingfilm
Paper towels
Ruler
Flat ended spatula
Wide gauge drinking straw

1. Remove the gel from the fridge. Carefully prise the glass plate off the surface of the gel using the flat end of a spatula as a lever between the gel former and the glass plate.

2. Run the spatula around the margins of the gel, trimming any excess from the edge of the gel former.

3. Cover the surface of the gel with cling film avoiding any creases or air bubbles.

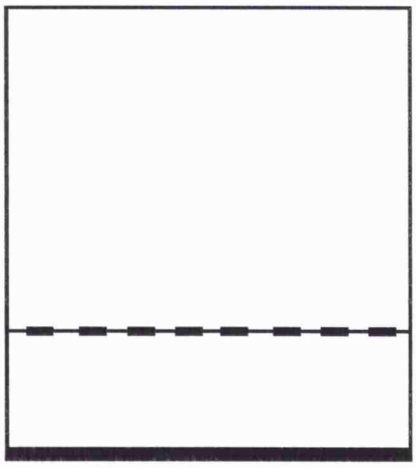
4. Mark out a 8cm running zone on the side of the gel former allowing at least 5cm from either end of the former. Most enzymes will migrate towards the positive electrode thus the wicks are usually placed closer to the cathodal edge of the gel. However it is necessary to determine this experimentally as different enzymes and different taxa behave differently, thus for initial screening runs, the wicks are often placed across the middle of the gel with both anodal and cathodal slices stained for enzyme activity.

5. Peel back the cling film to the beginning of the running zone. Using a ruler and the flat end of a spatula cut the gel through to the base, along the start of the running zone.

6. Pull one side of the gel back slightly causing a gap to open up. Using fine forceps pick up a filter paper wick, dab any excess sample extract off onto a paper towel and place in the gap at the start of the running zone. Repeat this for the remaining samples and the food dye marker, allowing at least 3mm between wicks.

7. When all the wicks are in place, place a straw between the narrowest portion of the gel and the gel former end wall. This is most easily done by a rolling action. The placing of the straw keeps the two parts of the gel in close contact maximising conductivity.

Loaded gel

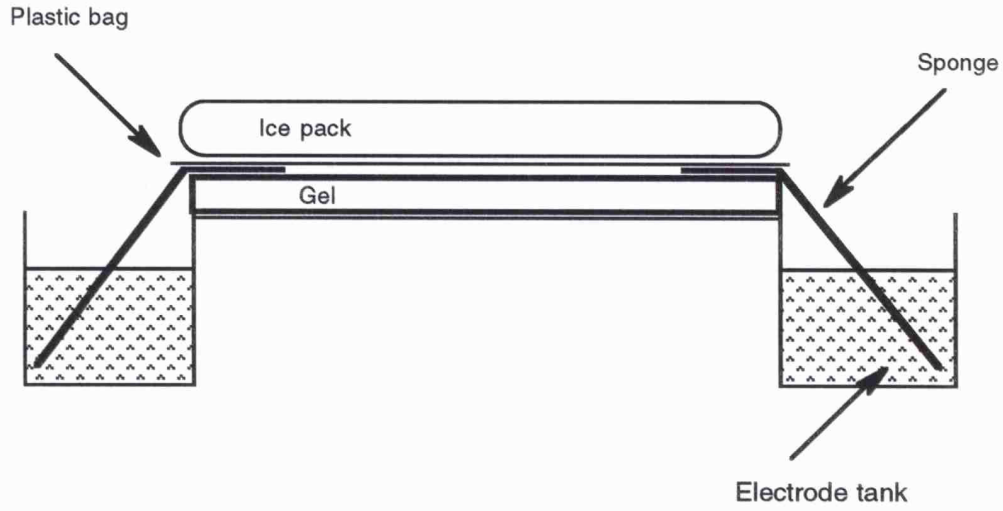


8. Dab a paper towel across the line of wells to absorb any excess extract squeezed up by the compressing force of the straw.

9. Pull the cling film back over the wicks but leave 4cm of gel exposed, expose a similar area of gel at the opposite end. Place the gel and gel former on the electrode rig and overlap the sponges of each tank with the exposed gel surface. Ensure the sponges are flat and evenly placed on the gel surface. Place any excess cling film at either end of the gel over the sponge-gel overlap.

10. Place 2 A4 plastic bags on top of the gel and place two blue ice packs side by side on top of the bags. The purpose of the plastic bags is to prevent the ice packs freezing the top of the gel.

Loaded Rig.



11. Place the entire rig in the fridge, connect to power pack and run for approximately 4hrs. Place a electricity hazard warning sign on the fridge door. Remember :

MOST ISOZYMES RUN TOWARDS THE POSITIVE TERMINAL

For $LiBO_3$ buffers run at constant voltage of 240V, this should give a current of approx. 70mA decreasing to approx. 25mA after 4hrs. For MC8 buffers run at constant current of 40 mA, this should give a voltage of approx. 150V. After the gels have been running for approximately 10 mins check that the dye is migrating in the correct direction.

E. STAIN PREPARATION

EQUIPMENT REQUIRED: 50ml conical flasks
Spatula
Fine balance
60°C water bath
Bunsen burner
50 ml blue top tubes

CHEMICALS REQUIRED: Tris HCL buffers
Enzyme staining reagents (see recipe section)

1. Decide which enzymes to stain for.

2. Lay out the recipe cards and conical flasks. Also make out a check list so that the chemicals can be crossed off as they are added. This is essential if more than 3 or 4 enzymes are to be stained for.

3. Weigh out chemicals according to instructions in recipe section. Many of the reagents used in the staining recipes are very hazardous, treat with appropriate caution ie: fume hood and gloves etc. Agar solutions should be made up and placed in a 60°C water bath in blue top tubes until required.

4. Place the staining trays on a bench, in front of the relevant conical flask and label carefully. For agar based stains, translucent staining trays should be used, enabling the gel to be photographed while still in the tray.

F. GEL SLICING AND STAINING

EQUIPMENT REQUIRED: Gel slicer
4lb fishing line
Thin glass plate (2-3mm)
Enamel tray

1. Place the gel slicer on top of the upturned enamel tray - raising the slicer off the bench makes slicing easier.

2. Switch off the power pack, wait till voltage drops to zero. Remove the gel and the gel former from the electrode rig.

3. Cut the gel using a spatula at the end of the running zone ie: 8 cm from the wicks. Discard the portion of gel in front of the running zone.

4. Cut a notch in the top right hand corner of the gel to facilitate orientation.

5. Carefully lift up the running zone area of the gel, supporting it underneath and keeping it as flat as possible. It may be necessary to use a spatula to separate the running zone strip from the cathodal strip.(Note when separating two pieces of gel with wicks at their interface, the wicks will tend to stick to the piece of gel that is not lifted.) Place the running zone portion of gel on a paper towel and gently dab the upper and lower surface dry.

6. Remove any wicks still attached to the gel.

7. Lift the gel up and place top side up on the gel slicer. Check that there are no air bubbles between the gel and the slicer. Lay the glass plate on top of the gel - do not squash the gel.

8. Hold the fishing line tight, across the farthest end of the gel slicer from the worker. Pull the line back towards you, maintaining a steady pace and downward tension until the line has sliced fully through the length of the gel.

9. Invert the slicer, glass plate and gel, and using a spatula, prize the gel slicer free. Carefully lift up the thin slice off the gel and supporting it underneath, transfer it to the relevant staining tray.

10. Dab the newly exposed surface of the remainder of the gel with a paper towel, place on the gel slicer and repeat steps 7-10 for the remaining slices. From a 7mm thick gel it should be possible to get 4 slices plus the very top slice which is not used because it has uneven running characteristics.

11. Add reagents to gels in staining trays according to instructions in the recipe section. For non-agar based stains carefully rock the staining tray so that the gel slice is free from the base of the tray. Cover the tray with cling film and place in an incubator in the dark at 38^{o}C. For agar based stains add the agar solution very slowly and mix gently avoiding air bubbles being formed. Pour onto the gel slice evenly ensuring the entire surface is covered. Cover the tray with cling film and place in the incubator as above. The dye chemicals used in most enzyme stains are highly light sensitive and thus once chemicals are added to the staining trays, they should be placed in the dark as soon as possible.

12. The gel slices should be left in the incubator until the bands have developed, the time this takes varies from enzyme to enzyme and taxon to taxon but is usually between 10 minutes and 1 hour. After a few isozyme runs it will become apparent how long to leave gels staining. Beware of over staining gels where the background stains up and band definition deteriorates.

13. When the bands have appeared, pour off the staining solution into a suitably labelled container for subsequent disposal by designated method eg:solvent waste system.

14.For non-agar based stains rinse the gel slices in water, place the gel on a glass plate or cling film, label, and photograph using a SLR camera with close up tubes, using illumination from a light box. Make a note of results in case of problems with film development. Agar based stains are best photographed while still in the staining tray.

15. Non-agar based stained gels can be stored in the fridge after being rinsed in 50% glycerol. Agar based stained gels can be stored in the fridge. All gels do however tend to deteriorate with time so photographed gels are preferred.

SECTION 3: ELECTRODE AND GEL BUFFER RECIPES

The two buffer systems given below, used with the general extraction buffer have provided good results from a wide range of taxa for a number of enzyme systems. Many other buffer recipes exist, but these two certainly represent a good starting point. All the buffers outlined below can be kept for up to 4 weeks in the fridge.

Lithium Borate System

Electrode Buffer	1 litre	2 litre	3 litre	4 litre
H_3BO_3	11.9g	23.8g	35.7g	47.6g
LiOH	1.2g	2.4g	3.6g	4.8g

Dissolve in H_2O, pH should be 8.1, adjust with dry constituents if necessary. Approx. 700ml should do 1 gel.

Gel Buffer	1 litre	2 litre	3 litre	4 litre
Tris base	5.45g	10.9g	16.35g	21.8g
Citric acid	1.28g	2.56g	3.84g	5.12g

For each litre of buffer dissolve tris and citric acid in 100ml of electrode buffer and 900ml of H_2O. pH should be 8.3, adjust with dry constituents if necessary.

Lithium borate gels should be run at a maxiumum of 240V and 70 mA.

MULTIPLE GELS OFF THE SAME POWER SUPPLY: It is possible to run more than one gel at a time off the same power pack providing the pack is powerful enough. If two gels are to be run off a power pack without two independent output controls, it is important that the gels are of similar dimensions and identical buffers are used. Set the voltage as per normal, and double the current.

Morpholine Citrate System

Electrode Buffer	1 litre	2 litre	3 litre	4 litre
Citric acid	8.4g	16.8g	25.2g	33.6g

For each litre of buffer dissolve the citric acid in 900ml H_2O and adjust pH to 8.0 with N-(3-aminopropyl)-morpholine (approx. 17 ml per litre). Make up to 1 litre using H_2O. Approx. 700ml should do 1 gel.

Gel Buffer: Dilute the electrode buffer with H_2O as follows:

For 300ml gel : 12ml electrode buffer: 288ml H_2O

For 350ml gel : 14ml electrode buffer: 336ml H_2O

Morpholine citrate gels should be run at a maximum of 240 V and 40 mA.

EXTRACTION BUFFER RECIPE

$LiBO_3$ Gel Buffer	50ml
KCl	37mg
$MgCl_2.6H_20$	10mg
EDTA tetrasodium salt	18mg
PVPP	25mg
Triton x100	0.5ml
2-mercaptoethanol	1.25ml

Place in a 100ml conical flask, foil wrap and keep in the fridge.

TRIS STAINING BUFFERS

0.1M Tris HCL = 12.11g Trizma base per litre of H_2O.

Adjust to required pH using conc. HCL. Make sure the pH meter used is tris compatible.

SECTION 4: ENZYME STAINING RECIPES

NOTE:

1. Below is a compilation of isozyme staining recipes that have shown successful results on taxa examined at Leicester University Botany Department using the enclosed protocol and buffer recipes. Recipes exist for many other enzyme systems and are to be found in the texts mentioned in the introduction.
2. Recommended slice number is a guide based on results from relatively few taxa and may not be appropriate for all. Slice 1 refers to the bottom slice, slice 4 to the highest used slice (slice 5 the actual top slice should be discarded due to uneven running characteristics). The slices closest to the bottom of the gel generally produce the best results, however some enzymes are less affected by slice position (those with recommend slice number 1-4) and these can be stained for with the upper slices.
3. Beware of interpreting 'ladders' of bands as multiple isozymes, numerous evenly spaced bands may be due to break down products.
4. 'Negative' bands appearing on gels whose staining recipe involves the use of MTT are usually considered to be Superoxide dismutase.

AAT (GOT)

0.1M Tris/HCL pH8.5	50ml
a-Ketoglutaric acid	18mg
Aspartic acid	65mg
PVP 40T	250mg
EDTA Na_2 salt	25mg
Disodium hydrogen phosphate	710mg
Pyridoxal-5-phosphate	1mg
Fast blue BB salt	200mg

Add all the chemicals to the buffer solution except the fast blue and the pyridoxal-5-phosphate (this solution can be stored in the fridge for up to 3 weeks). After weighing out, foil wrap the fast blue and the pyridoxal-5-phosphate and add to the buffer only immediately prior to staining.
Recommended slice = 1-3

ADH

0.1M Tris/HCL pH8.0	50ml
Absolute ethanol	2-5ml
ATP	25mg
NAD	10mg
MTT	10mg
PMS	3mg

Combine the NAD, MTT and PMS in 1ml of H_20, and add to the buffer along with the ATP and absolute ethanol just before staining. Make sure that the staining dish is covered in the incubator as ethanol evaporation can cross contaminate neighbouring staining dishes. This enzyme can show environmentally induced variation. Recommended slice =1-4

ALD (FBA)

Solution 1.

Agar	333mg
0.1M Tris/HCL pH8.0	25ml

Solution 2.

0.1M Tris/HCL pH8.0	25ml
Arsenic acid sodium salt	75mg
Glyceraldehyde-3-phosphate dehydrogenase	90ul
Fructose-1,6-diphosphate sodium salt	200mg
NAD	10mg
MTT	10mg
PMS	3mg

Heat solution 1. till it goes clear and place in a water bath at 60°C. Add the arsenic acid and the fructose-1,6-diphosphate to solution 2. Combine the NAD, MTT and PMS in 1ml of H_20, and add to solution 2 along with the G_3PDH just before staining. Remove solution 1 from the water bath, then gently mix into solution 2. Pour carefully onto the gel and allow agar to set. Recommended slice = 1-3

α **EST** (Non specific)

0.1M Tris/HCL pH7.5	50ml
α-Napthyl acetate solution	3ml
Fast blue BB salt	50mg

α-napthyl acetate solution consists of 1% α-napthyl acetate in 50% acetone. This is made by dissolving the α-napthyl acetate in the acetone, then adding the water. Add this to the tris buffer along with the fast blue immediately prior to staining. Staining can be carried out at ambient temperature in the light. Note some recipes suggest fast blue RR salt as the dye , however this is much less soluble than the BB salt. Recommended slice = 1-3

GDH

0.1M Tris/HCL pH7.5	50ml
L-Glutamic acid sodium salt	210mg
ATP	25mg
NAD	10mg
MTT	10mg
PMS	3mg
$MgCl_2$	50mg

Add the glutamic acid and the $MgCl_2$ to the buffer. Combine the NAD, MTT and PMS in 1ml of H_20, and add to the buffer along with the ATP just before staining. Recommended slice = 1-4

G6PDH

0.1M Tris/HCL pH7.5	50ml
Glucose-6-phosphate sodium salt	50mg
NADP	10mg
MTT	15mg
PMS	3mg

Add the glucose-6-phosphate to the buffer, Combine the NADP, MTT and PMS in 0.5 ml of 10% $MgCl_2$ and add to the buffer just before staining. Resolution of this enzyme can be improved by the addition of 15mg NADP per 300ml of gel buffer (added just before degasing) and 15mg of NADP per 300ml of electrode buffer (negative tank only). Recommended slice = 1-4

GPI

Solution 1.

Agar 333mg	
0.1M Tris/HCL pH8.0	25ml

Solution 2.

0.1M Tris/HCL pH8.0	25ml
Fructose-6-phosphate	40mg
NADP	7mg
MTT	12mg
PMS	3mg
Glucose-6-phosphate dehydrogenase	15µl

Heat solution 1. till it goes clear and place in a water bath at 60°C. Add the fructose-6-phosphate to solution 2. Combine the NADP, MTT and PMS in 0.5 ml of 10% $MgCl_2$ and add to solution 2 along with the G_6PDH just before staining. Remove solution 1 from the water bath, then gently mix into solution 2. Pour carefully onto gel and allow agar to set. Recommended slice = 1-2.

HEX

Solution 1.

Agar 333mg	
0.1M Tris/HCL pH8.0	25ml

Solution 2.

0.1M Tris/HCL pH8.0	25ml
Glucose	100-200mg
NADP	10mg
MTT	15mg
PMS	3mg
EDTA Na_2	25-50mg
Glucose-6-phosphate dehydrogenase	20-40µl
ATP	125mg

Heat solution 1. till it goes clear and place in a water bath at 60°C. Add the glucose and the EDTA to solution 2 . Combine the NADP, MTT and PMS in 0.5 ml of 10% $MgCl_2$ and add to solution 2 along with the ATP and the G_6PDH just before staining. Remove solution 1 from the water bath, then gently mix into solution 2. Pour carefully onto gel and allow agar to set. Recommended slice = 1-4.

IDH

0.1M Tris/HCL pH8.0	50ml
Isocitric acid	100mg
NADP	10mg
MTT	15mg
PMS	3mg

Add the isocitric acid to the buffer. Combine the NADP, MTT and PMS in 0.5 ml of 10% $MgCl_2$, and add to the buffer just before staining. Resolution of this enzyme can be improved by the addition of 15mg NADP per 300ml of gel buffer (added just before degasing) and 15mg of NADP per 300ml of electrode buffer (negative tank only). Recommended slice = 1-3.

LDH

0.1M Tris/HCL pH8.0	50ml
Lactic acid lithium salt	100mg
NAD	10mg
MTT	10mg
PMS	3mg

Add the Lactic acid to the buffer. Combine the NAD, MTT and PMS in 1ml of H_20, and add to the buffer just before staining. Recommended slice = 1-4

MDH

0.1M Tris/HCL pH8.5	50ml
Malic acid	750mg
NAD	10mg
MTT	10mg
PMS	3mg

Dissolve the malic acid in minimum volume 5M NaOH, neutralise to pH 6.5-7.5 by adding 5M NaOH (note when pH 5.5 is reached add remaining NaOH very slowly, when the titration point is reached one drop can cause a sudden pH jump) then add it to the buffer. Combine the NAD, MTT and PMS in 1ml of H_20, and add to the buffer just before staining. 4% Sucrose added to the gel buffer can sharpen MDH isozymes. Recommended slice = 1-3

ME

0.1M Tris/HCL pH8.0	50ml
Malic acid	750mg
NADP	10mg
MTT	15mg
PMS	3mg
$MgCl_2$	50mg

Dissolve the malic acid in minimum volume 5M NaOH, neutralise to pH 6.5-7.5 by adding 5M NaOH (note when pH 5.5 is reached add remaining NaOH very slowly, when the titration point is reached one drop can cause a sudden pH jump), then add it to the buffer. Add the $MgCl_2$ directly to the buffer. Combine the NADP, MTT and PMS in 0.5 ml of 10% $MgCl_2$, and add to the buffer just before staining. Resolution of this enzyme can be improved by the addition of 15mg NADP per 300ml of gel buffer (added just before degasing) and 15mg of NADP per 300ml of electrode buffer (negative tank only). Recommended slice = 1-3

SKDH

0.1M Tris/HCL pH8.0	50ml
Shikimic acid	60mg
NADP	10mg
MTT	15mg
PMS	3mg

Add the shikimic acid to the buffer. Combine the NADP, MTT and PMS in 0.5 ml of 10% $MgCl_2$, and add to the buffer just before staining. Resolution of this enzyme can be improved by the addition of 15mg NADP per 300ml of gel buffer (added just before degasing) and 15mg of NADP per 300ml of electrode buffer (negative tank only). Recommended slice = 1-2

6PGD

0.1M Tris/HCL pH8.0	50ml
6-Phosphogluconic acid barium salt	50mg
NADP	10mg
MTT	15mg
PMS	3mg
$MgCl_2$	50mg

Add the 6-phosphogluconic acid and the $MgCl_2$ to the buffer. Combine the NADP, MTT and PMS in 0.5 ml of 10% $MgCl_2$ and add to the buffer just before staining. Resolution of this enzyme can be improved by the addition of 15mg NADP per 300ml of gel buffer (added just before degasing) and 15mg of NADP per 300ml of electrode buffer (negative tank only). Recommended slice = 1-3

PGM

0.1M Tris/HCL pH7.5	50ml
Glucose-1-phosphate, sodium salt	100mg
NADP	10mg
MTT	15mg
PMS	3mg
ATP	25mg
Glucose-6-phosphate dehydrogenase	20-40µl

Add the glucose-1-phosphate to the buffer, Combine the NADP, MTT and PMS in 0.5 ml of 10% $MgCl_2$ and add to the buffer along with the G_6PDH and the ATP just before staining. Recommended slice = 1-3

SECTION 5: ISOZYME INFORMATION

ENZYME	NUMBER OF ISOZYMES	SUB-UNIT STRUCTURE	SUBCELLULAR COMPARTMENT	FUNCTION
AAT (GOT)	4	Dimer	C,P,MT,MB.	Degradation of aminio acids
ACO	1-3	Monomer	C,MT	TCA Cycle
ACP	2-4	Monomer Dimer	Various	Hydrolysis of phosphate esters
ADH	1-3	Dimer	C	Glycolysis
ALD	2	Tetramer	C,P	Glycolysis
EST	2-10	Monomer Dimer	C	Hydrolysis of carboxylic ester bonds
G6PDH	2	Dimer	C,P.	Calvin cycle
GPI (PGI)	2	Dimer	C,P.	Glycolysis
GDH	1	Hexamer	C	Biosynthesis and degradation of amino acids
HEX	2-3	Monomer	C,P,MT	
IDH	1	Dimer	C	TCA CYCLE

ENZYME	NUMBER OF ISOZYMES	SUB-UNIT STRUCTURE	SUBCELLULAR COMPARTMENT	FUNCTION
LDH	1	Tetramer	C	Glycolysis
MDH	3	Dimer	C,MT,MB	Oxidative phosphorylation
ME	1	Tetramer	C	TCA Cycle
MPI		Monomer		Glycolysis
PGM	2	Monomer	C,P.	Glycogen and starch degradation. Sucrose formation
PGD	2	Dimer	C,P.	Hexose monophosphate shunt
PO	2-13	Monomer Dimer	C,CW	Various, including lignin synthesis
SKD	1-2	Monomer	C,P.	Synthesis of constituents with aromatic rings
SOD	3	Dimer Tetramer	C,P. MT	Inactivates superoxide radicles
TPI	2	Dimer	C,P.	Calvin cylcle, glycolysis
XDH		Monomer Dimer		Purine metabolism

SUBCELLULAR COMPARTMENT ABBREVIATIONS

C	Cytosol
CW	Cell wall
MB	Microbody
MT	Mitochondria
P	Chloroplast

ENZYME ABBREVIATIONS

AAT	Aspartate Aminotransferase =GOT
ACO	Aconitase Hydratase
ACP	Acid Phosphatase
ADH	Alcohol Dehydrogenase
ALD	Aldolase =FBA
EST	Esterase
GDH	Glutamate Dehydrogenase
G6PDH	Glucose-6-phosphate Dehydrogenase
GPI	Glucose-6-phosphate Isomerase =PGI
HEX	Hexokinase
IDH	Isocitrate Dehydrogenase
LDH	Lactate Dehydrogenase
MDH	Malate Dehydrogenase NAD
ME	Malate Dehydrogenase NADP
MPI	Mannose-6-phosphate Isomerase
PGM	Phosphoglucomutase
PGD	Phosphogluconate Dehydrogenase
PO	Peroxidase
SKD	Shikimate Dehydrogenase
SOD	Superoxide Dismutase
TPI	Triose-phosphate Isomerase
XDH	Xanthine Dehydrogenase

SECTION 6: TROUBLESHOOTING GUIDE

A. PROBLEMS WITH GEL

1. PROBLEM: Gel surface is slimy and wet.
   FAULT: 1. Gel is undercooked.
   2. Gel has not been left to cool for long enough.
   3. Incorrect volume of buffer added to the gel

2. PROBLEM: Gel sticks to glass plate, is brittle and difficult to slice.
   FAULT: 1.Gel is overcooked.

3. PROBLEM: Large air bubbles trapped under the glass plate when it is placed on the gel.
   FAULT: 1. Gel not poured into gel former correctly - see protocol.
   2. Gel is too viscous due to undercooking or being left in the flask for too long after heating and before pouring.

4. PROBLEM: Air bubble appears in the gel a few minutes after glass plate has been put in place.
   FAULT: 1.Poor seal of starch around the top of the gel former resulting in air being sucked under the glass plate as the gel cools and contracts. Ensure when the glass plate is laid on the gel that there is a starch overlap all the way around the top of the former. If there are any obvious gaps, use a spatula to scrape excess overlapped starch from elsewhere and paste it across the junction of the glass plate and the gel former at these sites. If a bubble starting to form is noticed, placing a weight on top of the glass plate can often squeeze it back out again, giving time to reseal the gap.

ELECTROPHORETIC PROBLEMS

1. PROBLEM: Voltage too high for a given current.

   FAULT: 1. Poor contact between gel and sponges.
   2. Sponges old and have become less absorbent.
   3. Not enough electrode buffer in electrode tank.
   4. Electrode buffer not made up correctly - too low an ionic strength.
   5. Electrode buffer has been reused. It is possible to reuse electrode buffer providing the +ve and -ve electrode tanks are mixed between runs. However it becomes necessary to run the gels for longer, each time to attain the same migration distances.
   6. Sponges were still damp with water from being rinsed at the end of the last run. The liquid holding capacity of the cellulose sponges is high and the dilution factor to the electrode buffer considerable if the sponges are not 'brittle dry'. If 2 hours prior to electrophoresis the sponges are still damp, place them in a 40^{o}C incubator to allow them to dry. Alternatively rinse them thoroughly in electrode buffer prior to use.

2. PROBLEM: Voltage too low for a given current.

   FAULT: 1. Ionic strength of the electrode buffer is too high.
   2. If new sponges are being used and are not thoroughly rinsed in hot water, a precipitate is formed on the electrodes and the running characteristics of samples can be altered! Thus when using new sponges for the first time carefully check the current :voltage ratio. If it is clearly outside the expected range, remove the gel from the electrode tanks, rinse the sponges thoroughly in hot water, then in electrode buffer. Wash the electrodes in 50% HCl till any white coating is removed, rinse with water, dry, and then reload the gel. Note do not leave the HCl on the electrodes for too long otherwise corrosion of the wires may occur.

3. PROBLEM: Uneven migration of samples, concave when viewed from origin

   FAULT: 1. Uneven cooling of the gel, gel colder in centre than at edges.
   2. Uneven compression of sponges by cooling system.

4. PROBLEM: Uneven migration of samples, convex when viewed from origin.

   FAULT: 1. Uneven cooling of the gel, gel colder at the edges than at the centre.
   2. Uneven compression of sponges by cooling system.

5. PROBLEM: Uneven migration of samples, one side runs faster than the other.

   FAULT: 1. Uneven cooling of the gel, gel colder at one edge than the other.
   2. Uneven compression of sponges by cooling system.
   3. Electrodes corroded or coated unevenly.
   4. Gel part separated at origin.

6. PROBLEM: Uneven migration of samples, sample front wavy

   FAULT: 1. Uneven compression of sponges by cooling system.
   2. Electrodes corroded or coated unevenly.
   3. Gel part separated at origin.

PROBLEMS WITH GEL SLICING

1. PROBLEM: Grooves present in the gel slices.

   FAULT: 1.Dirt on the slicing line, use a new section of line.
   2. Sample wick caught in the gel, carefully check all have been removed prior to slicing.

2. PROBLEM: Difficulty getting a 4th slice separate from the very top layer.

FAULT: 1. Gel squashed during slicing, ensure that the glass plate used is not a thick and heavy one. A 4th slice can be obtained from a thin gel by removing the glass plate all together and guiding the slice wire by hand initially until it is embedded in the gel and then slicing as normal.

2. A tapering edge to the gel caused by the gel stretching during the slicing can cause the slicing line to slip over the gel rather than biting into it. Cutting off 1-2 cms from the end of the gel creating a vertical face for the wire to cut into can overcome this problem. Avoid slicing starting from different ends of the gel so that one end of the gel remains unstretched.

3. PROBLEM: Thick ridges are present in some slices resulting in gaps in other slices.

FAULT: Air bubbles between the gel and the gel slicer cause uneven cutting. Check and gently tap out any air bubbles before each slice is taken.

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