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Genetic structure and morphological variation of British populations of the hybrid *Potamogeton* x *salicifolius*

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Potamogeton x *salicifolius* Wolfg. is one of the three most frequently recorded *Potamogeton* hybrids in the British Isles and Europe. It is thought to be the hybrid between *P. lucens* and *P. perfoliatus*. Its scattered distribution suggests that it has arisen several times in Britain. Most British populations of *P.* x *salicifolius* can be identified by their morphological characteristics, which are intermediate between those of the putative parents *P. lucens* and *P. perfoliatus*. However, the population at the Ouse Washes, Cambridgeshire, differs from other populations in its greater similarity to *P. lucens*. A genetic study of eight British populations, using six isozyme systems, revealed that most populations consist of a single multi-enzyme phenotype. This suggests that they were the result of a single hybridization event and are therefore maintained through vegetative reproduction. By contrast, the Ouse Washes population consists of three multi-enzyme phenotypes. This variation is likely to have resulted from multiple hybridization events, although we cannot exclude the possibility that the plants are partially fertile. The isozyme systems studied were unable to identify *P. lucens* and *P. perfoliatus* unambiguously, and consequently did not provide evidence for their putative parentage of *P.* x *salicifolius*. However, at a local level the banding patterns of the hybrids were generally consistent with the local multi-enzyme phenotypes of these putative parents. *Botanical Journal of the Linnean Society*, 2004, **144**, 99–111.

ADDITIONAL KEYWORDS: genetic variability – hybrid origin – isozymes – PAGE – population structure – vegetative propagation.

INTRODUCTION

Potamogeton is one of two genera in the family Potamogetonaceae, the other being the monotypic *Groenlandia*. It is found in most areas of the world, except arid and polar regions, making it one of the more important genera of aquatic vascular plants. An important feature of *Potamogeton*, as is the case with many aquatic genera, is the prevalence of interspecific hybrids. Within the family Potamogetonaceae alone there are at least 50 hybrids worldwide (Wiegleb & Kaplan, 1998). The evidence for hybrids usually comes from studies of their morphology and reproductive biology. Hybrids are usually sterile, or only partially fertile, and even when they have originated from separate hybridization events, they usually share a strong morphological identity that make them clearly distinguishable from their parental species (Stace, 1975; Rieseberg & Ellstrand, 1993).

Potamogeton hybrids vary considerably in their frequency and distribution. In the British Isles some are found at numerous localities, whereas others are restricted to single location (Hollingsworth, Preston & Gornall, 1995, 1996; Preston, 1995; Preston, Bailey & Hollingsworth, 1998; Preston & Pearman, 1998; Fant, Preston & Barrett, 2001a,b; King *et al.*, 2001). The frequency of hybridization will depend on the ecological and geographical distribution of parents, the degree of genetic compatibility between the parental species, and the degree to which the resulting hybrids are sufficiently viable to become established in the wild. However, the frequency of hybrids in the wild might be governed by more than just the frequency of hybridization. It is possible that the observed distribution of some hybrids could have been attained through vegetative propagation and

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long-distance dispersal following an initial hybridization event (Preston, 1995). Hence variation in the frequency and distribution may reflect varying capability to reproduce asexually. There is also the possibility that some hybrids are fertile or partially fertile, and are therefore reproducing sexually and therefore able to spread by seed.

Allozymes, which are co-dominant, have simple Mendelian inheritance and are direct gene products, are useful markers for population studies, and have been used successfully in determining the origin of hybrids in many genera (Gallez & Gottlieb, 1982), including *Potamogeton* (Hollingsworth *et al*., 1995, 1996; Fant *et al*., 2001a,b; Kaplan, Plackova & Stepanek, 2001; Iida & Kadono, 2002). The banding pattern of a hybrid should be a composite of bands inherited from both of its parental species because many of these species are polyploid. However, the number of bands could be quite numerous (Les, 1983; Les & Philbrick, 1993; Hollingsworth, Preston & Gornall, 1998). For monomeric enzymes, a simple F_1 hybrid banding pattern will simply contain a mixture of bands from both parents. However, the banding pattern will become increasingly complex, depending on the quaternary structure of the enzyme, with additional, intermediate bands arising from heteromeric products (Wendel & Weeden, 1989).

Previous studies involving *P.* x *schreberi* (Hollingsworth *et al*., 1995), the Lake Shinji (Japan) (Iida & Kadono, 2001) population of *P. anguillanus* (*P. perfoliatus* x *P. malaianus*), and East Anglia (UK) and Czech populations of *P.* x *fluitans* (Fant *et al*., 2001b; Kaplan *et al*., 2001), and eight British populations of *P.* x *suecicus* (Hollingsworth *et al*., 1996) found that these *Potamogeton* hybrid populations comprised single clones, suggesting that they resulted from a single hybridization event and are maintained through vegetative propagation. The presence of variability within a hybrid population, as was found in *P.* x *sudermanicus* (Fant *et al*., 2001a), the Lake Biwa (Japan) population of *P. anguillanus* (Iida & Kadono, 2000, 2002), five British populations of *P.* x *suecicus* (Hollingsworth *et al*., 1996), and the Moors River (UK) population of *P.* x *fluitans* (Fant *et al*., 2001b), suggests either multiple hybridization events or sexual reproduction. As most hybrids are sterile, or at least only partially fertile, the former is often the more likely possibility. Variation in this instance originates from genotypic difference in the parental species involved in the original crosses. Variation between populations will depend upon whether they arose from vegetative or sexual reproduction. At sites that are geographically quite distant, variation between populations would be expected as it is likely that each population is a result of a separate hybridization event.

P. x *salicifolius* is one of Britain's commoner *Potamogeton* hybrids, having been identified since 1970 in at least 15 river catchments in England and southern Scotland (Preston, Pearman & Dines, 2002). It therefore provides a suitable hybrid to investigate the relationship between genetic variation within and between widely separated populations. In this study, populations of the putative hybrid *P.* x *salicifolius* will be examined, both to test the current view that this taxon arose from the hybridization between *P. lucens* and *P. perfoliatus* and to determine the levels of diversity maintained within these populations. Like many *Potamogeton* taxa, its morphological characteristics can vary considerably according to its growing conditions (Kaplan, 2002). In faster flowing waters, such as the Rivers Tweed and Avon, the leaves are long and narrow, much like those of a willow (hence the name '*salicifolius*'). In shallow ditches the leaves are generally shorter and almost as broad as long. At one site, the Ouse Washes, Cambridgeshire, *P.* x *salicifolius* resembles *P. lucens* so closely that it can only be reliably distinguished by a single character, the lack of petioles (Preston, 1995). Unfortunately, even within *P. lucens* there is some variability in this characteristic, especially in the new growth, which often has very short petioles. The strong resemblance that this population has to one of its putative parents distinguishes it from other populations of *P.* x *salicifolius*, and perhaps suggests that it may have backcrossed to *P. lucens*. However, as yet there has been no report of fruit formation on any *P.* x *salicifolius* plant. A detailed morphological study was therefore carried out to investigate the relationship of the hybrids to their putative parents.

MATERIAL AND METHODS

Samples of *P.* x *salicifolius* were collected from eight individual sites or river systems (Table 1). These populations varied considerably in size, from individual clumps at Martham Broad, Wicken Fen, River Earn and River Kennet, to scattered plants occurring over a distance of several kilometres in the River Tweed and its tributary, the Teviot. Where possible, samples of the putative parental species, *P. lucens* and *P. perfoliatus*, were also collected from these sites. Samples were collected at least 15 m apart to minimize sampling the same individual ramet twice, and two samples were collected from each ramet to ensure that they comprised a single clone. This was particularly important at sites where clumps were large, and it was difficult to distinguish individual ramets. Samples were collected using a grapple hook, and stored in plastic bags filled with water.

Table 1. List of sites from which *Potamogeton* x *salicifolius* and its putative parents were collected, with numbers of ramets sampled and different multi-enzyme phenotypes identified at each site

Species	Site	Grid reference	No. of ramets	No. of MEP (total no. for the site)
Martham Broads, v.c. 27				
P. perfoliatus	Martham Broad	TG/63 463202	2	1 (1)
P. x *salicifolius*	Martham Broad	TG/63 463202	3	1 (1)
Ouse Washes, v.c. 29				
P. lucens	Counter Drain (Sutton Gault)	TL/52 42-79-	7	3
	Counter Drain (Mepal)	TL/52 43-81-	16	2
	Counter Drain (Mepal)	TL/52 44-82-	9	2
	Counter Drain (Welches Dam)	TL/52 47-85-	8	1 (5)
P. perfoliatus	Counter Drain (Blockmoor Fen)	TL/52 41-80-	7	2
	Counter Drain (Mepal)	TL/52 43-81-	5	2
	Vermuden's Drain (Mepal)	TL/52 43-82-	2	2
	Counter Drain (Welches Dam)	TL/52 47-85-	4	1 (4)
P. x *salicifolius*	Counter Drain (Blockmoor Fen)	TL/52 41-80-	25	2
	Counter Drain (Sutton Gault)	TL/52 42-79-	6	1
	Counter Drain (Mepal)	TL/52 43-81-	22	2
	Vermuden's Drain (Mepal)	TL/52 44-82-	13	2
	Counter Drain (Welches Dam)	TL/52 47-85-	6	1 (3)
River Avon, v.c. 11				
P. lucens	Sopley Bridge	SZ/40 14-97-	3	2
	Near Christchurch	SZ/40 15-94-	1	1 (2)
P. perfoliatus	Sopley Bridge	SZ/40 14-97-	4	2 (2)
P. x *salicifolius*	Sopley Bridge	SZ/40 14-97-	6	1 (1)
River Piddle, v.c. 9				
P. lucens	Wareham	SY/30 92-87-	2	1 (1)
River Earn, v.c. 88				
P. x *salicifolius*	Nr Forteviot (south-west of Perth)	N0/37 04-18-	3	1 (1)
River Frome, v.c. 9				
P. lucens	Wool Bridge	SY/30 844871	2	1 (1)
P. perfoliatus	Wool Bridge	SY/30 844871	2	2
	East Stoke	SY/30 86-86-	4	2 (3)
P. x *salicifolius*	Nr Bindon Abbey	SY/30 85-86-	7	1
	East Stoke	SY/30 86-86-	6	1 (1)
River Kennett, v.c. 22				
P. perfoliatus	Reading	SU/41 66-71-	3	2 (2)

Table 1. *Continued*

Species	Site	Grid reference	No. of ramets	No. of MEP (total no. for the site)
P. x *salicifolius*	Reading	SU/41 668710	1	1 (1)
River Wye, v.c. 36				
P. perfoliatus	Hereford	SO/32 49-38-	4	3
		SO/32 49-39-	1	1 (3)
P. x *salicifolius*	Hereford	SO/32 49-38-	10	1
	Hereford	SO/32 49-39-	4	1 (1)
Tweed Catchment, v.c. 68, 80 & 81				
P. lucens	River Teviot (Castle ruin, Kelso)	NT/36 71-33-	5	2
	River Tweed (Twizel)	NT/36 88-44-	2	1
	River Tweed (Norham)	NT/36 90-48-	2	1
	River Tweed (Union Bridge, Horncliff)	NT/36 93-51-	3	1 (2)
P. perfoliatus	River Tweed (2 km from Wark)	NT/36 85-38-	2	2
	River Tweed (Twizel)	NT/36 88-44-	2	1
	River Tweed (Norham, near Island)	NT/36 90-47-	1	1
	River Tweed (Norham)	NT/36 90-48-	1	1
	River Tweed (Union Bridge, Horncliff)	NT/36 93-51-	3	2 (2)
P. x *salicifolius*	River Teviot (Castle ruin, Kelso)	NT/36 71-33-	7	1
	River Tweed (Near Spouston)	NT/36 75-35-	3	1
	(River Tweed Floating, Nr Wark)	NT/36 82-38-	1	1
	River Tweed (Coldstream Bridge)	NT/36 85-40-	3	1
	River Tweed (Twizel)	NT/36 87-43-	1	1
	River Tweed (Twizel)	NT/36 88-44-	3	1
	River Tweed (Norham, near Island)	NT/36 90-47-	1	1
	River Tweed (Norham)	NT/36 90-48-	3	1 (1)
Wicken Fen, v.c. 29				
P. lucens	Wicken Lode & Monks Lode	TL/52 55-70- to 56-69-	5	1 (1)
P. perfoliatus	Wicken Lode & Monks Lode	TL/52 54-70- to 56-69-	5	1 (1)
P. x *salicifolius*	Monks Lode	TL/52 566699	1	1 (1)

ISOZYMES

Samples collected for isozyme studies were placed in plastic bags filled with water and stored in the dark until they could be taken back to the laboratory. Six enzyme systems were used to investigate the population diversity and parentage: AAT (EC 2.6.1.1), ADH (EC 1.1.1.1), GDH (EC 1.4.1.2), ME (EC 1.1.1.40), PGD (EC 1.1.1.44), and SKD (EC 1.1.1.25). Extractions were made using non-flowering, growing tips and the enzymes were resolved using a discontinuous polyacrylamide system as described in Fant *et al*. (2001a,b).

MORPHOLOGICAL MEASUREMENTS

Samples of *P.* x *salicifolius* and its putative parents were collected from throughout the Ouse Washes. Some samples were also collected from other sites for comparison. The main characters that were investigated are those that commonly separate the two parental species. They included total leaf length and width, plus width near the apex and near the base of the leaf, size of mucro and petiole, degree to which leaf wraps around the stem, number of veins, and the length of the stipule and its ridges (Table 2; Fig. 1).

DATA ANALYSIS

Differences between species and between populations of the hybrid for the morphological characters were examined using Analysis of Variance (ANOVA) on Genstat 5 Release 3.2. A Principal Component Analysis was also performed with the same data using Systat Version 9.

RESULTS

ISOZYME STUDIES

P. x *salicifolius* was sampled at eight sites; at two of these sites only a single ramet was located but at the remaining six sites between three and 72 ramets were sampled. At six sites the hybrids were present as a unique multi-enzyme phenotype (Tables 1, 3). The exceptions are the Ouse Washes, where three multi-enzyme phenotypes were identified, and the Rivers Tweed and Earn, which shared the same multi-enzyme phenotype. There was therefore no detectable variation in five of the six sites from which more than one plant was collected. This is in contrast to the parental species, *P. lucens* and *P. perfoliatus*, where many sites contained more than one multi-enzyme phenotype. *P. perfoliatus* produced the most multi-enzyme phenotypes, with up to 12 different banding patterns being identified in AAT alone. Overall, 17 multi-enzyme phenotypes were identified in *P. perfoliatus*, from 50 ramets at eight different river systems; this is compared with 11 multi-enzyme phenotypes from 58 ramets from five river systems in *P. lucens*. In most cases, each multi-enzyme phenotype was restricted to a single locality. Comparisons of multiple samples collected from a single 'ramet' did not reveal any variation in their banding patterns, suggesting that the clumps sampled comprised a single clone. The only localities that shared a multi-enzyme phenotype were Wicken Fen and Ouse Washes, but these are less than 20 km apart. The Ouse Washes had the highest number of multi-enzyme phenotypes for all taxa examined, three of *P.* x *salicifolius*, four of *P. perfoliatus* and five of *P. lucens*; this was double the number that was found at any other site, although sample sizes at the Ouse Washes were considerably larger than at other sites.

Table 2. Characters used to distinguish *Potamogeton perfoliatus*, *P. lucens*, and their putative hybrid *P.* x *salicifolius* (see Fig. 1)

Morphological character	Description
Leaf length (mm)	Distance from base of petiole to tip
Leaf width – maximum	Widest distance across leaf
– tip	Width 5 mm from tip
– base	Width 10 mm from base
Wrap	Number of degrees the base of leaf wraps about the stem
Mucro	Length of mucro if present
Petiole	Length of petiole if present
Veins	Number of major lateral veins
Stipule – full length	Length of stipule which protects leaf
– length of ridges	Length of ridges on the stipule

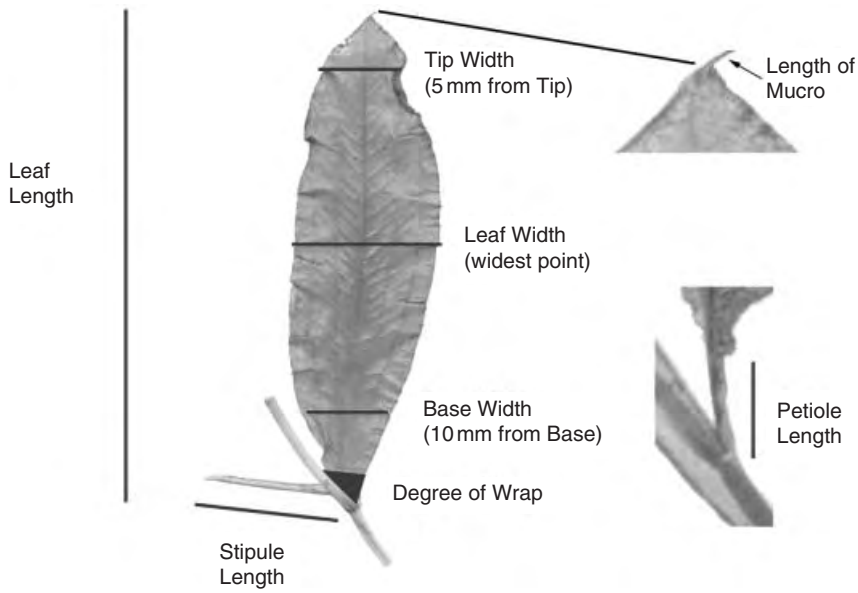

Figure 1. Leaf of *Potamogeton* x *salicifolius* (and *P. lucens* with petiole) showing measurements used for morphometric analysis.

Table 3. Phenotypic differences found in *Potamogeton* x *salicifolius* and both the putative parental species (see Figs 2, 3)

Species	No.	AAT	ADH	GDH	ME	PGD	SKD
Martham Broad, v.c. 27							
P. perfoliatus	2	X	–	–	L		J
P. x *salicifolius*	3	I	F	I	M	A	G
Ouse Washes, v.c. 29							
P. lucens	16	A	A	C	C	A	A
	11	A	B	A	A	B	B
	3	B	B	B	A	C	B
	1	C	B	B	A	B	B
	9	A	B	C	B	B	A
P. perfoliatus	5	M	J	N	–	J	H
	2	N	L	N	–	K	H
	7	O	K	M	B	M	I
	4	M	J	M	B	L	J
P. x *salicifolius*	23	I	F	C	B	F	B
	40	I	D	H	C	A	B
	9	I	E	C	B	F	D
Rivers Avon & Piddle, v.c. 9 & 11							
River Avon							
P. lucens	3	F	A	E	E	E	A
	1	G	A	E	F	E	A
P. perfoliatus	2	T	S	R	O	N	M
	2	U	R	S	L	P	N
P. x *salicifolius*	6	L	H	K	L	H	E
River Piddle							
P. lucens	2	F	A	F	E	E	D
River Earn, v.c. 88							
P. x *salicifolius*	3	K	H	C	I	G	F
River Frome, v.c. 9							
P. lucens	2	F	A	G	G	E	D
P. perfoliatus	2	V	Q	T	B	O	O
	1	S	Q	T	B	O	O
	3	R	Q	T	B	O	O
P. x *salicifolius*	13	L	I	L	K	I	G
River Kennet, v.c. 22							
P. perfoliatus	2	W	–	–	–	N	F
	1	W	–	–	–	P	U
P. x *salicifolius*	1	M	I	C	K	G	
River Wye, v.c. 36							
P. perfoliatus	1	Q	O	P	D	M	L
	2	R	–	P	L	Q	L
	2	P	P	Q	L	M	L
P. x *salicifolius*	14	J	D	J	J	F	E
Tweed Catchment, v.c. 61, 80 & 81							
River Teviot							
P. lucens	3	D	C	D	D	D	C
	2	E	C	D	D	D	D
P. x *salicifolius*	7	K	H	C	I	G	F
River Tweed							
P. lucens	7	E	C	D	D	D	D
P. perfoliatus	5	P	M	O	N	M	K
	4	R	N	N	N	Q	F
P. x *salicifolius*	15	K	H	C	I	G	F
Wicken Fen, v.c. 29							
P. lucens	5	A	B	C	B	B	A
P. perfoliatus	5	M	J	M	B	L	J
P. x *salicifolius*	1	H	G	C	H	A	E

The most widespread multi-enzyme phenotype of *P.* x *salicifolius* in the Ouse Washes occurred throughout the river system, whereas the remaining two had a more localized distribution, being restricted to smaller areas. A drainage ditch that feeds into the Ouse Washes at Blockmoor Fen contained two multi-enzyme phenotypes, one that was unique to the site and one that was more widespread. The relative proportions of each multi-enzyme phenotype in this ditch were seen to change over time. Initially the unique multi-enzyme phenotype made up the largest proportion (86% of the 14 plants sampled) of the population at this site. On a subsequent visit, a year after the site had been dredged, the proportions of individuals at this location shifted in favour of the more widespread multi-enzyme phenotype (73% of 11 sampled). It is unlikely that such a shift in proportions could be attributed to sampling variation. Of the three multi-enzyme phenotypes of the hybrid that were identified in the Ouse Washes, one was found consistently to produce a banding pattern similar, or identical, to that of *P. lucens*. This multi-enzyme phenotype was attributed to the hybrid rather than to *P. lucens* owing to its characteristic lack of petioles and the distinctive hybrid-banding pattern it produced for AAT.

The enzyme systems used in this study were unable to reveal any bands that were unique to either of the two parental species; in most instances the two species shared many of the bands, differing only in their composition and intensity (Fig. 2). As few bands could be identified as being specific to one or the other parental species it was difficult to say with any certainty that this hybrid had to be derived from a cross between these two species. This is further complicated by the fact that the parental species are polyploid. However, when a comparison of the allozyme banding patterns is restricted to only the local taxa it provides some support that *P.* x *salicifolius* may have arisen locally from crosses between *P. lucens* and *P. perfoliatus* in the area; this is particularly true for AAT, ADH, PGD, and SKD (Fig. 3). The five sites at which the hybrid and both putative parental species were collected were the Rivers Frome, Avon, Tweed and Teviot, Wicken Fen, and the Ouse Washes. The hybrids at these five locations all produced different multi-enzyme phenotypes, discussed in detail below.

With SKD, a monomeric enzyme, each band represents a single allele, and hence determining the parentage of an F_1 hybrid should be a matter of matching each band to one found in its parents. This enzyme system did not produce any unique bands that could equivocally be attributed to one or the other species. Nonetheless, in the cases of the Rivers Frome, Avon, Tweed and Teviot and Wicken Fen the banding patterns do support the origin of the hybrid as possibly arising from crosses between the local *P. lucens* and *P. perfoliatus* (Fig. 3). The evidence for the Ouse Washes is less conclusive with the two banding patterns of the hybrid being most similar to *P. lucens*, although one lacked a slower band that was found in all *P. lucens* examined.

With dimeric enzymes the banding pattern can depend on whether the band is homomeric, derived from a single allele, or heteromeric, derived from two alleles. Consequently, a hybrid will not necessarily inherit all the bands found in its parental species and will occasionally acquire novel heteromer bands. Nonetheless, it is possible to identify and exclude the putative parents that could have been the source of the banding pattern seen in the hybrids. With AAT, the main differences between *P. lucens* and *P. perfoliatus* were in the number and size of the faster bands. Hybrids from the Rivers Frome and Avon and Wicken Fen produced more faster bands than found in *P. lucens*, but they were similar to those of *P. perfoliatus* (Fig. 3). At the Ouse Washes the banding pattern of the hybrid had fewer bands compared with other hybrids, with most of the bands in the hybrid being found in *P. lucens*, except for one band that was not found in either parental species. The enzyme ADH produced a complicated banding pattern with only minimal distinction between taxa, but like AAT most of the differences were in the faster bands. In the hybrid, the faster bands were attributable to either *P. perfoliatus*, in the case of the Rivers Avon, Tweed and Teviot and the Ouse Washes, or *P. lucens* in the River Frome and Wicken Fen (Fig. 3). With the enzyme PGD the hybrids from the Rivers Frome and Avon, Wicken Fen and Ouse Washes had bands that were equivalent to ones in both *P. perfoliatus* and *P. lucens* (Fig. 3). Those bands in the parents that were not found in the hybrid are intermediate to those inherited by the hybrid and therefore may be heteromers. Similarly, the extra bands in the hybrids from Scotland could also be heteromers that are unique to this hybrid's genotype, although without better understanding of the genetics of these bands it is difficult to say with any certainty.

Morphological analysis

The similarity of the Ouse Washes hybrids to *P. lucens* prompted a more extensive survey of their morphological characters of this population. The characters selected for this study were shown to differ significantly between the two parental species, and were able to distinguish hybrid species (Table 4). *P. lucens* generally produced larger leaves, in both length and width, with a prominent mucro and obvious petiole. This was accompanied by larger stipules, which had a greater degree of ridging along their length. *P. perfoliatus* had a more cordate leaf, being broadest

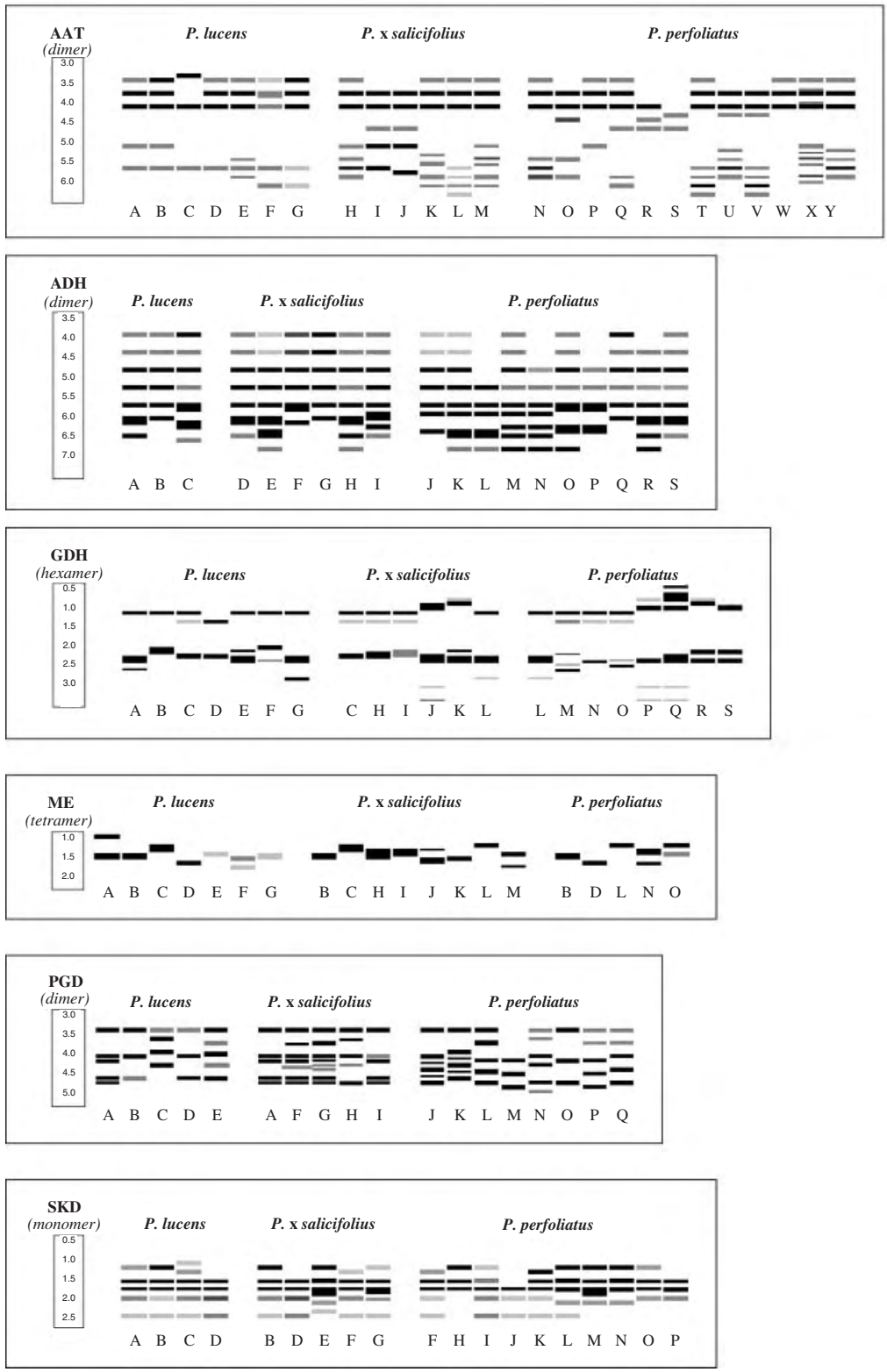

Figure 2. Diagrammatic representation of the different banding patterns found in the two parental species (*Potamogeton lucens* and *P. perfoliatus*) and its putative hybrid (*P.* x *salicifolius*). Letters are used to distinguish different phenotypes (see Table 1). Scale is distance travelled in centimetres down the gel, with anode at the bottom.

nearer the base, with a greater number of veins. The leaves were also sessile and wrapped around 80% of the stem. Interestingly, *P.* x *salicifolius* did not have intermediate characteristics for these measurements, but rather resembled one or the other parent. The leaves were of similar length and shape to *P. lucens*, as was the size and make-up of the stipules, whereas the absence of petioles, and the usual absence of a mucro, was more similar to *P. perfoliatus. P.* x *salicifolius* leaves were narrower than in both parents, at both the widest point and near the tip, although its base was only half as wide as *P. perfoliatus*, giving it a more oblong–elliptical shape.

Using principal component analysis (PCA) on these morphological measurements, 70% of the variation could be explained by two principal components. A graph of these showed that *P. lucens* and *P. perfoliatus* formed two distinct clusters (Fig. 4). *P.* x *salicifolius* also formed a distinct cluster that was intermediate between the two parental clusters. There was some overlap with *P. lucens*, although all of the overlapping individuals were from the Ouse Washes. The differences between the Ouse Washes and other *P.* x *salicifolius* populations were shown to be significant for all characters examined (Table 5). As there is no replication of genotypes across sites, it is difficult to determine to what extent the effects of environment vs. genotype plays in these phenotypic differences. Nonetheless, it would appear that the plants from the Ouse Washes had longer and wider leaves, with more veins per leaf, as well as larger stipules and greater ridging. The similarity of the Ouse Washes hybrids to *P. lucens* might suggest that these plants are not hybrids, but rather a morphological variant of the parent. However, these individuals were still shown to differ significantly from *P. lucens* having no petiole ($P > 0.001$), leaves that wrapped around the stem ($P > 0.001$), more major veins ($P > 0.001$), leaves that are broader at the base ($P > 0.001$), shorter stipules ($P > 0.05$), and less ridging on these stipules ($P > 0.001$), as well as a lack of fruits. For these morphological characters these individuals are more like *P. perfoliatus* than *P. lucens.*

DISCUSSION

Potamogeton x *salicifolius* is a relatively common hybrid found at scattered sites throughout Britain. The numbers of individuals found at sites varied considerably, with some sites, Wicken Fen, Reading and

Table 4. Analysis of variance of the leaf morphological characteristics used to distinguish the three *Potamogeton*. Values reflect mean value for those species ± standard deviation

Taxon	*N*	Length (mm)	Width (mm)	Tip (mm)	Wing (mm)	Wrap (°)	Mucro (mm)	Petiole (mm)	Veins (no.)	Stipule (mm)	Ridge (mm)
P. lucens	40	132.5 (±5.2)	41.5 (±1.3)	11.0 (±0.4)	12.2 (±0.7)	13.8 (±2.7)	5.40 (±1.4)	3.9 5(±0.4)	5.7 (±0.1)	53.8 (±1.9)	35.3 (±1.6)
P. perfoliatus	16	63.1 (±2.7)	35.1 (±2.5)	11.5 (±0.9)	33.1 (±2.2)	303.4 (±5.9)	0.0 (±0.0)	0.0 (±0.0)	12.8 (±0.6)	10.8 (±1.1)	1.43 (±1.4)
P. x *salicifolius*	66	122.4 (±3.7)	32.7 (±1.4)	10.3 (±0.4)	15.3 (±0.5)	62.8 (±2.1)	3.6 (±0.8)	0.0 (±0.0)	6.0 (±0.2)	43.9 (±1.6)	19.7 (±1.5)
F Prob.		<0.1% (***)	<0.1% (***)	0.5% (**)	<0.1% (***)	<0.1% (***)	3% (*)	<0.1% (***)	<0.1% (***)	<0.1% (***)	<0.1% (***)

Table 5. Analysis of variance of difference in *Potamogeton* x *salicifolius* across sites. Values reflect mean value for those species ± standard deviation

Taxon	*N*	Length (mm)	Width (mm)	Tip (mm)	Wing (mm)	Wrap (°)	Mucro (mm)	Petiole (mm)	Veins (no.)	Stipule (mm)	Ridge (mm)
Ouse Washes	45	130.6 (±3.9)	38.93 (±1.1)	10.3 (±0.4)	16.66 (±0.5)	64.9 (±2.1)	5.11 (±1.0)	0.0 (±0.0)	6.4 (±0.2)	49.9 (±1.5)	25.84 (±1.1)
Other	21	105.3 (±6.5)	19.64 (±1.3)	7.3 (±0.6)	12.29 (±0.4)	55.1 (±5.8)	0.33 (±0.1)	0.0 (±0.0)	5.4 (±0.2)	31.3 (±2.0)	6.67 (±1.8)
F Prob.		<0.1% (***)	<0.1% (***)	<0.1% (***)	<0.1% (***)	1.9% (*)	0.3% (**)		<0.1% (***)	<0.1% (***)	<0.1% (***)

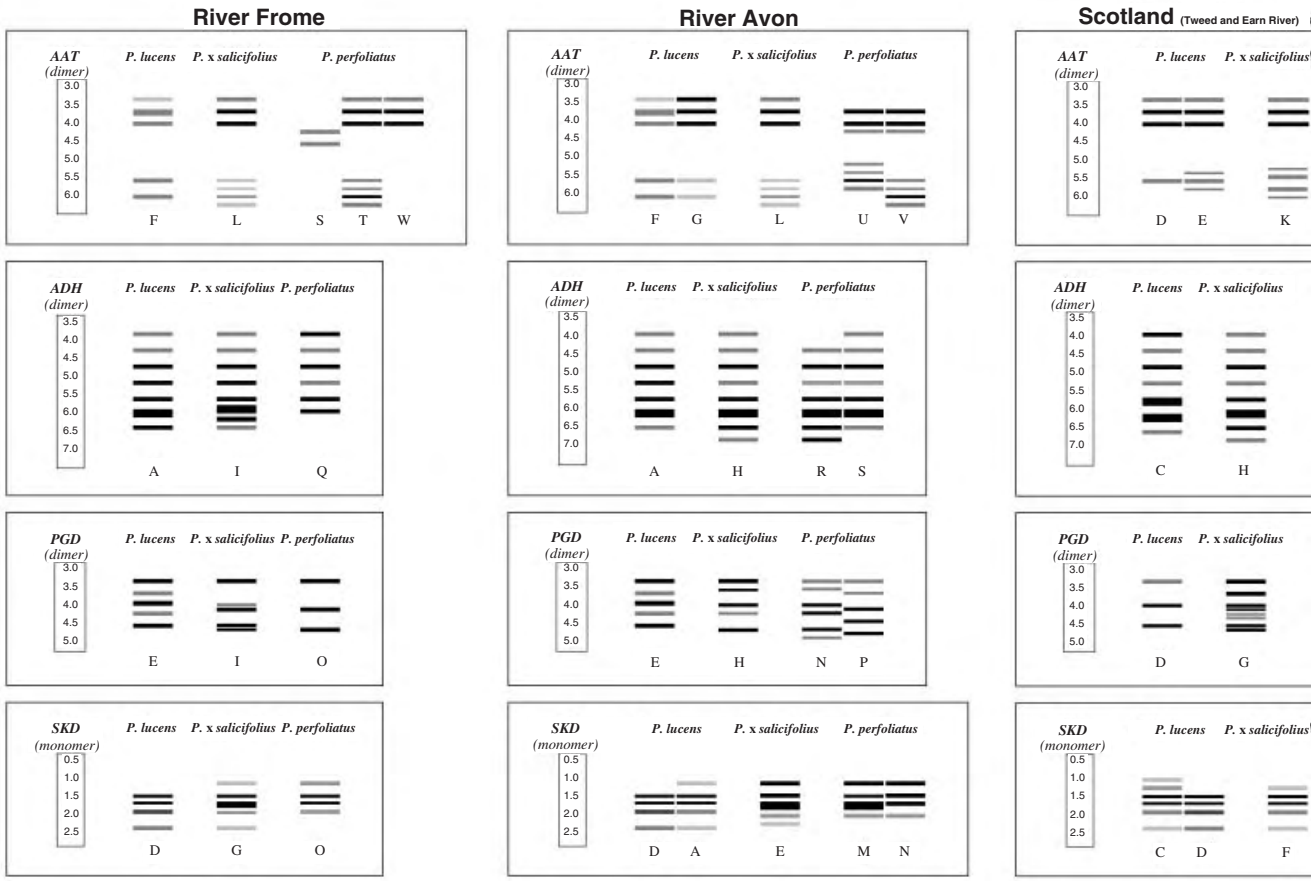

Figure 3. The banding patterns of *Potamogeton lucens*, *P. perfoliatus* and *P.* x *salicifolius* divided by locality. Letters are used to distinguish different phenotypes (see Table 1). Scale is distance travelled in centimetres down the gel, with anode at the bottom.

Martham Broad, apparently containing just a single clump of plants, whereas at others, clumps could be found distributed throughout the sections of the river system. Plants tended to be more numerous at sites where they were growing in free-flowing water. Sites at which plants were less common were characterized as having fairly still water, or sluggish rivers. The hybrid may be at a selective advantage in relatively rapidly flowing water, and it is likely that the current of the river could aid vegetative dispersal both by dislodging fragments from the plants and by carrying them downstream.

The morphological evidence supported the parental origin of *P.* x *salicifolius* as resulting from a cross between *P. lucens* and *P. perfoliatus*. Excluding material collected from the Ouse Washes, *P.* x *salicifolius* was distinct from, yet also intermediate between, both *P. lucens* and *P. perfoliatus* for all morphological characters measured. This was reflected in the principal component analyses, in which the hybrids fell between the two parents with no, or little, overlap. The exception to this was the *P.* x *salicifolius* from the Ouse Washes, which had characteristics very much like that of *P. lucens*. These individuals still formed a separate cluster, albeit with considerable overlap with *P. lucens*.

The allozyme evidence identified ten different multi-enzyme phenotypes of *P.* x *salicifolius*. Each site contained a single genotype, except the Ouse Washes, which contained three. The lack of variability at most sites suggests that their hybrid populations are the result of a single hybridization event, and they persist through vegetative reproduction. This is consistent with population studies of other *Potamogeton* hybrids (Hollingsworth *et al*., 1995, 1996; Iida & Kadono, 2000, 2002; Fant *et al*., 2001a,b; Kaplan *et al*., 2001; King *et al*., 2001). Single multi-enzyme phenotypes have colonized very large areas, as is the case with the *P.* x *salicifolius* from along a 22-km stretch of the River Tweed. Interestingly, this multi-enzyme phenotype was also found in the River Earn 100 km away. It is possible that the similarity of the hybrid at the River Earn is the result of the parental species being identical to

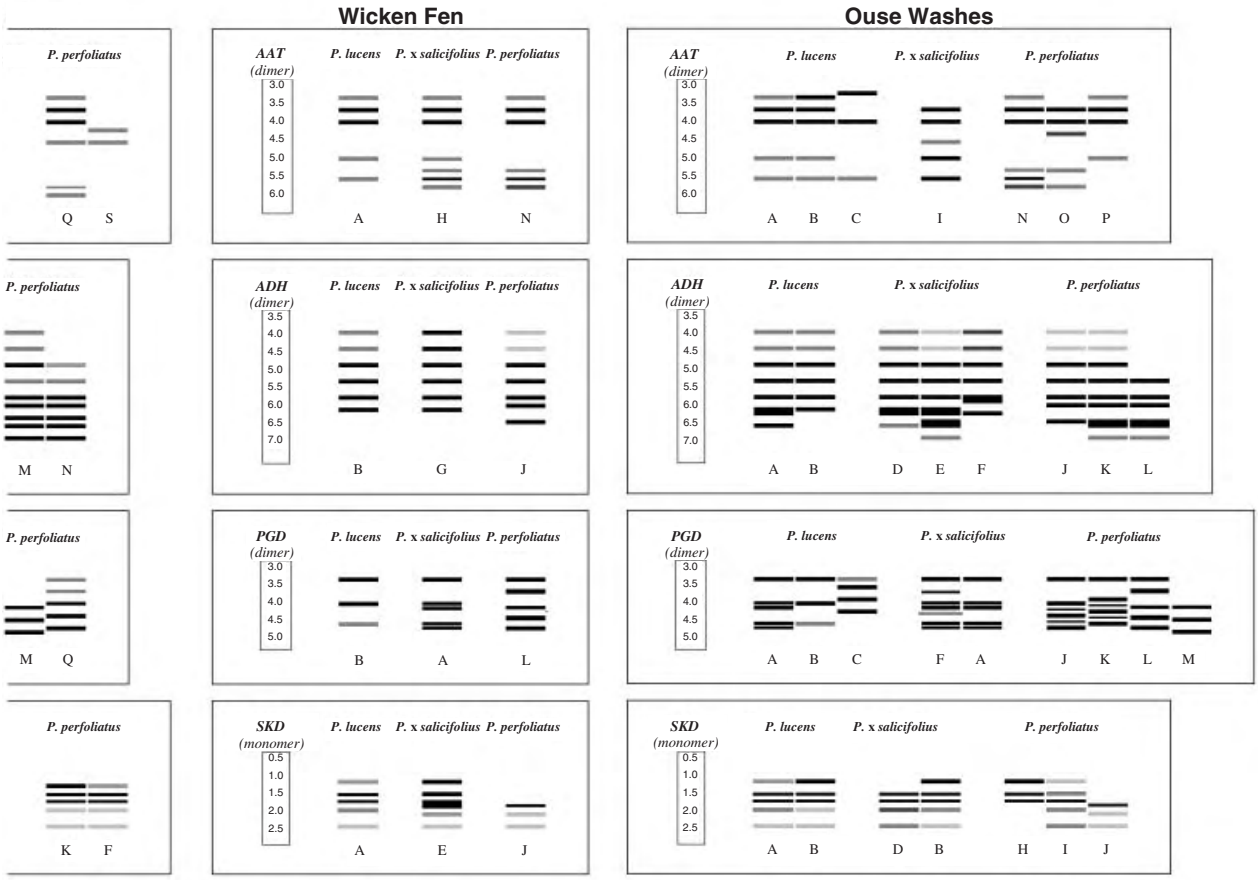

Figure 3. *Continued*

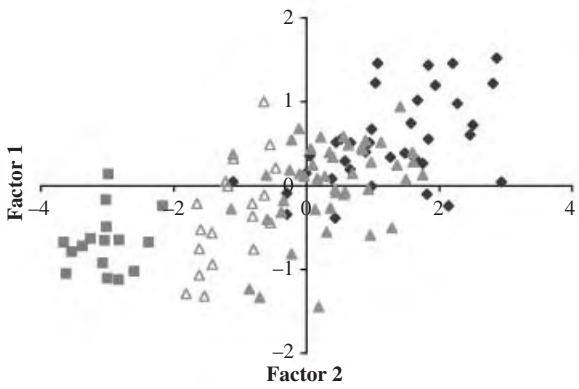

Figure 4. PCA plots comparing *Potamogeton* x *salicifolius* from the Ouse Washes (▲) and elsewhere (△), and its putative parents *P. lucens* (◆) and *P. perfoliatus* (■).

those in the Rivers Tweed and Teviot. Alternatively, the similarity reflects lack of resolving power by the isozymes. The other possibility is that this population arose through vegetative propagation of the populations in the Rivers Tweed or Teviot, or vice versa. This would involve the hybrid spreading large distances from vegetative propagules.

In the Ouse Washes, three multi-enzyme phenotypes of *P.* x *salicifolius* were identified. The greater level of diversity found in the Ouse Washes may in part be due to the environmental and ecological conditions in this area. The Ouse Washes is the location of an important wetland habit for many migratory birds and as part of its management policy is flooded annually to create an ideal habitat. The presence of waterfowl, which consume, damage and disperse pondweed, would undoubtedly aid in the spread and propagation of the pondweeds throughout the area, possibly bringing in material from neighbouring areas. In addition, the annual disturbance resulting from flooding, as well as boat traffic, would equally help with the spread and establishment of propagules. This combination of factors may provide conditions that help to maintain high levels of diversity within the area (Richards, 1990; Grace, 1993).

At most sites from which *P. lucens* was collected, only two multi-enzyme phenotypes were identified. These multi-enzyme phenotypes were usually evenly

distributed throughout the river systems, although in some areas only a single multi-enzyme phenotype could be found. *P. perfoliatus* proved to be a more common species, being found in all of the waterways in which the hybrid grew. In some areas the ramets grew in great masses, but in general they tended to produce smaller clumps than *P.* x *salicifolius* and *P. lucens*. Many of the sites examined consisted of up to three multi-enzyme phenotypes of *P. perfoliatus*.

The allozyme data of both the parental species and their hybrid suggest that the differences between the three taxa were not that substantial. In the case of GDH and ME some of the multi-enzyme phenotypes identified were found both in *P. lucens* and *P. perfoliatus*, as well as the hybrid. There was also significant overlap with SKD, where genotypes could be found in all taxa, varying only in the combinations and relative intensity of the bands. The differences with AAT, ADH, and PGD were more obvious, although not substantially so. Consequently, it is not surprising that all bands found in the hybrid could be interpreted as being derived from one or, in many cases, both parents. When the data were divided into regional groups, the banding pattern of local multi-enzyme phenotypes of *P.* x *salicifolius* better matched the variability found in the local parents. Unfortunately, the differences between *P. perfoliatus* and *P. lucens* were still not large enough to demonstrate unequivocally the putative origin of *P.* x *salicifolius*.

The Ouse Washes was the only site that was not monomorphic, and the allozyme evidence suggests that the hybrid has arisen at least three times in this area. One interesting characteristic of the more common multi-enzyme phenotype was that it also shared a number of allozyme banding patterns with *P. lucens*. The only exception to this was AAT, for which this multi-enzyme phenotype produced the same banding pattern as was found in all hybrids. This result is similar to what might be expected of a backcross, with a mixture of banding patterns that were completely parental, but resembling more closely one parent. Backcrossing could also explain why morphologically many of the hybrid clumps found within the Ouse Washes are almost indistinguishable from *P. lucens* for many characters. Alternatively, the same results could be achieved from the segregation of an F_1. It is known that these hybrids do produce flowers, but there are no records of fruit forming on these plants. It is also possible that these individuals are just variants of *P. lucens*, or even aneuploids, but as their banding patterns for AAT and many of the morphological characters were identical to other *P.* x *salicifolius*, this seems unlikely.

In this study it has been shown that *Potamogeton*, like many aquatic genera, shows great morphological variation, prolific clonal propagation, and limited sexual reproduction. As a result, most of the populations examined, and especially the hybrid populations, contained a limited number of multi-enzyme phenotypes. Consequently, the levels of diversity within populations were limited, but levels of diversity were found to be higher between populations. However, given the limited number of loci used in this study, the numbers of clones detected could be an underestimate of the numbers of clones present.

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