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Short communication

Chloroplast evidence for the multiple origins of the hybrid *Potamogeton* × *sudermanicus* Hagstr.

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Abstract

A previous study of the sole British population of *Potamogeton* × *sudermanicus* Hagstr. supported the putative origin of this taxon as a result of hybridization between *Potamogeton acutifolius* Link and *Potamogeton berchtoldii* Fieber. In this study, the *TrnL* (UAA) chloroplast region was used to identify the maternal parent of this hybrid. It was found that the two multi-enzyme phenotypes identified in previous studies had different chloroplast sequences. These sequences were shown to correspond to those of the two parental species. This is further evidence that this population is the result of at least two hybridization events and that the plants attributed to this hybrid are not an aneuploid of either of the parental species.

Keywords: *Potamogeton*; Hybrids; Genetic variability; *TrnL*; Chloroplast

1. Introduction

Until recently, the evidence for the prevalence of hybrids within the genus *Potamogeton* was based on morphological characters and the sterility of putative hybrid individuals (Fryer, 1890; Hagström, 1916; Ogden, 1943; Dandy, 1975; Preston, 1995; Wiegleb and Kaplan, 1998; Kaplan, 2001). Given the levels of morphological variability (Kaplan, 2002) and the prevalence of aneuploidy in *Potamogeton*, some have argued that more convincing evidence was needed. (St. John, 1925; Fernald, 1932; Les and Philbrick, 1993). Subsequently studies of *Potamogeton* hybrids have employed various biochemical and molecular techniques to confirm the hybrid origins of numerous *Potamogeton* populations (Haynes and Williams,

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1975; Hellquist and Hilton, 1983; Hollingsworth et al., 1995, 1996; Iida and Kadono, 2000; Fant et al., 2001a,b; Iida and Kadono, 2001, 2002; Kaplan, 2001; King et al., 2001). Such studies have reinforced existing views of the importance of hybrids within the genus *Potamogeton*.

In *Potamogeton*, hybrids are more likely to occur between species within a section than from species in different sections. Hybrids are most common between broad-leaved species, section *Potamogeton* (Preston, 1995). Hence, the Dorset population of *Potamogeton* × *sudermanicus* is of special interest as its parents are linear-leaved species (section *Graminifolii*) which hybridize less frequently (Hellquist and Hilton, 1983; Preston and Pearman, 1998).

The British populations of *P.* × *sudermanicus* originates from the crossing of *Potamogeton acutifolius* and *Potamogeton berchtoldii* (Fant et al., 2001b). In their study, Fant et al. (2001b) identified two multi-enzyme phenotypes at the only known British site, suggesting that there may have been more than one instance of hybridization between these two species. These hybrids grow in the small ditches of grazing marshes by the River Frome near Wareham, Dorset. *P. acutifolius* is also found at this site but not *P. berchtoldii*, although the latter is found north of Wareham. Recent surveys of the area have found that *P. acutifolius* seems to be declining, whereas *P.* × *sudermanicus* may be increasing its range (Preston and Pearman, 1998; Fant et al., 2001b). Preston and Pearman (1998) suggest that the hybrids most likely arose at a site where both parents grew rather than from the long-distance dispersal of pollen or seed. The lack of one parent and apparent reduction in range of the second suggests that the hybrid may be a more successful competitor at this site than one or both parents under current environmental conditions.

The aim of this study was to use a conserved region of the chloroplast genome to determine which species was the maternal parent in these hybridizations (Spooner et al., 1991; Taberlet et al., 1991; Cruzan and Arnold, 1994; Giannattasio and Spooner, 1994; Demesure et al., 1995; Dumolin-Lapegue et al., 1997). Rieseberg (1995) suggested that as intraspecific pollination is more likely to succeed than interspecific pollination, hybrids tend to be more common at sites where one species is in the minority. In these situations, the minor species is usually the maternal parent, and therefore, the resulting hybrids will inherit its cytoplast. The *TrnL* (UAA) primers were chosen for this study due to their success in evaluating interspecific variation (Gielly and Taberlet, 1996). It was hoped that these primers would be able to distinguish difference between the two parental species and therefore identify the maternal parent of the hybrid.

2. Materials and methods

Samples of *P.* × *sudermanicus* and some *P. acutifolius* were collected from Wareham, Dorset, and additional samples of *P. acutifolius* were also collected from Limpenhoe Marshes in East Anglia. No *P. berchtoldii* was located at the site where the hybrid was identified, so samples were collected at two sites north of Wareham in the River Piddle, adjacent to the location where the hybrid was found. All samples were collected at a minimum of 25 m apart, unless ramets could be clearly distinguished. The collected leaf tissue was stored in plastic bags, with water, and placed in Styrofoam ice boxes, until they could

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Berchtoldii         GAGCCTTGGTATGGAAACCTACTAAGTGTTAACTTCCAAATTCAGAGAAACCCTGGTAAT
Sudermanicus-B      ************************************************************
Sudermanicus-A      ************************************************************
Acutifolius         ************************************************************

Berchtoldii         TAAAAATGGGCAATCCTGAGCCAAATCCCTGTTTTGAGAAAAAAGGGTTTATTTTCTCTA
Sudermanicus-B      ************************************************************
Sudermanicus-A      ************************************************************
Acutifolius         ************************************************************

Berchtoldii         TTTCGAATTTTCTATTATATAGAAATTCGAAAATAAGAATTTAAAAAAGGGATAGGTGCA
Sudermanicus-B      ************************************************************
Sudermanicus-A      ****************************T*********************************
Acutifolius         ****************************T*********************************

Berchtoldii         GAGACTCAATGGAAGCTGTTCTAACGAATGAAGTTGTTTGCATTGGTAGCAGGAATCCTT
Sudermanicus-B      ************************************************************
Sudermanicus-A      ****************************************G*********************
Acutifolius         ****************************************G*********************

Berchtoldii         CTATGAAAATTACAGAAAGGAAGGATGACCCTATATACCTAATACATACTGACATATCAA
Sudermanicus-B      ************************************************************
Sudermanicus-A      ************************************************************
Acutifolius         ************************************************************

Berchtoldii         ACGATTAATCATAATCACGACTTCAATTTAGAATTTATTTATATAGAATTGAAATTCTAT
Sudermanicus-B      ************************************************************
Sudermanicus-A      ************************************************************
Acutifolius         ************************************************************

Berchtoldii         GAAAAATTTACAAGTGATTGTGAATCTATGACAATTCAAGTTGAAGGAAGAATCAGATAT
Sudermanicus-B      ************************************************************
Sudermanicus-A      ************************************************************
Acutifolius         ************************************************************

Berchtoldii         TCAGTGATCAAATCATTCATTCCGGAGTCTTATTTATCATTAAAACAAAAATGATTAATC
Sudermanicus-B      ************************************************************
Sudermanicus-A      ********T***************************************************
Acutifolius         ********T***************************************************

Berchtoldii         GGACGAGAATAAAGAGAGAGTCCC
Sudermanicus-B      ************************
Sudermanicus-A      ************************
Acutifolius         ************************
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Fig. 1. Comparison of *P. acutifolius* and *P. berchtoldii* sequences to those found in *P.* × *sudermanicus*.

Table 1
Location, size and chloroplast genotype of the *P.* × *sudermanicus*, and its parents, *P. acutifolius* and *P. berchtoldii*

Species	Location and sites	Grid reference	Number of ramets	Phenotype
P. berchtoldii	Wareham	SY/30 92-88-	2	*P. berchtoldii*
P. × *sudermanicus*	Swineham	SY/30 93-86-	1	*P. berchtoldii*
	West of Redcliffe Farm	SY/30 92-86-	1	*P. acutifolius*
P. acutifolius	West of Redcliffe Farm	SY/30 92-86-	1	*P. acutifolius*
	Limpenhoe Marshes	TG/63 39-02-	1	*P. acutifolius*

be transported to the laboratory to be frozen at −80 °C. As previous studies identified two multi-enzyme phenotypes of *P.* × *sudermanicus* at this site, the phenotype of each sample was determined and one of each was sequenced.

DNA was extracted using the CTAB protocol described by Doyle and Doyle (1990). The *TrnL* (UAA) intron was amplified by the polymerase chain reaction using universal primers described in Taberlet et al., 1991. The PCR was performed in 25 μl reaction mixture containing 2 ng DNA, 20 mM Tris, 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 μm of each primer, 200 μm dNTP, and 0.5 U Taq Polymerase (Gibco-BRL). Amplification conditions were as follows: 1 cycle of 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 2 min; followed by 1 cycle of 72 °C for 10 min. The length of the products were analysed on a 1.5% (w/v) agarose gel and excised and purified using QIAquick PCR Purification Kit™. The products were sequenced using the forward primer: sequencing was carried out at the Sequencing Facility at the University of Cambridge with an ABI 3100 genetic analyzer. The sequences were aligned and compared using the multiple sequence alignment web site http://prodes.toulouse.inra.fr/multalin/multalin.html.

3. Results

The intron of both the species and hybrid was 504 bp long. *P. berchtoldii* (EMBL accession number AJ438310) and *P. acutifolius* (EMBL accession number AJ438309) were shown to vary at three sites; one transversion and two transitions. No variation was identified within the parental species. The two multi-locus phenotypes of the hybrid were also shown to differ in their chloroplast sequences, with one having a sequence identical to *P. berchtoldii* and the other being identical to *P. acutifolius* (Fig. 1; Table 1). Interestingly, the multi-enzyme phenotype that Fant et al. (2001b) identified as having additional bands characteristic of *P. acutifolius* was shown to possess the *P. berchtoldii* chloroplast.

4. Discussion

This study has confirmed that this population of *P.* × *sudermanicus* consists of two multi-locus phenotypes. It also suggests that the origins of these two hybrids are distinct, with different species acting as the maternal parent. This would suggest that any post-pollination barriers are not sufficient to prevent hybridization in either direction. We cannot rule out the possibility that one of these multi-locus phenotypes is a backcross, rather than an F1, although there is no morphological evidence to suggest that some hybrids are closer to one parent than others. The presence of hybrids with the chloroplast from both *P. berchtoldii* and *P. acutifolius* confirm that this population of *P.* × *sudermanicus* is derived from hybridization events, ruling out the possibility that these plants are aneuploid derivatives of either parent.

Together with previous studies, this chloroplast data would suggest that this population is the result of at least two hybrid events. Although it is likely that the two hybrids have resulted from reciprocal F1 crosses between *P. acutifloius* and *P. berchtoldii*, we cannot rule out the possibilty that one could be the result of hybrid pollen fertilising a parental species (or pollen from a parental species pollinating a hybrid). As we suspect that both parents

have been rare at various periods in the history of this populations, this would provide an increased chance of interspecific pollen succeeding in rare individuals (Rieseberg, 1995).

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