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Genetic evidence for hybridization between the native *Spartina maritima* and the introduced *Spartina alterniflora* (Poaceae) in South-West France: *Spartina* × *neyrautii* re-examined

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Abstract. *Spartina alterniflora*, a perennial grass native to the North American Atlantic coast, was introduced during the 19th century in western Europe (Southern England and western France) where it hybridized with the native *Spartina maritima*. In England, the sterile hybrid *S.* × *townsendii* gave rise by chromosome doubling to the highly fertile allopolyploid *Spartina anglica*, which has now invaded many salt marshes and estuaries in western Europe, and has been introduced in several continents. In South-West France, another sterile hybrid was discovered in 1892 in the Bidassoa Estuary, and named *Spartina* × *neyrautii*. According to their morphology, some authors suggested that *S.* × *neyrautii* and *S.* × *townsendii* result from reciprocal crosses. During the 20th century, the hybridization site was severely disturbed, and surviving of *S.* × *neyrautii* was questioned. In this paper, various *Spartina* populations are investigated in the Basque region (France and Spain), and compared to the hybrid taxa formed in England (*S.* × *townsendii* and *S. anglica*). The samples were analyzed using molecular fingerprinting (RAPD and ISSR) and Chloroplast DNA sequence (*trn*L-*trn*T spacer, *trn*L intron and *trn*L-*trn*F spacer). In the Bidassoa estuary, a hybrid isolated clone has been found, that displays additive species-specific nuclear markers of *S. maritima* and *S. alterniflora*, and that is subsequently considered as a surviving clone of *S.* × *neyrautii*. The molecular analyses indicate that *S.* × *neyrautii* and *S.* × *townsendii* share the same maternal (*S. alterniflora*), and paternal (*S. maritima*) parental species, but also that the two independent hybridization events have involved different parental (nuclear) genotypes in England and in South-West France.

Key words: Hybridization, *Spartina* × *neyrautii*, RAPD, ISSR, cpDNA.

Introduction

Spartina species are perennial grasses frequently concerned by human-caused dispersal outside their native range (Patten 1997). Since the 19th century, long distance introductions have led to several hybridization events involving related hexaploid species (Baumel et al. 2002a) that were initially geographically isolated. This has resulted in various ecological and evolutionary consequences including biological invasion and

introgressive hybridization and/or speciation, respectively (Ellstrand and Schierenbeck 2000). As a striking example, *Spartina alterniflora* Loiseleur, native to the eastern coast of the North America was introduced to the West European coast as well as to the western North American coast. In both regions, it has hybridized with indigenous species.

In England, *S. alterniflora* was introduced in the Southampton area in the early 1800s where it hybridized with the native *Spartina maritima* Fernald, resulting in a sterile perennial hybrid named *Spartina* × *townsendii* H. & J. Groves (Groves and Groves 1882). *Spartina* × *townsendii* still remains localized in the locality of Hythe (Hubbard 1965, Raybould et al. 2000). Chromosome doubling in the hybrid gave rise to a new fertile and robust rhizomatous allopolyploid species, *Spartina anglica* C.E. Hubbard, which has been actively colonizing British and French salt marshes and estuaries since its formation around 1890 (Hubbard 1965, Raybould et al. 1991, Guénégou and Levasseur 1993, Baumel et al. 2001). The ability of this species to trap large volumes of tidal sediments led to its deliberate introduction in several parts of the world (North Europe, Australia, New Zealand, China…) in conjunction with land reclamation. However, the rapid expansion of the invasive young allopolyploid is now considered to be a threat to coastal environments (Guénégou et al. 1991, Thompson 1991, Gray and Raybould 1997).

In France, *S. alterniflora* was also introduced during the 19th century in Brittany near Brest (Elorn Estuary), and in the Basque region near Hendaye (Bidassoa Estuary). In the latter site, *S. alterniflora* was recorded for the first time in 1806 (Chevalier 1923). In 1892, Neyraut discovered a new taxon near Hendaye (Foucaud 1897). Named *Spartina* × *neyrautii* Foucaud, it was first suspected to be the same hybrid as *S.* × *townsendii* found in England (Foucaud 1897). However, some authors (Chevalier 1923, Saint-Yves 1932) believed that *S.* × *neyrautii* was simply a variant of *S. alterniflora*. A careful morphological analysis conducted by Jovet (1941) led to the recognition of *S.* × *neyrautii* as a distinct taxon. Jovet (1941) considered *S.* × *neyrautii* and *S.* × *townsendii* as two different hybrid forms between *S. maritima* and *S. alterniflora*. The morphological differences observed between the two hybrid taxa was thought to be the results of reciprocal cross between the parental species in Southampton and Hendaye respectively (Arber 1934, in Marchant 1977).

In 1970, a botanical expedition found that the type locality of *S.* × *neyrautii* had been destroyed by land reclamation (Hubbard et al. 1978). The only surviving colony was growing in a small marsh area, bordering the San Sebastian airport, in the estuary of the river Bidassoa. Marchant (1977) who examined plants from this colony, showed that it was similar to the first description of *S.* × *neyrautii* (Foucaud 1897), and also that the anthers did not dehisce and contained aborted pollen grains. Marchant (1977) also determined that this taxon had $2n = 62$ chromosomes, like *S.* × *townsendii* and *S. alterniflora*. Meiotic chromosome behavior was irregular, as expected for a hybrid, with univalents, bivalents and multivalents. The pattern was similar to that observed in *S.* × *townsendii* (Marchant 1968); however, *S.* × *neyrautii* displayed a slightly higher frequency of multivalents. Moreover Marchant (1977) reported that "some *S. alterniflora* characters are more strongly emphasized in the morphology of *S.* × *neyrautii* than in any clone of *S.* × *townsendii,* again suggesting a different genetic basis". The molecular evidence for hybrid status of *S.* × *neyrautii* was provided by isozyme studies (Raybould et al. 1990) based on 11 enzyme systems. Electrophoretic patterns of *S.* × *neyrautii* was found to be completely identical with those of *S.* × *townsendii*.

In this paper, we address the following questions:

i) Is *S.* × *neyrautii* still present in the Basque region?
ii) Are morphological differences observed between *S.* × *neyrautii* and *S.* × *townsendii* the result of reciprocal crosses between

S. maritima and *S. alterniflora*, or is *S. alterniflora* the maternal genome donor of *S.* × *neyrautii* as found for *S.* × *townsendii* (Ferris et al. 1997)?

iii) To what extent are the parental genotypes of *S.* × *neyrautii* genetically different than those involved in the parentage of *S.* × *townsendii*?

iv) Is there evidence for chromosome doubling in the hybrid plants from South West France similar to that found in Southampton Bay where a new invasive allopolyploid species (namely *S. anglica*) was formed?

In order to explore these questions, a survey of *Spartina* populations was performed in the Basque region during summer 2000. The sampling was concentrated into this area as there are no any other known region (if we except southern England) where hybridization occurred between the indigenous *S. maritima* and the introduced *S. alterniflora*. All of the collected plants from the Bidassoa River and the adjacent estuaries were investigated using various molecular markers from both the nuclear and the chloroplast genomes, and ploidy levels were assessed by flow cytometry. These samples were compared to *S.* × *townsendii* and to the allopolyploid *S. anglica*. Various *S. alterniflora* accessions that have been introduced in Western Europe (England and France) were also analyzed by chloroplast and nuclear DNA markers.

Material and methods

Plant material

The material used in this study is presented in Table 1. It includes the parental species *S. alterniflora* and *S. maritima*, the English hybrid (*S.* × *townsendii*) from Southampton and its allopolyploid derivative *S. anglica*. As populations of *S. anglica* were previously found composed of only one major multilocus genotype over its distribution range (Baumel et al. 2001, 2002b), only two samples from France were used here for comparison. Ten sites have been sampled in the Basque region (from Guernika in Spain to Hossegor in France). Samples from southern England and Brittany have been also analyzed for comparison (Table 1). In the Basque region, sampling efforts were concentrated along the Bidassoa River where *S.* × *neyrautii* was expected: Plants were collected on both the French bank (Hendaye, Chingoudy Bay) and the Spanish bank (Irun Pheasant Island, and Plaiundi Park). The initial marsh near the San Sebastian airport where *S.* × *neyrautii* was recorded for the last time (Hubbard et al. 1978) has since then been reshaped following airport extension, and we were unable to localize any *Spartina* from this area. The plants collected in the Bidassoa Estuary were initially referred to as *S. alterniflora* prior to genetic analyses (see below). All the *Spartina* sampled in the Basque region were grown in the greenhouse at University of Rennes.

Table 1. Origins of the studied *Spartina* samples

Taxa	Sites
S. alterniflora	Hythe, Southampton water, England
	Marchwood, Southampton water, England
	Landerneau, Elorn River, France
	Hossegor Lake, France
	Ascain, Nivelle River, France
	Hendaye, Bidassoa River, France
	Chingoudy, Bidassoa River, France
	Irun Pheasant Island, Bidassoa River, Spain
	Plaiundi Park, Bidassoa River, Spain
	Zumaria, Urola River, Spain
	Orio, Oria River, Spain
S. maritima	Saint Briac, Frémur river, France
	Guernika, Oca River, Wildlife reserve of Urdaibai, Spain
S. × *townsendii*	Hythe, Southampton water, southern England
S. anglica	Baie des Veys, France
	Seine estuary, France

Molecular analyses

DNA extraction. DNA extracts were prepared according to the cetyltrimethylammonium bromide

(CTAB) method (Ausubel et al. 1995). Approximately 30 mg of fresh leaves were ground in 2% CTAB solution (1.4 M NaCl, 20 mM EDTA, 100 mM Tris HCl pH 8.0, 2% CTAB). The extracts were incubated for 1 hour at 65 °C, and then 500 µl of chloroform were added. After mixing, 500 µl of isopropanol were added to the supernatant for precipitation. After microcentrifugation the DNA pellet was washed with 70% ethyl alcohol then it was resuspended in the TE (Tris-EDTA) buffer.

Molecular fingerprinting. The *Spartina* samples were analyzed by RAPD (Randomly Amplified Polymorphic DNA; Williams et al. 1990) and ISSR (Inter Simple Sequence Repeat; Gupta et al. 1994) using primers generating species-specific markers that discriminate *S. maritima* from *S. alterniflora* (Baumel et al. 2001, Baumel et al. 2002b).

Genomic DNA was amplified using $(CTC)_6G$, $(AGC)_6G$ and $(TGC)_6C$ primers for ISSR markers, and the 10-mer random oligonucleotide primer OP-C01 (Operon Technologies) for RAPD markers. Each PCR volume of 20 µl for ISSR contains approximately 50 ng of genomic DNA, 1X incubation buffer (Qbiogen), 1.5 mM $MgCl_2$, 20 mM dNTP, 0.1 µM SSR primer and 1 unit of Taq polymerase (Qbiogen). PCR reactions were run on a Techne thermal cycler using the following program: 2 minutes at 94 °C, 30 cycles of: 30 seconds at 94 °C, 30 seconds at 58 °C, 2 minutes at 72 °C, 10 minutes of final extension at 72 °C.

Each PCR volume of 20 µl for RAPD contained approximately 50 ng of genomic DNA, 1X incubation Taq Buffer (Qbiogen), 1.5 mM of $MgCl_2$, 20 mM dNTP, 0.2 µM Operon primer and 1.25 units of Taq polymerase (Qbiogen). Reactions were placed in a Techne thermal cycler programmed as follows: 1 minute at 94 °C followed by 45 cycles of 1 minute at 94 °C, 1 minute at 36 °C and 2 minutes and 72 °C. An additional cycle of 15 minutes at 72 °C was used for final extension.

Chloroplast DNA analysis. Chloroplast markers were examined in plants that displayed hybrid RAPD and ISSR patterns (see below), in order to assess maternal inheritance. The *trn*T–*trn*L and *trn*L–*trn*F spacers and the *trn*L intron were sequenced and compared to those obtained for *S. maritima* and *S. alterniflora*. *Trn*L intron sequences were further compared with additional *S. alterniflora* samples from North America (Antilla et al. 2000).

Sequence amplification and sequencing have been performed using the a, b, c and f primers from Taberlet et al. (1991). Each reaction (final volume of 100 µl) contained 1 µl DNA template, 1X incubation buffer (Qbiogen), 7.5 mM $MgCl_2$, 10 µM each primer, 5 units Taq polymerase (Qbiogen). The amplifications were conducted as following: the thermocycler was preheated until 85 °C, and then PCR reactions were run according to the following program: two minutes at 94 °C followed by 30 cycles of 1 minute at 94 °C, 1 minute at 48 °C and 2 minutes at 72 °C. The program ended by a final extension step of 10 minutes at 72 °C.

PCR products were purified using Ultra-Free® MC Millipore membrane and both strands of the amplified fragments were sequenced. Direct sequencing was done using the ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystem, Foster city CA). Sequencing reactions were set up according to the supplier's recommendations and purified using ethanol precipitation at room temperature. Automated DNA sequencing was performed on a ABI PRISM™ 310 Genetic analyzer (PE Biosystem, Foster city CA). Sequences were aligned visually among species.

Flow cytometry analysis

The relative ploidy levels of *S. maritima*, *S. alterniflora*, *S.* × *townsendii*, *S. anglica* and putative hybrid plants were determined using flow cytometry, according to the method previously described by Misset and Gourret (1996). Fresh leaves were chopped with a razor blade in 2 ml of a 0.1 M Tris-HCl pH 7.00 0.1 M NaCl buffer, containing 2 mM $MgCl_2$ and 0.05% Triton X-100, in order to break cells and obtain free nuclei suspensions. The flow cytometer, a Partec CA II device with DPAC software, was used to measure the fluorescence emission of these nuclear suspensions stained with 20 µl of 4′,6-diamino-2-phenylindole (DAPI) added to the buffer as a fluorochrome of DNA. *Brassica napus* was used as standard, and chopped separately in the nuclear extracting buffer. During the whole session of measurements, channel 100 was adjusted to *B. napus*.

Results

PCR-based multilocus analysis

All the samples listed in Table 1 were first analyzed by the OP-C01 RAPD primer and the $(AGC)_6G$ ISSR primer. As these primers generated species-specific patterns differentiating *S. maritima* from *S. alterniflora* (Baumel et al. 2001, Baumel et al. 2002b), this preliminary screening allowed us to search for potential hybrid individuals. In the Bidassoa Estuary where *S.* × *neyrautii* was first described, the plants sampled in Chingoudy, Plaiundi Park and Irun Pheasant Island displayed unambiguous *S. alterniflora* patterns. However, one clone from Hendaye exhibited a hybrid pattern composed of additive diagnostic DNA fragments from both *S. maritima* and *S. alterniflora* (Fig. 1).

This putative hybrid was then compared to different accessions of the parental species (*S. maritima*, *S. alterniflora*), to *S.* × *townsendii* and to the allopolyploid *S. anglica* (Table 2) on the basis of 2 PCR-based multilocus markers (ISSR and RAPD). A total of 39 DNA markers have been generated over the different taxa. Their presence/absence in the analyzed samples are listed in Table 2. Each marker found in the putative hybrid from Hendaye belongs to either *S. alterniflora* or to *S. maritima*. Marker additivity confirms a hybrid origin of at least one *Spartina* clone in the Bidassoa River. The electrophoretic patterns of this hybrid are very similar to those of *S.* × *townsendii* and *S. anglica*, however, a few notable differences were encountered: Two RAPD markers (F and K in Table 2) are found only in *S. anglica* and *S.* × *townsendii*, and are absent in the hybrid from Hendaye. These markers, specific from both the *S. maritima* samples (Mar1 and Mar2) analyzed, indicate that the actual *S. maritima* parental genotype of the Hendaye hybrid has not been sampled. Another RAPD marker (H in Table 2) was also found only in the English hybrids (*S.* × *townsendii* and *S. anglica*), but this marker is present

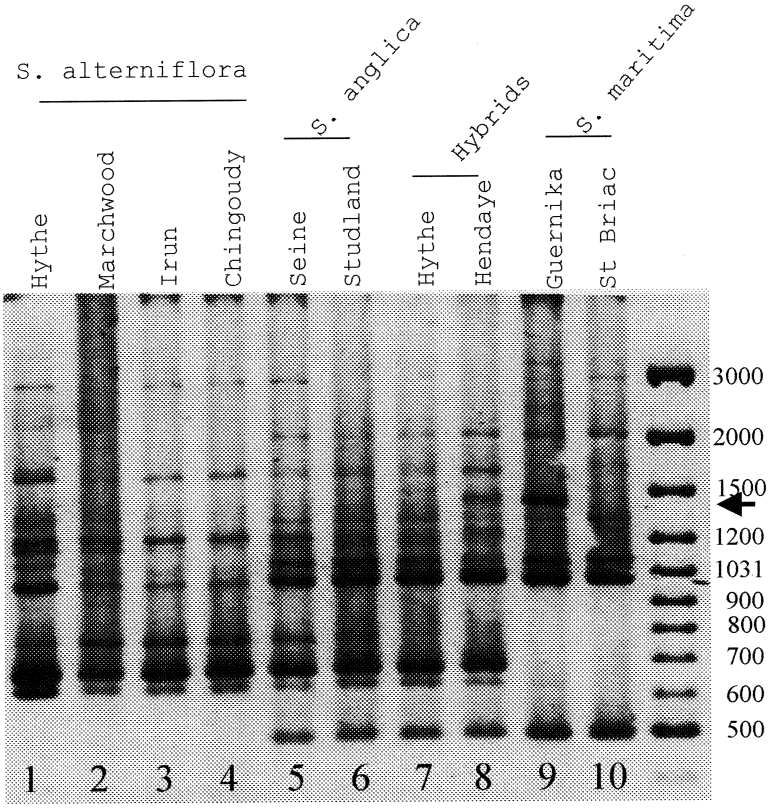

Fig. 1. ISSR electrophoretic patterns, $(TGC)_6C$ primer. Lane 1 to 4: 4 accessions of *S. alterniflora* from Hythe (England), Marchwood (England), Irun (Spain), and Chingoundy (France), respectively. Lane 5 to 6: two accessions of *S. anglica* from the Seine estuary (France) and Baie des Veys (France), respectively. Lane 7 to 8: the two hybrids, from Hythe, England- (*S.* × *townsendii*) and from Hendaye, France, respectively. Lane 9 to 10: two accessions of *S. maritima* from Guernika (Spain) and Saint Briac (France), respectively. The arrow indicates the size of the polymorphic band in *S. maritima*

Table 2. Multilocus comparison of the parental species, the first generation hybrids and the allopolyploid. Alt1, Alt2, Alt3, Alt4, Alt5: *S. alterniflora* from Hythe (England), Marchwood (England), Landerneau (France), Irun (Spain), and Hendaye (France) respectively. Hyb: hybrid from Hendaye (France). Tow: *S.* × *townsendii* from Hythe (England). Ang: *S. anglica*. Mar1: *S. maritima* from Oca River (Spain), Mar2: *S. maritima* from Frémur River (France)

Marker	Alt1	Alt2	Alt3	Alt4	Alt5	Hyb	Tow	Ang	Mar1	Mar2
Opc01										
A	1	1	1	1	1	1	1	1	0	0
B	0	0	0	0	0	1	1	1	1	1
C	1	1	1	1	1	1	1	1	0	0
D	0	0	0	0	0	1	1	1	1	1
E	0	0	0	0	0	1	1	1	1	1
F	0	0	0	0	0	0	1	1	1	1
G	1	1	1	1	1	1	1	1	0	0
H	1	1	1	0	0	0	1	1	0	0
I	1	1	1	1	1	1	1	1	0	0
J	1	1	1	1	1	1	1	1	1	1
K	0	0	0	0	0	0	1	1	1	1
$(TGC)_6C$										
A	0	0	0	0	0	1	1	1	1	1
B	1	1	1	1	1	1	1	1	0	0
C	0	0	0	0	0	1	0	0	1	0
D	1	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1	1
F	0	0	0	0	0	1	1	1	1	1
G	1	1	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1	0	0
I	0	0	0	0	0	1	1	1	1	1
$(CTC)_6G$										
A	0	0	0	0	0	1	1	1	1	1
B	0	0	0	0	0	1	1	1	1	1
C	1	0	0	0	0	0	0	0	0	0
D	1	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	1	1
F	1	1	1	1	1	1	1	1	0	0
G	1	1	1	1	1	1	1	1	1	1
H	0	0	0	0	0	1	1	1	1	1
$(AGC)_6G$										
A	1	1	1	1	1	1	1	1	0	0
B	0	0	0	0	0	1	1	1	1	1
C	0	0	0	0	0	1	1	1	1	1
D	1	1	1	1	1	1	1	1	0	0
E	1	1	1	1	1	1	1	1	0	0
F	1	1	1	1	1	1	1	1	0	0
G	0	0	0	0	0	1	1	1	1	1
H	1	1	1	1	1	1	1	1	0	0
I	0	0	0	0	0	1	1	1	1	1
J	1	1	1	1	1	1	1	1	0	0
K	1	1	1	1	1	1	1	1	0	0

only in the samples of *S. alterniflora* from England and from Landerneau in Brittany. Thus, the *S. alterniflora* plants from the Bidassoa River (France and Spain samples) are identical or close to the parental genotype of the Hendaye hybrid. Finally, one ISSR marker (C band generated by the $(TGC)_6C$ primer, Table 2) of *S. maritima* origin is polymorphic among the hybrids. The size of this fragment is approximately 1450 bp (Fig. 1). It is found in the hybrid sample from Hendaye and in the sample of *S. maritima* from the Oca River (Spain), but it is not observed in *S.* × *townsendii* nor in *S. anglica* (Fig. 1). These results consequently indicate that different genotypes of *S. alterniflora* and *S. maritima* have been involved in the hybridization events in the Basque region and in southern England.

Chloroplast DNA sequencing

Sequences of the *trn*T-*trn*L spacer, *trn*L intron and *trn*L-*trn*F spacer of the hybrid from Hendaye and from the different accessions of *S. maritima*, *S. alterniflora* (listed in Table 1) have been compared. No intraspecific variation was encountered: the samples of *S. maritima* from Saint Briac (France) and from Guernika (Spain) display the same sequences as previously reported in other accessions by Baumel et al. (2001) for *trn*T-*trn*L and *trn*L-*trn*F and by Ferris et al. (1997) for *trn*L intron. In *S. alterniflora*, all the French and Spanish samples display the same sequences as the samples from Marchwood and Hythe in England. These sequences are also identical to those previously reported by Ferris et al. (1997), Baumel et al. (2001) and Baumel et al. (2002a) for *trn*L intron, *trn*L-*trn*F spacer and *trn*T-*trn*L spacer respectively. *Spartina anglica* and *S.* × *townsendii* display the same *trn*L intron (Ferris et al. 1997), *trn*T-*trn*L, *trn*L-*trn*F and *rpl*16 intron (Baumel et al. 2001) sequences as *S. alterniflora*.

Table 3 shows 10 polymorphic sites between *S. alterniflora* and *S. maritima* that were scored among the 1650 nucleotides sequenced from the chloroplast genome. These species specific markers reveal that the hybrid from Hendaye has the same chloroplast sequences as *S. alterniflora*, as do *S.* × *townsendii* and *S. anglica* populations from England (Ferris et al. 1997) and from France (Baumel et al. 2001).

Flow cytometry

The ploidy level of the hybrid plant sampled in Hendaye was assessed by comparison to that of the four other taxa using flow cytometry. *Spartina alterniflora* (2n = 62), *S. maritima* (2n = 60), *S.* × *townsendii* (2n = 62) fluoresced approximately the same quantity which was also close to that of the standard *B. napus* (not shown). As expected from its chromosome number (2n = 124), the fluorescence of the allopolyploid *S. anglica* was twice that of the previously mentioned taxa. The hybrid from Hendaye appears to have the same nuclear DNA amount as the parental species *S. maritima* and *S. alterniflora* and as *S.* × *townsendii*, that is about half of the fluorescence provided by *S. anglica* (Fig. 2). Then, hybridization in Hendaye has not been followed by chromosome doubling, which is consistent with the sterility observed (important amount of aborted pollen and absence of seed, Baumel, personal observations).

Discussion

Our molecular investigations in *Spartina* populations from the Basque region revealed that the hybrid *S.* × *neyrautii* formed between *S. maritima* and *S. alterniflora*, is present in the estuary of the Bidassoa River. This hybrid displays the features that have been previously reported for *S.* × *neyrautii* (Marchant 1977), including morphological resemblance to *S. alterniflora*, pollen sterility, and it has the same ploidy level as *S.* × *townsendii*, the hybrid from southern England. As the closest site of *S. maritima* has been found farther South-West, in the mouth of the Oca River (Guernika, Spain), it is very unlikely that the hybrid we sampled in Hendaye is the result of a new hybridization

Table 3. Polymorphic sites from 3 chloroplast DNA sequences. The sequences of *S. alterniflora* and *S. maritima* have been obtained from the different accessions listed in Table 1

	*trn*T-*trn*L spacer 759 bp					*trn*L-intron 555 bp				*trn*L-*trn*F spacer 336 bp				
	298	492	541	Genbank accession no.		126	382	430	Genbank accession no.	7	189	190	197	Genbank accession no.
S. maritima	T	G	T	AF275669*		C	C	T	Z69911**	C	T	C	G	AF311341*
S. alterniflora	G	T	G	AF275667*		T	A	A	Z69914**	T	C	A	T	AF311340*
Hybrid (Hendaye)	G	T	G			T	A	A		T	C	A	T	

* Accession number from Baumel et al. 2001
** Accession number from Ferris et al. 1997

event between *S. maritima* and *S. alterniflora*. Rather, our findings suggest that the hybrid represents the last extant, or one of the few remnant clones of *S.* × *neyrautii* that appeared 110 years ago in the same place.

The maternally inherited chloroplast genome of this hybrid is identical to that of *S. alterniflora*, which is then the maternal parent of *S.* × *neyrautii*, as well as for *S.* × *townsendii* and *S. anglica* (Ferris et al. 1997). Therefore, earlier speculations that *S.* × *neyrautii* was the reciprocal hybrid to *S.* × *townsendii* (Marchant et al. 1977) are inconsistent. This follows the finding of Raybould et al. (1990) who found that *S.* × *neyrautii* and *S.* × *townsendii* displayed identical isozyme phenotypes.

Several different *S. alterniflora* individuals collected through western Europe were analyzed in this study. Chloroplast haplotypes were identical among the samples of *S. alterniflora* from South England, Brittany and South-West France. Three chloroplast haplotypes have been encountered along the Atlantic coast of North America (Antilla et al. 2000). The *trn*L intron sequence of the European samples matches with the TAA chlorotype recorded by Antilla et al. (2000) from Massachusetts. As expected from founding effects in introduced clonal plants, weak genetic variation is encountered in the European *S. alterniflora* populations. This is also observed for the nuclear markers used in this study. However, the minor RAPD variants differentiating the samples collected in the Basque region from those sampled in England and Brittany, suggest that in western Europe, *S. alterniflora* populations have resulted from at least two different introductions.

Chloroplast haplotypes were invariant between the two populations of *S. maritima* sampled from Brittany and Spain, and only one ISSR marker was polymorphic. A more extensive screening of *S. maritima* populations (Yannic 2001, G. Yannic, A. Baumel and M. Ainouche unpublished data) has revealed a striking lack of variation in western Europe, which is interpreted as a result of a predominantly asexual propagation of the species in its northern range limit.

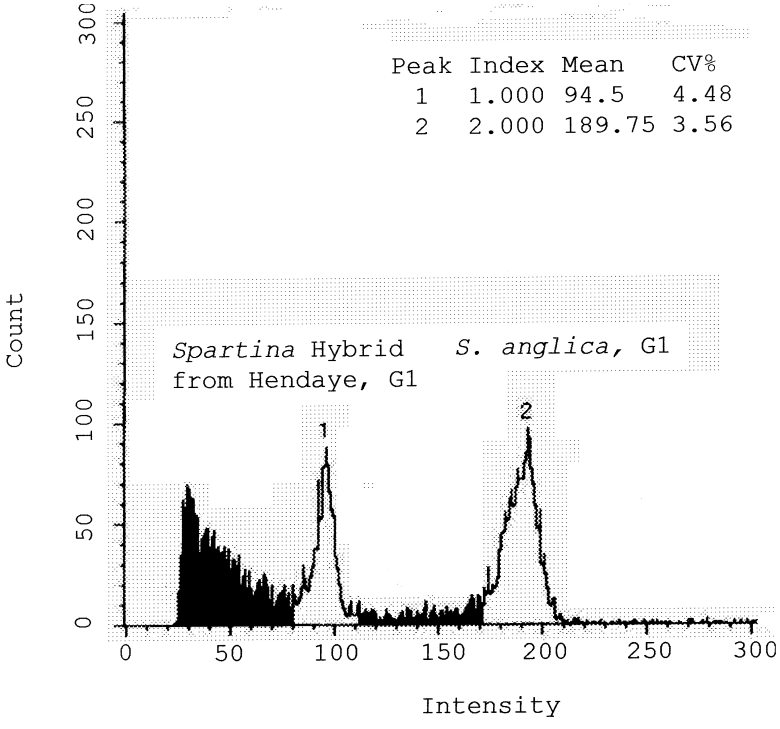

Fig. 2. Flow cytometry analysis of the hybrid from Hendaye and *Spartina anglica* (DAPI fluorescence, linear scale)

Despite the low variation found in the parental species *S. alterniflora* and *S. maritima*, three RAPD and one ISSR markers allow to distinguish *S.* × *neyrautii* from *S.* × *townsendii*. These differences inherited from different parental genotypes in Southampton and Hendaye respectively, might explain the morphological differences reported between the two hybrids (Marchant 1977). Accordingly, it is amazing that very few genetic differences may account for such phenotypic variation.

In conclusion, our study shows that *S.* × *neyrautii* and *S.* × *townsendii* share the same maternal (*S. alterniflora*), and paternal (*S. maritima*) species, but also that the two independent hybridization events have involved different parental (nuclear) genotypes in Southampton water and in the Bidassoa estuary. Considering the threatened situation of *S.* × *neyrautii* now represented by only a few surviving plants, careful attention should be paid for the conservation of this taxon.

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