Retrotransposons and Genomic Stability in Populations of the Young Allopolyploid Species *Spartina anglica* C.E. Hubbard (Poaceae)

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Spartina × *townsendii* arose during the end of the 19th century in England by hybridization between the indigenous *Spartina maritima* and the introduced *Spartina alterniflora*, native to the eastern seaboard of North America. Duplication of the hybrid genome gave rise to *Spartina anglica*, a vigorous allopolyploid involved in natural and artificial invasions on several continents. This system allows investigation of the early evolutionary changes that accompany stabilization of new allopolyploid species. Because allopolyploidy may be a genomic shock, eliciting retroelement insertional activity, we examined whether retrotransposons present in the parental species have been activated in the genome of *S. anglica*. For this purpose we used inter-retrotransposon amplified polymorphism (IRAP) and retrotransposons-microsatellite amplified polymorphism (REMAP) markers, which are multilocus PCR-based methods detecting retrotransposon integration events in the genome. IRAP and REMAP allowed the screening of insertional polymorphisms in populations of *S. anglica*. The populations are composed mainly of one major multilocus genotype, identical to the first-generation hybrid *S.* × *townsendii*. Few new integration sites were encountered in the young allopolyploid genome. We also found strict additivity of the parental subgenomes in the allopolyploid. Both these findings indicate that the genome of *S. anglica* has not undergone extensive changes since its formation. This contrasts with previous results from the literature, which report rapid structural changes in experimentally resynthesized allopolyploids.

Introduction

Allopolyploidy is a well-known process in plant speciation involving the duplication of an interspecific hybrid genome. However, evolution of the two duplicated parental genomes (i.e., the homoeologous subgenomes) in the allopolyploid remains obscure in most cases because of the lack of information on the initial conditions (Wendel 2000). In most natural polyploids, the parental species have evolved or become extinct since the hybridization event. *Spartina anglica* (Poaceae) is among the few well known recent allopolyploids. The history of this species has been well documented on the basis of morphological (Marchant 1967; Hubbard 1968, pp. 358–359), cytogenetic (Marchant 1968), and isozyme (Guénégou, Citharel, and Levasseur 1988; Gray, Benham, and Raybould 1990; Raybould et al. 1991*a*, 1991*b*) data. *Spartina anglica* arose around 1890 in southern England (Southampton Water), after hybridization between the indigenous *S. maritima* Fernald (2n = 60) and the introduced Eastern North American *S. alterniflora* Loiseleur (2n = 62). Chromosome doubling in the sterile hybrid (*Spartina* × *townsendii*) gave rise to the new allopolyploid species *S. anglica* (2n = 120, 122, and 124, according to Marchant 1968). This species is a fertile, perennial plant that has been actively colonizing the salt marshes and estuaries of Britain and France since 1890 and 1906, respectively. Both significant seed production and extensive lateral clonal growth characterize this rhizomatous species. As a nascent species (less than 150 years old), *S. anglica* is a particularly suitable model to trace allopolyploid genome evolution because its parental species are unambiguously identified and are both still extant.

Previous studies have shown no variation at isozyme loci in British (Raybould et al. 1991*a*) and French populations (M. T. Misset, personal communication). A recent study of several French populations (Baumel, Ainouche, and Levasseur 2001), using RAPD markers, also revealed low molecular variation, thus supporting a unique origin of the allopolyploid (i.e., unique hybridization event or multiple events involving identical parental genotypes). Both parental species (*S. alterniflora* and *S. maritima*) lack genetic variation in western Europe (Raybould et al. 1991*a*, 1991*b;* Yannic, Baumel, and Ainouche, unpublished data).

In this study, we focus on potentially rapidly evolving parts of the *S. anglica* genome. Because changes can occur at both single and multiple loci, dispersed components of the genome must be investigated to test the null hypothesis of strict parental genome additivity in the allopolyploid. It is well known that retroelements constitute an important fraction of the DNA content of many plants, particularly in grasses (Vicient et al. 2001). Their abundance, dispersion across the nuclear genome, and their insertional activity indicate that they play a significant role in plant genome evolution (Bennetzen 2000). Retrotransposons propagate by a replicational mechanism that has potential to remodel the genome (Kumar and Bennetzen 1999). In wild barley, *Hordeum spontaneum*, the retrotransposon *BARE*-1 generates genetic polymorphism and increases genome size by its activity (Kalendar et al. 2000). Studies on *BARE*-1 in *Hordeum* indicate that it is an active component of genome size variation in *Hordeum* species, which appears prone to adaptation to environmental stresses such as

Key words: allopolyploidy, retrotransposon, IRAP, REMAP, *Spartina anglica*, genome stress, genomic stability.

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Table 1
List and Origin of the Plant Material (* = Chromosome Numbers from Marchant 1968)

Species	Number of Samples	Origin
Spartina maritima (2*n* = 60*)	1	Saint Briac (France)
	1	Baden (France)
	1	Etel (France)
Spartina alterniflora (2*n* = 62*)	1	Marchwood (south England)
Spartina anglica (2*n* = 120, 122, 124*)	10	Bull Island (Ireland)
	10	Falley (south England)
	10	Key haven (south England)
	10	Studland (south England)
Spartina × *townsendii* (2*n* = 62*)	10	Hythe (south England)

drought (Vicient et al. 1999; Kalendar et al. 2000; Wendel and Wessler 2000).

Retrotransposon activity is regulated by various factors, and biotic and abiotic stresses can enhance transposition in natural populations (Wessler 1996; Kalendar et al. 2000). Although it is known that transposable elements can be involved in speciation, as suggested by the example of hybrid dysgenesis in *Drosophila* (Kidwell and Lisch 2000), little is known about their role during the first stages of homoploid hybrid and allopolyploid speciation in plants. Interspecific crosses could result in genome stress (McClintock 1984; O'Neill, O'Neill, and Marshall Graves 1998; Liu and Wendel 2000), increasing retroelement insertional activity. Because polyploid genomes contain duplicates of all genes, they are thought to be buffered from the deleterious consequences of transposition (Matzke, Mittelsten Scheid, and Matzke 1999).

The high copy number and general dispersion of retrotransposons throughout the genome, as well as the large local change caused by their insertion, provide an excellent basis for the development of DNA-based markers. Such markers allow investigation of genetic variation related to the distribution and activity of retrotransposons, both at the intraspecific and the interspecific levels. Retrotransposon marker systems rely on PCR to generate fingerprints. The fingerprints are multilocus profiles revealing polymorphisms in the insertion of members of given families of retrotransposons. The inter-retrotransposon amplified polymorphism (IRAP) technique (Kalendar et al. 1999) generates PCR products from retrotransposons inserted near enough to each other so as to allow efficient amplification and generally uses primers matching the outer segments of the long terminal repeats (LTRs). Retrotransposons-microsatellite amplified polymorphism (REMAP) (Kalendar et al. 1999) uses one LTR primer, together with a primer designed for annealing to the 3′ end of a stretch of simple sequence repeats (SSR), and detects retrotransposons inserted near to SSRs. Therefore, REMAP can amplify a pool of three different sequences: fragments situated between an LTR and a microsatellite locus, sequences situated between two microsatellite loci, and sequences situated between retroelements. Because many retroelements have the tendency to insert into regions rich in tandemly repeated DNA (Kalendar et al. 1999; Ramsay et al. 1999), as well as near to, or nested within, each other (San Miguel et al. 1996; Shirasu et al. 2000), REMAP and IRAP are efficient at detecting insertion events. In this study, fragments situated between microsatellite loci (Inter Simple Sequence Repeat or ISSR markers, Wolfe and Liston 1998) were also amplified in order to detect eventual variation affecting the repetitive fraction of the allopolyploid genome.

The goal of this study was to determine whether polyploid formation has led to retrotransposon activation, generating molecular variation in *S. anglica* populations. Because this clonal plant is characterized by vigorous vegetative propagation, mutations that have occurred since its appearance are likely to be fixed in populations. Our previous studies revealed a striking lack of genetic variation (Baumel, Ainouche, and Levasseur 2001), indicating that almost all the French populations of *S. anglica* are composed of the same major multilocus genotype. In this study, several samples from the center of origin of the species (Southampton salt marshes, England) have been analyzed by IRAP, REMAP, and ISSR markers. The plants examined include both the parental species (*S. maritima* and *S. anglica*) and the first-generation hybrid *S.* × *townsendii*.

Plant Material

A list of the *Spartina* samples analyzed in this study is presented in table 1. The samples of *S.* × *townsendii* are from the Hythe (UK) salt marshes, where this species was recorded for the first time in 1870. These samples were identified as *S.* × *townsendii* by morphological observation (sterile stamens) and by genome size estimated with flow cytometry (unpublished data). Four populations of *S. anglica* have been sampled near this site, in Falley and Keyhaven, separated by approximately 5 and 15 km, respectively, from Hythe. Studland is in the southeastern part of Pool Harbour, about 50 km from Hythe. The fourth population is at Bull Island in Ireland. Individual plants were collected in the field with roots, leaves, and rhizomes to allow both for DNA extraction (from leaves) and for transplanting into the greenhouse. Because *Spartina* species are present at each site as clonal plants, we tried to maximize the distance between samples to avoid collecting from the same individual. Ten individuals per population were then collected for *S. anglica* and *S.* × *townsendii*. The parental species, *S. maritima* and *S. alterniflora*, were

Table 2
Primers

Primer name LTR primers	Element origin in barley	Sequence	Used for
C0699	*Nikita*	CGC TCC AGC GGT ACT GCC	IRAP,
C0795	*BARE-1*	TCC CAT GCG ACG TTC CCC	IRAP, REMAP with $(GAG)_6C$ and $(GTG)_7A$ SSR primers
C0905	*Sukkula*	TTT GAA AAC TGG CGG CAA CG	IRAP
C0945	*Sabrina*	GCA AGC TTC CGT TTC CGC	IRAP
C0947	*Sabrina*	TTG TTC ACC CAC CGT CTA CTT GC	IRAP
9900	*Sukkula*	GAT AGG GTC GCA TCT TGG GCG TGA C	IRAP, REMAP with $(GTG)_7C$ and $(GTG)_7A$ SSR primers
SIRE 1	*Sire*	GCA GTT ATG CAA GTG GGA TCA GC	IRAP
Stowaway	*Stowaway*	CTT ATA TTT AGG AAC GGA GGG AGT	IRAP
SSR primers			
		$(GTG)_7C$	ISSR, REMAP with 9900 LTR primer
		$(CTC)_6G$	ISSR
		$(TGC)_6C$	ISSR
		$(AGC)_6G$	ISSR
		$(GAG)_6C$	REMAP with C0795 LTR primer
		$(GTG)_7A$	REMAP with 9900 and C0795 LTR primers

included for comparison. Three samples of *S. maritima* have been collected in different French salt marshes, and *S. alterniflora* has been collected in Marchwood (near Hythe, UK). As mentioned previously, these species lack genetic variation in western Europe; then, we might reasonably expect this sampling to be representative from the actual progenitors of *S. anglica*.

Methods

DNA Extraction

Samples of DNA were prepared by the Cetyltrimethylammonium bromide (CTAB) method (Ausubel et al. 1995, pp. 4.7.1–4.7.8). 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 extract was incubated for 1 h at 65°C, then 500 μl of chloroform were added. After mixing, 500 μl of 100% isopropanol was added to the supernatant for precipitation. After microcentrifugation, the DNA pellet was washed with 70% ethanol, then resuspended in the Tris-EDTA buffer.

IRAP and REMAP Procedures

The IRAP and REMAP procedures were based on earlier published methods (Kalendar et al. 1999; Schulman et al. 2002). The IRAP reactions of 20 μl contained: 0.075 M Tris-HCl pH 8.8, 0.02 M $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.01% Tween-20, 0.2 mM dNTPs, 0.2 μM each primer (presented in table 2), 1.5 U *Taq* polymerase, and 10 ng DNA template. The amplifications were performed on a Mastercycler Gradient (Eppendorf) using the following program: 2 min at 94°C; 33 cycles of 30 s at 94°C, 30 s at 58°C, 2 min at 72°C; termination by 10 min of final extension at 72°C.

The reaction conditions and amplification program for REMAP were similar to those of IRAP, except that we used less *Taq*1 polymerase (1 U per reaction) and fewer PCR cycles (29 instead of 33).

Inter Simple Sequence Repeat Procedure

ISSRs are PCR markers commonly displaying a high degree of polymorphism (Wolfe and Liston 1998). In order to determine the specific contribution of retrotransposons to the observed polymorphism, we performed ISSR amplifications on the same *Spartina* material used for IRAP and REMAP. We used the following SSR primers: $(GTG)_7C$, $(CTC)_6G$, $(TGC)_6C$, $(AGC)_6G$. Amplification conditions were the same as for IRAP but with only 30 cycles.

Electrophoresis and Data Analysis

The three fingerprinting techniques produce numerous bands. Therefore, high-resolution electrophoresis is required for their reaction products. PCR products were run on 2% agarose gels (RESULT LE, BIOzym, Landgraaf, The Netherlands) for 7–8 h at 85 volts, stained with ethidium bromide, and photographed with a 35-mm film-based reflex camera.

As some primer combinations have one primer in common, these combinations might amplify the same polymorphic band. This eventual bias in polymorphism estimates was checked by band size comparisons and by recording the absence or presence of these bands among the samples.

IRAP and REMAP data were analyzed in two ways. First, a table scoring the presence or absence of the bands observed on the gels was created in order to record the variation in *S. anglica* populations. Invariable, ambiguous and nonreproducible bands were not included. Second, reaction products for the allopolyploid and its parents were run on the same gel, and interspecific comparisons were performed. Under the assumption that bands of the same size are derived from the same loci in each of these species, seven possible outcomes could be scored, with a given band being present or absent in one, two, or three species.

Table 3
IRAP, REMAP and ISSR Multilocus Genotypes and Their Distribution in *Spartina anglica* Populations. The Last Column Indicates the Parental Origin of the Marker When it was Possible to Identify it: Mar for *S. maritima*, Alt for *S. alterniflora* and Mar/Alt for both; Ang Indicates that the Marker is Specific to the Allopolyploid. N = Number of Samples Recorded for Each Genotype, M = Number of Individuals Recorded for Each Mutation Relative to the Major Genotype

	Genotypes														Parental Contribution	
Markers	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV		M
IRAP																
C0795/C0945A	−	−	−	−	−	−	+	−	−	−	−	−	−	−	Ang	1
C0699/C0945A	+	+	+	+	+	+	+	+	+	+	−	+	+	+	Mar	1
C0947/C0905C	+	+	−	−	−	+	+	+	+	+	+	+	+	−	Mar	7
9900/C0905A	+	+	−	+	+	+	+	+	+	+	+	+	+	+	Mar	2
9900/C0699A	−	−	−	−	−	−	+	−	−	−	−	−	−	−	Ang	1
9900/C0699B	−	−	−	+	−	−	−	−	−	−	−	−	−	−	Ang	2
9900/C0699C	+	+	+	+	+	+	+	+	−	+	+	−	+	+	Mar/Alt	2
REMAP																
9900/$(GTG)_7$C	+	+	+	+	+	+	+	−	+	+	+	−	+	+	Alt	4
C0795/$(GAG)_6$C-A	+	+	+	+	−	−	+	+	+	+	−	+	+	+	Mar	8
C0795/$(GAG)_6$C-B	+	+	+	+	+	+	+	+	+	−	+	+	+	+	Alt	1
ISSR																
$(GTG)_7$C	+	+	+	+	+	+	+	+	+	+	+	−	−	+	Mar	4
$(AGC)_6$A	+	+	+	+	+	+	+	+	+	+	+	−	+	+	Mar	1
$(AGC)_6$C	−	+	−	−	+	−	−	−	−	−	−	−	−	−	Ang	3
Populations																
Bull Island	3	0	2	2	2	1	0	0	0	0	0	0	0	0		
Key Haven	4	0	0	0	0	0	1	3	0	0	0	1	1	0		
Studland	6	0	0	0	0	4	0	0	0	0	0	0	0	0		
Fawley	3	1	0	0	0	0	0	0	1	1	1	0	2	1		
N	16	1	2	2	2	5	1	3	1	1	1	1	3	1		
%	40	2.5	5	5	5	12.5	2.5	7.5	2.5	2.5	2.5	2.5	7.5	2.5		

Results

Adaptation of IRAP and REMAP to *Spartina*

In the absence of characterized native retrotransposons, primers matching retrotransposons from other grass genera were tested for use in *Spartina*. The approach seemed reasonable because active retrotransposon families appear to cross generic boundaries in the grasses (Vicient et al. 2001). Several LTR primers designed for barley were tested in single-primer amplifications. Only those producing numerous, strong bands and a weak background, as well as polymorphism between the parental species, were selected for IRAP and REMAP. Because the annealing conditions of IRAP, REMAP, and ISSR were stringent, reproducible band patterns were expected. Reproducibility was tested and polymorphism confirmed by multiple reactions and electrophoretic separations.

Retrotransposon primers used in this study are listed in table 2 and match the LTRs of different families of elements. Two primers were designed to anneal to sequences of the miniature transposable elements Sire and Stowaway. Retrotransposon primers were also tested as pairs for IRAP and, in combination with SSR primers, for REMAP (table 2). We selected the following LTR/LTR primers for IRAP: C0795/C0945, C0795/Sire, C0795/Stowaway, C0699/C0945, C0947/C0905, 9900/C0905, 9900/C0699. For REMAP, the following LTR/ISSR primers were used: C0795/$(GAG)_6$C, C0795/$(GTG)_7$A, 9900/$(GTG)_7$A, 9900/$(GTG)_7$C.

Genetic Variation in *S. anglica* Populations Examined by IRAP, REMAP, and ISSR

Forty samples of *S. anglica* and 10 samples of *S.* × *townsendii* were analyzed with eight IRAP and four REMAP primer combinations and with four SSR primers. Over 296 bands were generated by IRAP and REMAP markers in *S. anglica*. IRAP produced an average of 26 bands for each primer combination, whereas REMAP yielded an average of 22 bands per primer combination. The ISSR procedure generated 92 bands with an average of 23 bands per primer.

In *S. anglica*, we found seven polymorphic bands with IRAP, three with REMAP, and three with ISSR analysis. The primer combinations that revealed polymorphism are presented in table 3. These 13 polymorphic bands constituted 14 multilocus genotypes (table 3). Most samples (40%) belonged to one genotype. The other 13 genotypes were both of low frequency and very similar to the major genotype because the differences involve no more than absence or presence of 1-4 bands. Two IRAP bands were polymorphic in *S.* × *townsendii* (Hythe population), two samples each lacking one band. Except for these two samples, genotypes of the other eight accessions of *S.* × *townsendii* are similar to the major genotype of *S. anglica*. This major genotype is additive with respect to the parental bands. We assume that the major genotype is the founder of both hybrid *S.* × *townsendii* and *S. anglica* and that therefore the var-

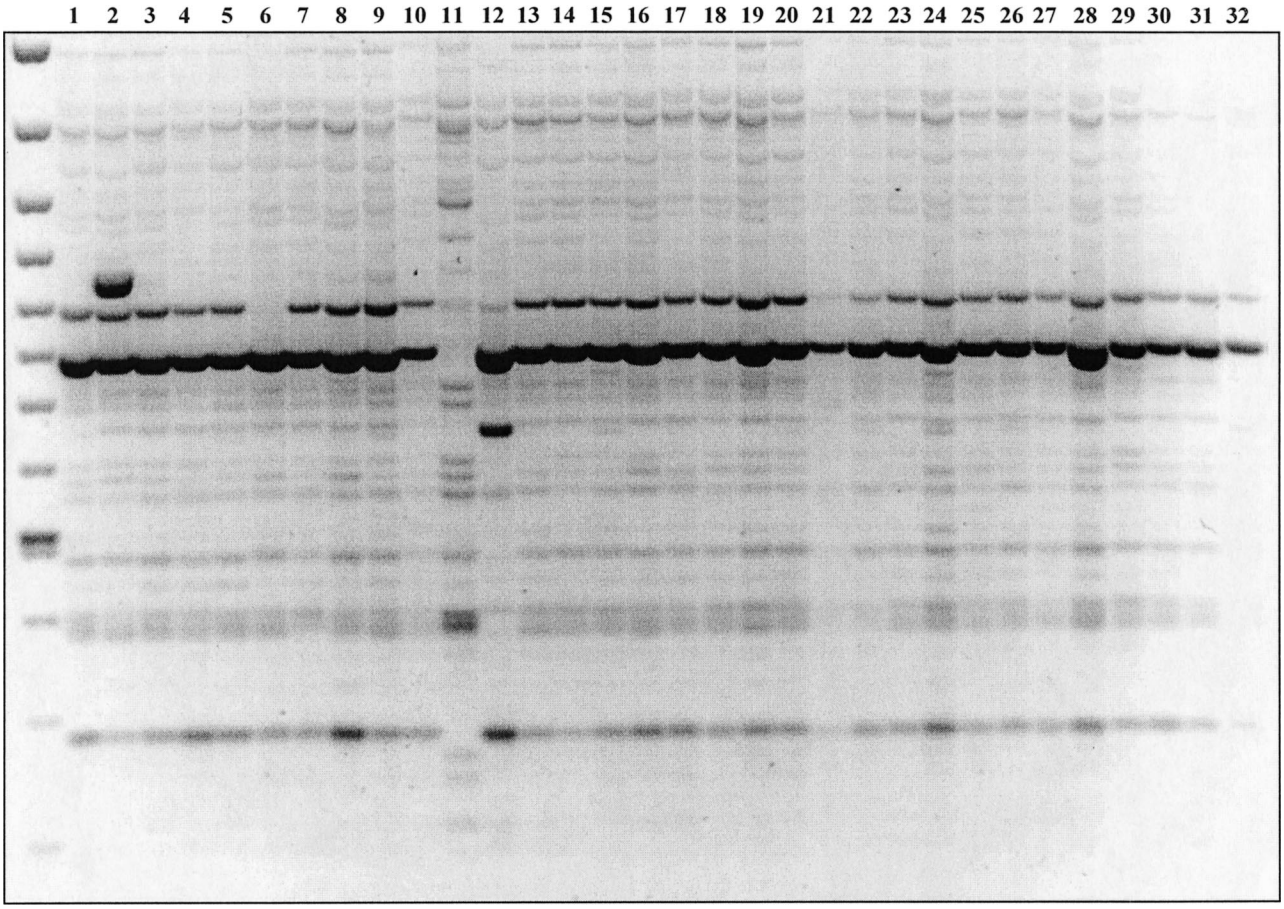

FIG. 1.—Electrophoretic pattern generated by IRAP, using primers 9900 and C0699. Lanes 1–10: *S. anglica* from Keyhaven, lane 11: *S. maritima*, lane 12: *S. alterniflora*, lanes 13–22: *S. anglica* from Studland, lanes 23–32: *S. anglica* from Bull Island.

iation observed in these species is the result of mutations.

Most polymorphisms (8/13) were restricted to one population, whereas five were recorded in more than one population. The two most frequent (C0947/C0905-C; C0795/$[GAG]_6$A-A) occurred in 17.5% and 20% of the samples, respectively, and are each found in two or three populations. Furthermore, excepting the major genotype, only two multilocus genotypes (6 and 13) were recorded in more than one population. Of these, genotype 6 was found at Bull Island and Studland, and genotype 13 was recorded at Keyhaven and Fawley. Of the polymorphic bands in *S. anglica* (table 3), 9 out of 13 represent band absence from the major genotype. Six belong to the *S. maritima* genome, two are specific to the *S. alterniflora* genome, and one is present in both parents. Only three new IRAP bands and one new ISSR band were observed in the allopolyploid *S. anglica*. Two out of three new IRAP bands occurred only in one individual from Keyhaven. These two IRAP bands could be amplified by most of the combinations that involve the *BARE*-1 and *Sukkula* LTR primers (C0795 and 9900, respectively). Their reproducibility was confirmed by independent DNA preparations from another leaf. These DNA fragments were robustly amplified (fig. 1).

We cloned and sequenced these two polymorphic IRAP bands and determined their identity by BLAST searching. Band K2 (accession AF 450249), amplified by the primer 9900, contains a region (nt 325–959) comprising about 40% of a the LTR of a *BARE*-1 retrotransposon (accession Z17327), as well as a region (nt 26–324) which may be protein-encoding on the basis of EST and protein database matches. The structure of this novel band indicates that at least two, nested *BARE*-1 integration events, as seen earlier in the barley and maize genomes (San Miguel et al. 1996; Shirasu et al. 2000), occurred to generate the amplifiable domain. The other sequence, B2 (accession AF 450250), was generated with *Sukkula* and Stowaway primers and contains an internal domain which may encode part of a proline-rich protein.

Comparison Between *S. anglica* and its Parents

IRAP, REMAP, and ISSR patterns were compared among the species by recording the presence or absence of PCR bands (table 4). The comparison was based on the major genotype of *S. anglica*, which is identical to that of *S.* × *townsendii*. The three data sets were congruent and produced the same result. Most markers of *S. maritima* and *S. alterniflora* were species-specific, and only a minority (15%) was found in both species. The three techniques, but particularly IRAP and REMAP, indicated that *S. maritima* contributed slightly

Table 4
Presence-Absence of Markers in the Three *Spartina* Species, Alt = *S. alterniflora*, Mar = *S. maritima*, Ang = *S. anglica*. Comparison is Based on 224 IRAP Markers, 100 REMAP Markers, and 108 ISSR Markers

Alt	+	+	−	+	−	−	+
Mar	+	−	+	−	+	−	+
Ang	+	+	+	−	−	+	−
IRAP %.....	14	35	43	3.5	4.5	0	0
REMAP % ...	15	33	41	8	3	0	0
ISSR %	16	36	39	4.5	4.5	0	0
Total %	15	34.5	41	5.5	4	0	0

more markers (41%) in the allopolyploid than *S. alterniflora* (34.5%). The first three combinations of markers, representing the highest share (90.5%) of the total number of bands, result from the additivity of the parental markers in the allopolyploid (table 4).

A minority of the marker bands (9.5%) was found only in one of the parental species. The major genotype does not exhibit any marker specific to *S. anglica* (i.e., not present in the parental species).

Discussion

LTR Primer Specificity

The IRAP and REMAP methods were first implemented in *Hordeum* (Kalendar et al. 1999). They were subsequently applied in *Hordeum vulgare* for fingerprinting and biodiversity studies (Kalendar et al. 2000; Vicient et al. 2001) and for mapping of traits in *H. vulgare* (Manninen et al. 2000) and *Aegilops tauschii* (Boyko et al. 2002). Within the Poaceae, *Spartina* belongs to the subfamily Chlorodoideae, and is not closely related to *Hordeum*, which is in the Pooideae subfamily (Hsiao et al. 1999; Kellog 2001). Nevertheless, most of the primers designed in *Hordeum* amplify bands in *Spartina*. This result is consistent with the evidence that the closely related retrotransposons are transcribed and translated in diverse grass species belonging to different subfamilies (Vicient et al. 2001). The primers produced reproducible patterns (fig. 1) and a greater number of bands than seen with RAPDs (Baumel, Ainouche, and Levasseur 2001) or ISSRs (this study). The abundance of marker bands indicates that retroelements closely related to many of those in barley are abundant in *Spartina* and display a similar genome organization (clustering, association with SSRs), similar to the pattern identified in barley (Shirasu et al. 2000).

IRAP and REMAP in *S. anglica* Populations

Fourteen genotypes were identified in the populations of *S. anglica* on the basis of 13 polymorphic bands. One genotype is most frequent in all populations, representing 40% of all samples. Most populations are composed of the same major genotype, identical to that of the first-generation hybrid *S.* × *townsendii*. The other genotypes encountered in this study differ only by the absence of 1-4 markers or, very rarely, by the appearance of a new band. Previous investigations in populations of *S. anglica* using multilocus RAPD markers (Baumel, Ainouche, and Levasseur 2001) also revealed a general lack of genomic variation. Taken together, these results support a dramatic genetic bottleneck at the time of formation of the species in Southampton, perhaps as a consequence of the unique origin of the species.

Evolution of the Parental Repetitive Sequences in the Allopolyploid

Both ISSR and the retrotransposon-based IRAP and REMAP reveal numerous markers that differentiate the parental genomes *S. alterniflora* and *S. maritima* as do the RAPD data. (Baumel, Ainouche, and Levasseur 2001). Our recent phylogenetic analysis (Baumel et al. 2002) indicates that although *S. alterniflora* and *S. maritima* belong to the same lineage, they contain divergent nuclear and chloroplast DNA sequences. This ability to differentiate the two parental genomes on the basis of several loci provides the opportunity to compare them with their homologues duplicated in the allopolyploid. A comparison of the three data sets (IRAP, REMAP, and ISSR) between *S. anglica* and its parents shows that the parental genomes remain largely unchanged in the allopolyploid, 90.5% of the markers showing additivity for the parental genomes. The remaining 9.5% of parental markers, found neither in the allopolyploid nor in *S.* × *townsendii*, might result from polymorphism in the parental species (i.e., markers not present in the genotypes involved in the parentage of *S.* × *townsendii*), or, alternatively, might result from parental marker loss in the hybrid and the allopolyploid, as reported in *Brassica* (Song et al. 1995) and wheat (Liu et al. 1998*a*, 1998*b*; Ozkan, Levy, and Feldman 2001), though a particular mechanism for these losses has to be elucidated. Aside from cryptic parental polymorphism and PCR artifacts, marker loss could occur from recombination, mutation, or deletion events, that separate or destroy PCR amplification sites. Because our analyses reveal very few mutations affecting the initial (major) genotype of *S. anglica*, we can conclude that the young allopolyploid genome has not undergone extensive change since its formation. *Spartina anglica* populations therefore are composed of the initial genotype that, in some samples, displays a few mutations that do not extensively affect the allopolyploid genome as a whole.

Nondeleterious mutations may be maintained in natural populations as a result of the vigorous clonal reproduction of *S. anglica*. Mutations may progressively lead to divergence between the parental (homoeologous) subgenomes present in the allopolyploid and their homologues in the parental species. Currently, however, such divergence is limited. Our multilocus investigations reject the hypothesis of rapid structural changes in *S. anglica* subgenomes. The results presented here and those published previously (Baumel, Ainouche, and Levasseur 2001) in the 150 year old *S. anglica* contrast with the rapid (1-3 generations old) and extensive changes reported in synthetic *Brassica* and *Triticum* allopolyploids (Song et al. 1995; Feldman et al. 1997; Liu

et al. 1998*a*, 1998*b;* Ozkan, Levy, and Feldman 2001; Shaked et al. 2001). These authors demonstrate that rapid, non-Mendelian changes, involving preferential sequence elimination or modification of methylation patterns, may occur in the earliest stages of polyploid stabilization. As a consequence, young polyploids generally are not assumed to be completely additive with respect to their progenitors (Wendel 2000). However, analysis of the young allopolyploid *B. juncea* revealed no structural changes when compared with its diploid progenitors (Axelson et al. 2000), in parallel with our data for *Spartina*. Furthermore, both genomic additivity and epigenetic stasis were found in artificial (resynthesized) *Gossypium* polyploids (Liu et al. 2001).

In *S. anglica*, 9 of 13 polymorphisms result from the lack of parental bands from one of the parents. Among these, six are from *S. maritima* and two are from *S. alterniflora*. One band is present in *S. maritima*, *S. alterniflora*, the hybrid *S.* × *townsendii*, and present or absent in *S. anglica*. The multiplex REMAP and IRAP methods, unlike the monoplex RBIP technique (Flavell et al. 1998), result in dominant markers, which means that the allele lacking the retrotransposon insertion cannot be directly scored. The assumption that a missing band means that an empty allele has been inherited, rather than that a mutation has eliminated PCR primer binding, has not been systematically examined and requires isolation and sequencing of the loci from the genome of the polyploid. However, previous extensive sequencing of the LTR termini (Suoniemi, Schmidt, and Schulman 1997), in which REMAP and IRAP primers lie, demonstrated that mutations are rare in these regions critical to retrotransposon function and hence to band polymorphism. However, the one band in *S. anglica* that is present in both parents but missing in a few allopolyploid individuals may be caused by such a mutation. In a previous study (Baumel, Ainouche, and Levasseur 2001), six of seven RAPD polymorphic bands also originated from *S. maritima*. On the basis of a limited number of polymorphic loci (16), this asymmetrical parental subgenome variation may indicate that the mutation rate is higher in the *S. maritima* subgenome of the allopolyploid *S. anglica* than in the *S. alterniflora* subgenome. Because *S. alterniflora* has been shown to be the maternal parent of *S. anglica* (Ferris, King, and Gray 1997), this would indicate that the paternal genome is preferentially modified. Similar findings have been reported in the young synthetic allopolyploids involving reciprocal crosses between the diploids *Brassica rapa* and *Brassica nigra* and between *B. rapa* and *Brassica oleracea* (Song et al. 1995).

Genome duplication in polyploids may buffer the potentially deleterious effects of mutations that might result from bursts of retroelement activity (Matzke and Matzke 1998). The genome stress of interspecific hybridization, in turn, may activate such bursts (McClintock 1984). We therefore expected that new retroelement insertions might be detectable in the young allopolyploid genome of *S. anglica*. In the allotetraploid *G. hirsutum*, Zhao et al. (1998) found that colonization of one subgenome by repetitive elements from the other subgenome followed the polyploidization event of about a million years ago. Overall, however, this genome showed remarkably few changes after the formation of the artificial *Gossypium* polyploids (Liu et al. 2001). The data of Liu and Wendel (2000) indicate that, after genetic introgression in *Zizania latifolia*, retroelement activity is ephemeral and rapidly repressed.

Of particular interest are the two new IRAP bands detected in the population from Keyhaven (sample K2), each generated by *Sukkula* or *BARE*-1 LTR primers. These new bands correspond to integrations of a retrotransposons, which likely have occurred since the origin of the allopolyploid. The integration of new retrotransposon copies indicates that the *BARE*-1 family of retrotransposons remains active in *S. anglica*. The two sites are 0.68% of the total number of retrotransposon insertion sites (296) scored in *S. anglica*. Liu and Wendel (2000) demonstrated retrotransposon activation in the rice-*Z. latifolia* introgression lines that they had constructed. To our knowledge, though, the new IRAP bands found in *S. anglica* represent the first new retrotransposon integration events documented to have occurred in a natural population during a defined period of time, in this case less than 150 years. New integration events are, however, often observed in plants regenerated from tissue culture (Hirochika et al. 1996), which is known to activate retrotransposons and which served in the isolation of the first such active element from plants (Grandbastien, Spielmann, and Caboche 1989).

On the basis of these data, one can attempt to estimate the number of integration events represented by these two new insertions. *Spartina anglica* has a base chromosome number of 10, as do other species in the Chloridoideae (Marchant 1968); given a genome size of 5.341×10^9 bp (2N = 122; http://www.rbgkew.org.uk/cval/homepage.html), the basic haploid genome is 4.378×10^8 bp or about twice that of rice (2.45×10^8). The *Arabidopsis thaliana* genome, the only plant genome for which complete information exists (Bevan et al. 1998), contains 3×10^4 genes of 4.8 kb each. Assuming a similar density in other plants (data on 1,000 genes indicates a slightly lower density in rice), genes in the *S. anglica* genome occupy about 1.44×10^8 bp or roughly one-third of the genome, compared with perhaps 3% in barley (Vicient et al. 2001). Hence, the repetitive fraction would constitute 4.36×10^9 bp in barley but 2.94×10^8 bp in the *S. anglica* basic genome. Barley contains about 8.7×10^4 *BARE*-1 LTRs (Vicient et al. 1999), the priming sites for IRAP and REMAP based on *BARE*-1 and about the same number of *Sukkula* elements (unpublished data). If the number of *BARE*-1 and *Sukkula* elements are reduced proportionately according to the fraction of repetitive DNA in the genome, then in *S. anglica*, the basic (haploid) genome might contain about 6,000 *BARE*-1 or *Sukkula* LTRs and the polyploid genome about 7.1×10^4. All marker combinations involving *BARE*-1 or *Sukkula* primers amplified a total of 154 bands from *S. anglica*. If all the BARE-1 and *Sukkula* LTRs could generate PCR products, one would expect 6,000 products from each haploid genome. The 154 bands observed represent 2.6% (154/6,000) of the total

expected if the each haploid (x) chromosome set were to give products of identical size (because of the retroelements being identically organized in each genome). If, however, the constituent genomes are fully distinct regarding their retrotransposon organization, 154 bands would represent only 0.02% of the total possible (154/ 7.1×10^4).

Taking these calculations on retrotransposon sampling efficiency, repetitive DNA fraction, and retrotransposon copy number into account, two insertion events would represent between 76 (2/0.026) and 929 (2/ 0.0022) insertion events for these two retrotransposon families. This implies that the two novel insertions detected may represent between 75 and 1,000 retrotransposon integration events in the less than 150 years since the formation of the *S. anglica* genome. Although this suggests that the complement of *BARE*-1 and *Sukkula* elements grew by at most 1% in the *S. anglica* genome during the speciation event, it is nevertheless consistent with the hypothesis of activation by polyploidization, but such ploidy-specific variation, if any, has changed the genome of the allopolyploid very little. These calculations, in addition to being subject to sampling error, may be underestimates of retrotransposon activity in the *S. anglica* genome for two reasons: first, ancient element families, conserved between relatively distant genera, may be less active than more recently evolving, native elements; furthermore, nested integration events such as seen in other grasses (San Miguel et al. 1996; Shirasu et al. 2000) might not have been detected.

Recent progress in molecular analysis of allopolyploid systems resulted in significant insights into polyploid genome evolution (Wendel 2000). One of the best-investigated models in this respect is genus *Gossypium*. Contrasting results have been derived from different portions of this genome, revealing independent evolution of the parental subgenomes for low- and single-copy sequences (Cronn, Small, and Wendel 1999; Small and Wendel 2000) but nonindependent and concerted evolution of repetitive sequences (Wendel, Schnabel, and Seelanan 1995; Zhao et al. 1998). As mentioned previously, insights from experimentally resynthesized allopolyploids also reveal conflicting results: either genomic or phenotypic instability after polyploidization (Song et al. 1995; Feldman et al. 1997; Liu et al. 1998*a*, 1998*b;* Comai et al. 2000) or genomic stasis (Axelson et al. 2000; Liu et al. 2001). All natural alloplyploid models investigated to date are more or less stabilized species. Insights from early stages of the polyploid species formation have been exclusively recorded from studies of experimentally created polyploids. *Spartina anglica* is to our knowledge the first natural nascent species investigated in this respect. Our results indicate overall additivity and overall structural stasis of the parental subgenomes in this allopolyploid, concerning both low-copy (Baumel, Ainouche, and Levasseur 2001) and repetitive (this study) sequences, although some changes might be encountered in local populations, as noticed in Keyhaven. Comparisons among different natural young systems are needed for a better understanding of the short-term evolutionary process after duplication of a hybrid genome under natural selection pressures.

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