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Research

Rapid structural and epigenetic reorganization near transposable elements in hybrid and allopolyploid genomes in *Spartina*

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Summary

• Transposable elements (TE) induce structural and epigenetic alterations in their host genome, with major evolutionary implications. These alterations are examined here in the context of allopolyploid speciation, on the recently formed invasive species *Spartina anglica,* which represents an excellent model to contrast plant genome dynamics following hybridization and genome doubling in natural conditions.

• Methyl-sensitive transposon display was used to investigate the structural and epigenetic dynamics of TE insertion sites for several elements, and to contrast it with comparable genome-wide methyl-sensitive amplified polymorphism analyses.

• While no transposition burst was detected, we found evidence of major structural and CpG methylation changes in the vicinity of TE insertions accompanying hybridization, and to a lesser extent, genome doubling. Genomic alteration appeared preferentially in the maternal subgenome, and the environment of TEs was specifically affected by large maternal-specific methylation changes, demonstrating that TEs fuel epigenetic alterations at the merging of diverged genomes.

• Such genome changes indicate that nuclear incompatibilities in *Spartina* trigger immediate alterations, which are TE-specific with an important epigenetic component. Since most of this reorganization is conserved after genome doubling that produced a fertile invasive species, TEs certainly play a central role in the shock-induced dynamics of the genome during allopolyploid speciation.

Introduction

An important paradigm that emerged from the genomic era includes: first, the high level of genomic redundancy following duplications in eukaryotic genomes; second, the permeability of the species barrier, as revealed by the widespread occurrence of reticulate evolution; and third, the importance and evolutionary significance of the repetitive compartment (most particularly transposable elements) that modulates genome plasticity (Lynch, 2007). Merging differentiated genomes into a single nucleus (hybridization) and multiplying the chromosome sets (polyploidy) are increasingly recognized as major evolutionary phenomena resulting in diversification and speciation (Mallet, 2007; Rieseberg & Willis, 2007). As such, both short-term and long-term effects of hybridization and polyploidy received a strengthened attention (Comai, 2005; Soltis, 2005; Otto, 2007). Over the past decade, evidence has accumulated revealing that allopolyploid (i.e. merged and doubled) genomes are particularly dynamic at both the structural and expression levels (Levy & Feldman, 2004; Chen, 2007). Accordingly, large-scale to small-scale chromosomal rearrangements, including sequence elimination and/or activation of transposable elements (Chen *et al.*, 2007; Otto, 2007), as well as significant epigenetic alterations, including methylation repatterning and chromatin remodeling (Liu & Wendel, 2003; Rapp & Wendel, 2005), are commonly reported in allopolyploid species. The differential gene expr-

ession resulting from epigenetic changes provides new, putatively adaptive, phenotypes (Schranz & Osborn, 2004), but may also participate in re-establishing compatibility among products of merged genomes (Rieseberg, 2001). Drastic genetic and epigenetic changes are considered to participate in the rapid stabilization of allopolyploid genomes (Lim *et al.*, 2007; Leitch & Leitch, 2008).

Because transposable elements (TEs) are a major component of eukaryotic genomes (Gaut & Ross-Ibarra, 2008) and are highly mutagenic and silenced by various overlapping epigenetic mechanisms, they represent likely candidate sequences playing a pivotal role fuelling genome reorganization in response to changes in the cellular environment (McClintock, 1984). According to the 'genome shock' hypothesis of Barbara McClintock (1984), genetic incompatibilities unmasked by hybridization are assumed to induce a programmed response involving TEs, leading to various genomic alterations. Since many TEs have been shown to be activated in relation to abiotic and biotic stress (Kalendar *et al.*, 2000; Grandbastien *et al.*, 2005), hybridization (Madlung & Comai, 2004) and polyploidy (Petit *et al.*, 2007), they may play a central role, both structural and epigenetic, during the hybridization/polyploidization as well as the diploidization processes.

As most data gathered to date were generated from experimentally resynthesized allopolyploid model systems, understanding how nascent species react to hybridization and polyploidy in natural populations is a crucial, underexplored topic in the context of current concerns about mechanisms generating biodiversity. The genus *Spartina* offers an excellent opportunity to unravel the early genome changes following allopolyploid speciation in natural conditions (Ainouche *et al.*, 2004a). Indeed, *Spartina anglica* is one of the best historically documented case of a recent – about a century year old – and ecologically successful allopolyploidization event (Ainouche *et al.*, 2009 and references therein). In addition, the maternal (*Spartina alterniflora*) and paternal (*Spartina maritima*) species as well as F_1 homoploid hybrids, *Spartina* × *neyrautii* and *Spartina* × *townsendii*, still exist on the initial hybridization sites (see Materials and Methods section). Various fingerprinting methods detected no significant structural rearrangements in the young allopolyploid, whose genome was nearly additive to the parental ones (93.5%) and, in contrast to the rapid changes observed in several experimentally resynthesized allopolyploids, *S. anglica* illustrates structural genomic stasis following allopolyploidy (Ainouche *et al.*, 2004a). In particular, Baumel *et al.* (2002) showed a lack of TE activation during allopolyploidization and revealed low polymorphism of TE insertions among populations of *S. anglica*. However, consistent methylation changes of randomly assayed sequences were detected after hybridization and were transmitted after polyploidization (Salmon *et al.*, 2005), suggesting that epigenetic mechanisms represent a core component of the immediate genome response to allopolyploidy.

In this paper, we focus on the potentially highly dynamic TE component of the genome to unravel the early genomic mechanisms associated with hybridization and genome duplication in *Spartina*. In particular, we explore the pivotal role of TEs in putatively linking the lack of structural reorganization with the substantial methylation repatterning observed in the CpG fraction of the genome of hybrids and allopolyploids. Using methyl-sensitive transposon display (MSTD), we aimed at first, testing for the structural stability of the TE component in response to hybridization in two independently formed hybrids (*S.* × *neyrautii* and *S.* × *townsendii*) sharing the same maternal and paternal genome donors, and second, assessing the methylation changes of CpG sites around TE insertions during the early stage of allopolyploid speciation. Comparing these data with a methyl-sensitive amplified polymorphism (MSAP) assay previously obtained from the same plant material (Salmon *et al.*, 2005), we contrasted the specific response of genomic sequences close to TE insertions against the response of random sequences.

Materials and Methods

Model species and plant material

The introduction of the American *Spartina alterniflora* Loiseleur ($2n = 62$) into the native range of the European *S. maritima* (Curtis) Fernald ($2n = 60$) *c.* 1830 resulted in the independent formation of F_1 homoploid hybrids between *S. alterniflora* as a maternal parent and *S. maritima* as a paternal parent (Ainouche *et al.*, 2004b; Fig. 1). These hybrids, *S.* × *neyrautii* Foucaud and *S.* × *townsendii* H. Groves & Groves ($2n = 62$), appeared in Hendaye (France) *c.* 1892 and Southampton Bay (UK) *c.* 1870, respectively. Being sterile, they survived by clonal propagation and are still observable where they have formed. *c.* 1892, the hybrid *S.* × *townsendii* gave rise to the fertile allopolyploid *S. anglica* C. E. Hubbard ($2n = 122, 124$). This young species reached a worldwide distribution and is considered as a

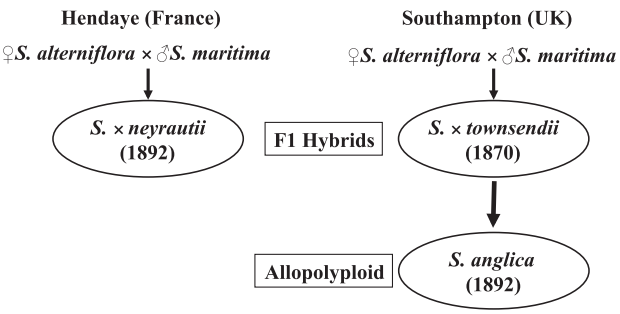


Fig. 1 Origin of *Spartina* × *neyrautii*, *Spartina* × *townsendii* and *Spartina anglica*.

severe invasive species (Ainouche *et al.*, 2009). Various molecular analyses provided an accurate picture of the genetic context of the species formation, revealing that both parental species and the derived allopolyploid are genetically depauperate in Europe (Baumel *et al.*, 2001, 2003; Yannic *et al.*, 2004). Despite consistent morphological plasticity (Thompson *et al.*, 1991), populations of the neopolyploid *S. anglica* lack interindividual genetic variation (Ainouche *et al.*, 2004a).

Five *Spartina* species were analysed in this study: the maternal (*S. alterniflora*) and paternal species (*S. maritima*), two independently formed F_1 hybrids (*S.* × *neyrautii* from Hendaye, France, and *S.* × *townsendii* from Hythe, UK), and the derivative allopolyploid (*S. anglica*). All plants used in this study were maintained together in common conditions in the glasshouse under natural photoperiod in pots of 3–7 l containing an equal mix of sand, mold and soil, and they were automatically watered daily (6–10 min each morning). Analyses were performed on young leaves from well-developed plants. DNA was extracted from fresh leaves according to Baumel *et al.* (2001). Plants had already been characterized by various molecular analyses: accessions of *S. alterniflora* from Marchwood–Eling and of *S. maritima* from Saint Armel investigated here were characterized as representative of the most common genotype (Baumel *et al.*, 2003; Yannic *et al.*, 2004) and the accession of the allopolyploid *S. anglica* from Saint-Armel analyzed here was characterized as the major genotype encountered in western Europe (Baumel *et al.*, 2001; Ainouche *et al.*, 2004a).

Methyl-sensitive transposon display

Sequence-specific amplified polymorphism is a high-resolution TE-anchored PCR strategy allowing the simultaneous detection of multiple insertions and thus representing a transposon display (Waugh *et al.*, 1997). Briefly, the amplifications of digested genomic DNA, specifically targeting TE insertions, generate a pool of labeled fragments containing the termini of inserted copies of a given TE and its flanking genomic region. Polymorphic fragments (i.e. presence or absence) reveal insertion polymorphisms and changes at insertion sites, including rearrangements, indels and mutations of flanking restriction sites, but the former seem to predominate over other processes (Waugh *et al.*, 1997). The transposon display was performed here in four steps: digestion, ligation of adaptors, preamplification and selective-amplification (for a detailed protocol see the Supporting Information, Notes S1).

Using restriction enzymes with differential sensitivity to DNA methylation at the digestion step, a methyl-sensitive derivative of this multilocus transposon display was exploited (Fig. 2). The isoschizomers *Msp*I and *Hpa*II are widely used for methyl-sensitive displays (Shaked *et al.*, 2001; Cervera *et al.*, 2002; Takata *et al.*, 2007). Both enzymes recognize the same tetranucleotide sequence (5′-CCGG-3′), but present different sensitivities to DNA methylation. *Msp*I and *Hpa*II were used here on the same samples in parallel to provide a methyl-sensitive transposon display (MSTD). *Hpa*II is sensitive to the methylation of any cytosine at both strands (5′-CCGG-3′), while *Msp*I cuts methylated internal cytosine (5′-C^{5m}CGG-3′), thus allowing assessment of the methylation status of internal cytosine at restriction sites (CpG methylation). However, *Msp*I is sensitive to methylation of the external cytosine (5′-^{5m}CCGG-3′). Hence, methylation of external cytosine on both strands (CpCpG methylation: 5′-^{5m}CCGG-3′ and 5′-$^{5m}C^{5m}$CGG-3′) may not produce bands with this MSTD. Since *Hpa*II cleaves when the external cytosine is methylated on one strand, while *Msp*I does not, hemi-methylated CpCpG sites can be detected with this MSTD (described later). The

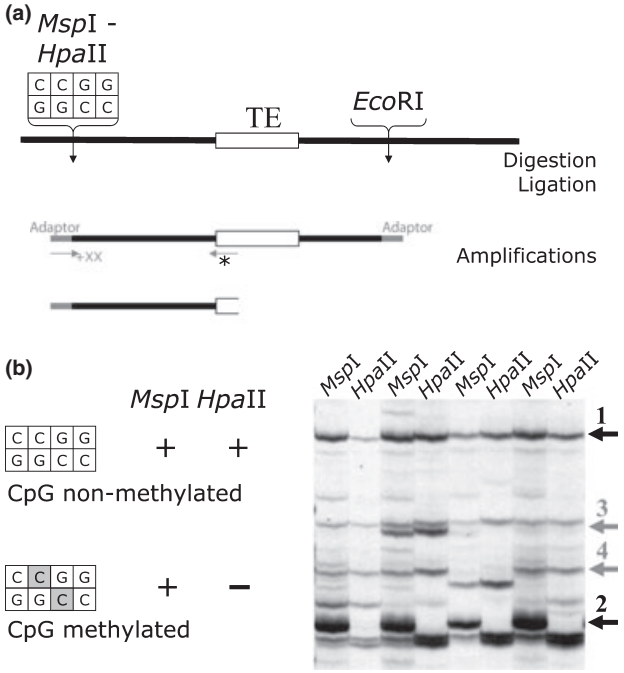


Fig. 2 Principle of methyl-sensitive transposon display (MSTD). (a) Schematic representation of the transposon display technique. This transposable element (TE)-anchored PCR strategy allows the simultaneous detection of labeled fragments (*) containing the termini of inserted copies of a given TE and its flanking genomic region up to the nearest *MspI*/*Hpa*II site. (b) Methylation sensitivity of isoschizomer enzymes (*Msp*I and *Hpa*II) and an example of MSTD banding pattern, allowing investigation of the methylation state of restriction sites flanking insertions of TEs. Arrow 1 shows an example of CpG nonmethylated band (present with *Msp*I and *Hpa*II). Arrow 2 shows an example of CpG methylated band (present with *Msp*I, but absent with *Hpa*II). Arrow 3 shows an example of (nonmethylated) new band. Arrow 4 shows an example of band loss. Note: methyl-sensitive transposon display (MSTD) is similar to methyl-sensitive amplified polymorphism (MSAP), except that MSTD is anchored in TE extremities and specifically amplifies TE insertion sites, while MSAP is adaptor-anchored only and amplifies random genomic loci.

present study focuses on the CpG methylation of the genome, which was shown to be essential for the coordination of stable transgenerational inheritance of various epigenetic marks in plants (Mathieu *et al.*, 2007).

The preselective amplification by PCR was carried out by using a single selective nucleotide. Selective amplifications were then performed with TE-specific primers (see next section on transposable elements investigated) radioactively labeled with phosphorus (^{33}P). The whole procedure was replicated twice on different DNA extractions of the same individuals to check for reproducibility of all profiles.

Transposable elements investigated

Ins2, a class II nonautonomous hAT DNA transposon of 367 bp isolated from the bronze locus in Maize (Ralston *et al.*, 1988), was successfully amplified in *Spartina* with the TE-specific primer 5′-GCCCGTTTAGCACGAAAAA-3′. The sequence of *Cassandra*, a class I terminal-repeat retrotransposon in miniature of *c.* 800 bp isolated in *S. alterniflora*, was available in Genbank (AY603377) from the study of Kalendar *et al.* (2008). The TE-specific primer 5′-CTTAACCCCAGAGTTCTCTGCAG-3′, located at the 5′ end of the TE was successfully used for MSTD amplification across the *Spartina* species investigated. The partial sequence of *Wis-like*, a class I Ty1/copia-LTR retrotransposon of *c.* 5 kb, was isolated from *S. anglica* (Baumel *et al.*, 2002). After alignment with available *Wis*-like sequences across Poaceae, the primer 5′-TGAGTGAATCCTCGACATCG-3′ was designed in the mostly conserved portion of the 5′ end of the terminal repeat and successfully used for MSTD amplification across *Spartina* species.

Data analysis

No MSTD fragments of strongly higher intensity, indicative of amplification between priming and internal restriction sites, were observed. Only clearly identifiable and reproducible bands were manually scored as present (1) or absent (0) and translated into a *Msp*I-MSTD data matrix and a *Hpa*II-MSTD data matrix. The *Msp*I-MSTD bands that were also present in *Hpa*II-MSTD profiles corresponded to nonmethylated internal cytosine at the restriction sites and *Msp*I-MSTD bands that were absent in *Hpa*II-MSTD profiles represented methylated internal cytosine. Hemimethylated external cytosine may not be transmitted through generation and is less important than CpG methylation in maintaining TE silencing (Lippman *et al.*, 2003; Huettel *et al.*, 2006). Accordingly, the 13 MSTD bands (2.6%) absent in *Msp*I-MSTD, but present in *Hpa*II-MSTD profiles were not included in the analysis. Similarly, 18 MSAP bands (4.4%) in the dataset of Salmon *et al.* (2005) that were absent in *Msp*I-MSAP but present in *Hpa*II-MSAP profiles were not included here.

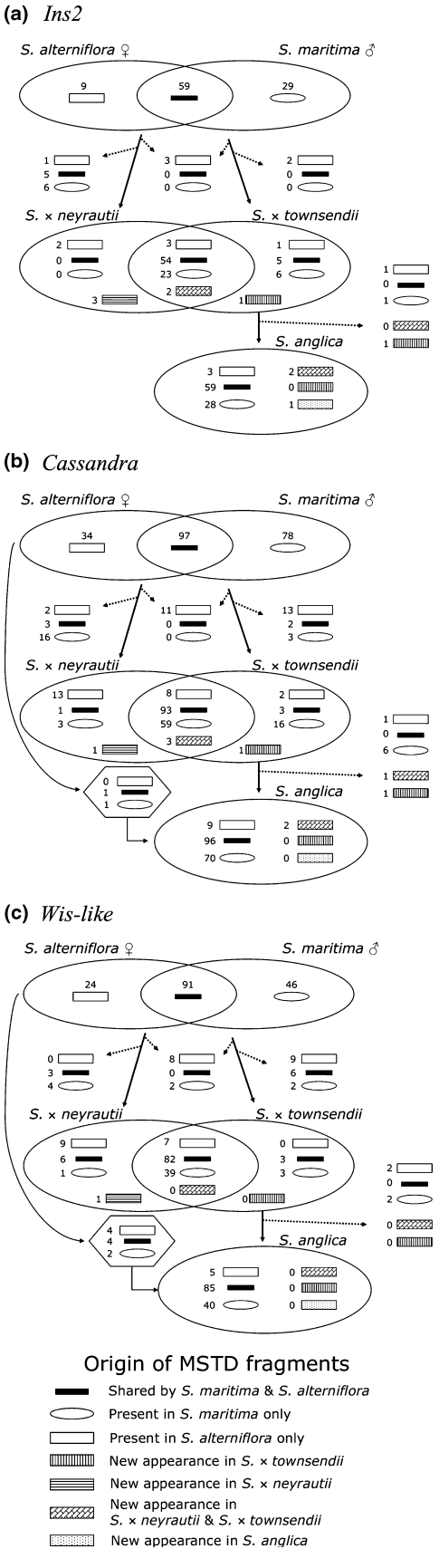
All proportions were presented with 95% confidence interval calculated by the score method with continuity correction, following Newcombe (1998). The MSTD datasets were analysed separately for each TE (*Ins2*, *Cassandra* and *Wis-like*) and were also pooled into a combined MSTD dataset. For both MSTD and MSAP, differences in the proportion of bands and the proportion of methylated bands, but also differences in the proportion of bands showing departure from the expected profiles in the hybrids and the allopolyploid, were tested by multiple Yates' one-sided χ^2 test, using SPLUS (Insightful Corp.). Significance was assessed at $\alpha = 0.05$ with sequential Bonferroni correction to account for multiple comparison (Rice, 1989).

Results

Investigation of the structural and CpG methylation dynamics of TE insertion sites was performed on 480 reliable MSTD fragments (Fig. 3; Tables 1 and 2). Three TEs were investigated, *Ins2* (a nonautonomous hAT DNA transposon), *Cassandra* (a terminal-repeat retrotransposon in miniature) and *Wis-like* (a Ty1/copia-LTR retrotransposon), and these generated 104, 214 and 162 MSTD bands, respectively. Polymorphism of *Msp*I-MSTD bands among species was recorded to explore the genome structural dynamics in relation to TEs while polymorphism of *Hpa*II-MSTD bands described the methylation changes in CpG sequences flanking TE insertions (Fig. 2). In order to contrast specific reaction of TEs against genome-wide sequences during hybridization and genome doubling, the MSTD dataset was compared with 392 MSAP bands obtained on the same plants (Salmon *et al.*, 2005) and reanalysed. Methyl-sensitive transposon display is similar to MSAP, except that MSTD is anchored in TE extremities and specifically amplifies TE insertion sites, while MSAP is adaptor-anchored only and amplifies random genomic loci. For clarity, we describe the effect of hybridization and genome doubling on structural changes in or near insertion sites and the methylation status of CpG regions flanking TE insertions.

Distribution of TEs in the parents and the F_1 hybrids

Multiple χ^2 tests with sequential Bonferroni correction revealed a different distribution of MspI-MSTD and MspI-MSAP bands between the parental banding patterns. The MSTD bands shared by both parental species were significantly more numerous for *Ins2*, *Cassandra* and *Wis-like* than corresponding MSAP fragments (Fig. 4a). Parent-specific MSTD bands were significantly less abundant in the maternal species (*S. alterniflora*) than in the paternal species (*S. maritima*) for *Ins2*, *Cassandra* and *Wis-like* (Fig. 4b). By contrast, MSAP bands were evenly distributed between the maternal and the paternal parents.



Banding patterns in the homoploid F_1 hybrids (*S.* × *neyrautii* and *S.* × *townsendii*) were expected to be additive with respect to parental patterns (*S. alterniflora* and *S. maritima*). Compared with the expected additivity of the parental *Msp*I-MSTD profiles, *S.* × *neyrautii* showed 15 missing and five new bands for *Ins2*, 32 missing and four new bands for *Cassandra*, 17 missing and one new band for *Wis-like* as well as 40 missing and 10 new MSAP bands. *Spartina* × *townsendii* showed five missing and three new *Msp*I-MSTD bands for *Ins2*, 29 missing and four new bands for *Cassandra*, 27 missing but no new bands for *Wis-like* as well as 38 missing and six new MSAP bands. Departure from additivity was not significantly different among TEs or MSAP in the two hybrids (Fig. S1). When combining the three TEs into a single dataset (Fig. 5a), *Msp*I-MSTD did not show significantly different departure from additivity between hybrids: 15.5% were not transmitted from the parent to *S.* × *neyrautii* and 14.3% were not transmitted to *S.* × *townsendii* (χ^2, $P = 0.89$). Only 12 new *Msp*I-MSTD bands were revealed in hybrids (2.5%), so most of the departure from additivity was caused by missing bands. Although not significant, combined MSTD showed slightly more departure from additivity than MSAP (χ^2, $P = 0.30$ for *S.* × *neyrautii* and $P = 0.25$ in *S.* × *townsendii*).

The parental contribution to the hybrids was assessed by examining the origin of the MSTD bands (Fig. 6a). In *S.* × *neyrautii*, combined *Msp*I-MSTD bands of *S. alterniflora* (maternal)-origin showed 37.3% departure from additivity, while MSTD bands of *S. maritima* (paternal)-origin showed 18.3% departure from additivity. In *S.* × *townsendii*, maternal-specific MSTD bands showed 68.7% departure from additivity, while paternal-specific MSTD bands showed 4.6% departure from additivity. Combined *Msp*I-MSTD bands of maternal origin thus showed significantly more departure from additivity than bands of paternal origin. Note that contrasted patterns were observed in *S.* × *townsendii* and *S.* × *neyrautii*: departure form additivity of *Msp*I-MSTD bands of maternal origin was significantly more pronounced in *S.* × *townsendii* than in *S.* × *neyrautii*, while the opposite was observed for *Msp*I-MSTD bands of paternal origin. Like MSTD bands, genome-wide departure from additivity in both *S.* × *neyrautii*

Fig. 3 Representation of *Msp*I-methyl-sensitive transposon display (MSTD) fragments in the parental species *Spartina alterniflora* and *Spartina maritima*, the homoploid F_1 hybrids *Spartina* × *neyrautii* and *Spartina* × *townsendii*, and the natural allopolyploid *Spartina anglica*. (a) *Ins2*; (b) *Cassandra*; (c) *Wis-like*. The parental origin of each *Msp*I-MSTD band is represented by symbols according to the panel below. Shared MSTD bands are represented in overlapping ovals and lost fragments are indicated with dotted arrows. Reappearance of parental MSTD bands in the polyploid is presented as a hexagon.

Table 1 Distribution of methyl-sensitive transposon display (MSTD) and methyl-sensitive amplified polymorphism (MSAP) bands in *Spartina* F_1 hybrids relative to their parental lines

	Band status presence/methylation*		Band score							
			Spartina × *neyrautii*				*Spartina* × *townsendii*			
Band origin	In parents	In hybrids	Ins[1]	Cas[1]	Wis[1]	MSAP[2]	Ins[1]	Cas[1]	Wis[1]	MSAP[2]
Parental bands transmitted to the F_1 hybrids										
S. maritima (P)[3]	+/NM	+/NM	13	35	24	110	14	38	24	114
S. alterniflora (M)[4]	+/NM	+/NM	1	9	7	83	0	4	2	79
Shared[5]	+/NM	+/NM	43	58	48	123	49	63	46	127
S. maritima (P)[3]	+/M	+/M	7	22	12	6	9	30	14	7
S. alterniflora (M)[4]	+/M	+/M	2	3	2	5	2	2	0	5
Shared[5]	+/M	+/M	6	21	19	9	7	22	17	6
S. maritima (P)[3]	+/M	+/NM	2	2	2	0	4	2	2	0
S. alterniflora (M)[4]	+/M	+/NM	1	2	1	0	1	1	1	0
Shared[5]	+/M	+/NM	3	2	2	0	2	1	5	0
S. maritima (P)[3]	+/NM	+/M	1	3	2	1	2	5	2	1
S. alterniflora (M)[4]	+/NM	+/M	1	7	6	1	1	3	4	1
Shared[5]	+/NM	+/M	2	13	19	2	1	10	17	2
Deviation from additivity										
S. maritima (P)[3]	+/NM	−/nr	2	7	3	9	0	2	3	5
S. alterniflora (M)[4]	+/NM	−/nr	1	10	4	21	2	19	11	25
Shared[5]	+/NM	−/nr	5	3	1	1	0	1	5	2
S. maritima (P)[3]	+/M	−/nr	4	9	3	1	0	1	1	0
S. alterniflora (M)[4]	+/M	−/nr	3	3	4	6	3	5	6	6
Shared[5]	+/M	−/nr	0	0	2	2	0	1	1	0
New[6]	−/nr	+/NM	3	2	0	9	1	2	0	5
New[6]	−/nr	+/M	2	2	1	1	2	2	0	1

*+, Presence in *Msp*I-derived profiles; −, absence in *MspI*-derived profiles; NM, CpG nonmethylated as revealed by the presence of the band in *Hpa*II-derived profiles; M, CpG methylated as revealed by the absence of the band in *Hpa*II-derived profiles; nr, not relevant.
[1]Bands generated by methyl-sensitive transposon display (MSTD) for the nonautonomous hAT DNA transposon *Ins2* (Ins), the terminal-repeat retrotransposon in miniature *Cassandra* (Cas) and the Ty1/copia-LTR retrotransposon *Wis-like* (Wis).
[2]Bands generated by methyl-sensitive amplified polymorphism (MSAP) according to Salmon *et al.* (2005).
[3]Bands originating from the paternal parent (P) *Spartina maritima*.
[4]Bands originating from the maternal parent (M) *Spartina alterniflora*.
[5]Bands shared by both parents *S. maritima* and *S. alterniflora*.
[6]bands newly detected in the hybrids *S.* × *neyrautii* or *S.* × *townsendii*.

and *S.* × *townsendii* was significantly more important for *Msp*I-MSAP bands of maternal origin (23.3% and 26.7%, respectively) than paternal origin (7.9% and 4.1%, respectively), but the levels of parent-specific departure from additivity were similar in both hybrids. Departure from additivity of maternal bands was thus more distinctively pronounced for MSTD than MSAP in *S.* × *townsendii*.

Effect of genome doubling in *S. anglica*

Banding patterns of the allopolyploid (*S. anglica*) were expected to be similar to the progenitor hybrid (*S.* × *townsendii*). Compared with *S.* × *townsendii*, *S. anglica* exhibited few differences in the *Msp*I-MSTD banding patterns. Three *Ins2* bands (4.1%), eight *Cassandra* bands (4.3%) and four *Wis-like* bands (2.8%), present in the hybrid, were missing in the polyploid. Only one new *Msp*I-MSTD band was observed with *Ins2* in *S. anglica*. In addition, two *Cassandra* and 10 *Wis-like* fragments of parental origin (2.5%), missing in *S.* × *townsendii*, were observed in the allopolyploid (Fig. 3). Reappearance of parental fragments in allopolyploids has previously been reported in different studies (Shaked *et al.*, 2001; Salmon *et al.*, 2005) and may be explained by methylation changes of the *Eco*RI site or methylation of the external cytosine at the *Msp*I site in the hybrid. This also roughly corresponds to the background noise of transposon display (Melayah *et al.*, 2004), so those bands were not included in further analysis.

Departure from the expected pattern (i.e. similarity with *S.* × *townsendii*) was not significantly different among the TEs surveyed and not significantly different from MSAP (Fig. S1). Combining the *Msp*I-MSTD bands from the three TEs into a single dataset (Fig. 5a), 16 *Msp*I-MSTD bands out of 431 showed a nonsimilar pattern in the allopolyploid *S. anglica* compared with *S.* × *townsendii* (3.7%). Therefore, in comparison with genome doubling, hybrid-

Table 2 Distribution of methyl-sensitive transposon display (MSTD) and methyl-sensitive amplified polymorphism (MSAP) bands in the *Spartina anglica* allopolyploid relative to its F_1 hybrid progenitor *Spartina* × *townsendii*

	Band status presence/methylation*		Band score			
			S. anglica			
Band origin	In F_1 hybrid	In polyploid	Ins[1]	Cas[1]	Wis[1]	MSAP[2]
Hybrid bands transmitted to the polyploid						
S. maritima (P) [3]	+/NM	+/NM	16	36	26	115
S. alterniflora (M)[4]	+/NM	+/NM	1	5	3	80
Shared[5]	+/NM	+/NM	50	62	47	125
S. × *townsendii* F_1 hybrid[6]	+/NM	+/NM	1	2	0	2
S. maritima (P) [3]	+/M	+/M	8	28	12	6
S. alterniflora (M)[4]	+/M	+/M	0	4	5	6
Shared[5]	+/M	+/M	4	28	36	9
S. × *townsendii* F1 hybrid[6]	+/M	+/M	1	1	0	1
S. maritima (P) [3]	+/M	+/NM	2	4	4	0
S. alterniflora (M)[4]	+/M	+/NM	0	1	1	0
Shared[5]	+/M	+/NM	4	4	3	0
S. × *townsendii* F_1 hybrid[6]	+/M	+/NM	0	0	0	0
S. maritima (P) [3]	+/NM	+/M	2	1	0	0
S. alterniflora (M)[4]	+/NM	+/M	2	1	0	0
Shared[5]	+/NM	+/M	1	3	3	0
S. × *townsendii* F_1 hybrid[6]	+/NM	+/M	0	0	0	0
Deviation from similarity						
S. maritima (P) [3]	+/NM	−/nr	0	2	1	1
S. alterniflora (M)[4]	+/NM	−/nr	1	1	1	1
Shared[5]	+/NM	−/nr	0	0	0	0
S. × *townsendii* F_1 hybrid[6]	+/NM	−/nr	0	1	0	3
S. maritima (P) [3]	+/M	−/nr	1	4	1	0
S. alterniflora (M)[4]	+/M	−/nr	0	0	1	0
Shared[5]	+/M	−/nr	0	0	0	0
S. × *townsendii* F_1 hybrid[6]	+/M	−/nr	1	0	0	0
New[7]	−/nr	+/NM	1	0	0	1
New[7]	−/nr	+/M	0	0	0	1

*+, presence in *Msp*I-derived profiles; −, absence in *MspI*-derived profiles; NM, CpG nonmethylated as revealed by the presence of the band in *Hpa*II-derived profiles; M, CpG methylated as revealed by the absence of the band in *Hpa*II-derived profiles; nr, not relevant.
[1]Bands generated by methyl-sensitive transposon display (MSTD) for the nonautonomous hAT DNA transposon *Ins*2 (Ins), the terminal-repeat retrotransposon in miniature *Cassandra* (Cas) and the Ty1/copia-LTR retrotransposon *Wis*-like (Wis).
[2]Bands generated by methyl-sensitive amplified polymorphism (MSAP) according to Salmon *et al.* (2005).
[3]Bands originating from the paternal parent (P) *Spartina maritima*.
[4]Bands originating from the maternal parent (M) *Spartina alterniflora*.
[5]Bands shared by both parents *S. maritima* and *S. alterniflora*.
[6]bands newly detected in the hybrid, *S.* × *townsendii*.
[7]bands newly detected in the allopolyploid, *S. anglica*.

ization resulted in at least five times higher departure from the expected *Msp*I-MSTD patterns (χ^2, 9.45, $P = 0.002$). Comparatively, MSAP provided 44 nonadditive bands out of 386 in *S.* × *townsendii* (11.4%) and five missing as well as two new bands out of 351 in *S. anglica* (2.0%). Both MSTD and MSAP showed similar levels of departure from parental additivity at genome doubling (χ^2, 1.44, $P = 0.230$) and showed significantly more departure from the expected additive pattern after hybridization than after genome doubling (χ^2, 23.80, $P < 0.001$).

Together, these data show that while no transposition burst was detected, some restructuring occurred in the vicinity of TE insertions during hybridization and, to a lesser extent, genome doubling, preferentially affecting the maternal genome. Accordingly, the TE component exhibited specific alteration compared with random loci.

Methylation states of sequences flanking TE insertions

The methylation state of CpG sequences flanking the TE insertions was assessed by comparing *Hpa*II-MSTD with *Msp*I-MSTD profiles. The numbers of methylated MSTD bands were not significantly different among *Ins2*, *Cassandra* and *Wis-like* in parental species but were, however,

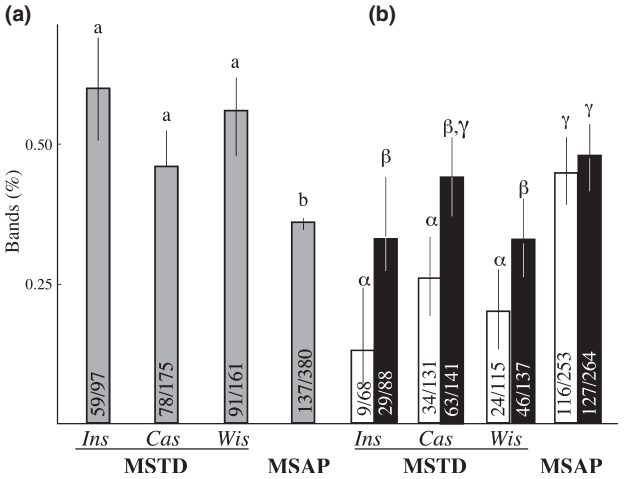


Fig. 4 Distribution of *Msp*I-methyl-sensitive transposon display (MSTD) and *Msp*I-methyl-sensitive amplified polymorphism (MSAP) bands in the parental species. (a) Proportion of bands shared by the maternal (*Spartina alterniflora*) and paternal (*S. maritima*) species (tinted bars) relative to the total number of parental bands. (b) Proportion of parent-specific bands relative to their total number within species; *alterniflora* specific, open bars; *maritima* specific, closed bars. Band numbers are shown for each TE: *Ins2* (*Ins*), *Cassandra* (*Cas*) and *Wis-like* (*Wis*), and for genome-wide MSAP. Error bars indicate 95% confidence intervals. Proportions with distinct lower case letters are significantly different according to multiple χ^2 tests.

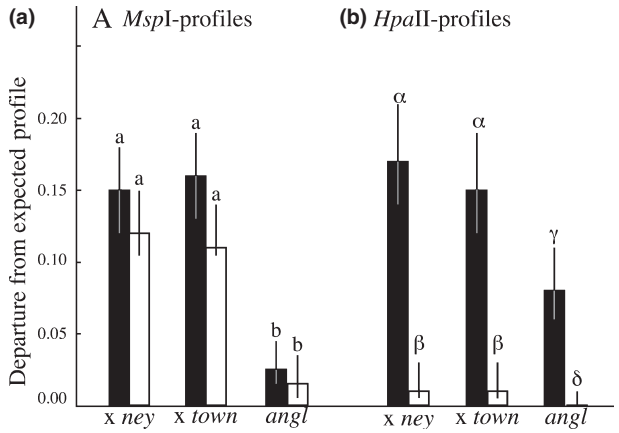


Fig. 5 Structural (a) and CpG methylation changes (b) during allopolyploid speciation in *Spartina*, as assessed by departure from the expected profile for the three transposable elements (TEs) combined (*Msp*I- and *Hpa*II-MSTD, respectively) and for genome-wide sequences (*Msp*I- and *Hpa*II-MSAP, respectively). For hybrids, *Spartina* × *neyrautii* (× *ney*) and *Spartina* × *townsendii* (× *town*), the expected profile is the additivity of the parents (*Spartina alternifolia* and *Spartina maritima*). For the allopolyploid, *Spartina anglica* (*angl*), the expected profile is similarity to the hybrid *S.* × *townsendii*. Error bars indicate 95% confidence intervals. Proportions of structural/methylation changes with distinct lower case letters are significantly different according to multiple χ^2 tests. MSTD (methyl-sensitive transposon display), closed bars; MSAP (methyl-sensitive amplified polymorphism), open bars.

slightly higher in hybrids and allopolyploid for *Cassandra* and *Wis-like* than for *Ins2* bands (Fig. S2). Overall, 34.7% of MSTD bands were CpG methylated in *S. alterniflora*, 36.0% in *S. maritima*, 32.7% in *S.* × *neyrautii*, 36.7% in *S.* × *townsendii* and 34.2% in *S. anglica*. By contrast, MSAP showed genome-wide methylation of CpG as low as 8.8% in *S. alterniflora*, 6.9% in *S. maritima*, 8.9% in *S.* × *neyrautii*, 7.5% in *S.* × *townsendii* and 6.4% in *S. anglica*. The numbers of methylated bands were thus significantly higher for combined MSTD (i.e. in regions flanking the investigated TEs) than for MSAP (i.e. in random genomic sequences) in each species (Fig. 7).

Effect of hybridization on methylation states

In order to evaluate CpG methylation changes following hybridization in regions surrounding TE insertions, the methylation state of *Msp*I-MSTD bands transmitted from the parental species to the F_1 hybrids was assessed and departure from additivity of the *Hpa*II-MSTD profiles was recorded (Table 1, Fig. 5b). In *S.* × *neyrautii*, 12.4% *Ins2* *Hpa*II-MSTD bands, 16.4% *Cassandra* *Hpa*II-MSTD bands and 22.2% *Wis-like* *Hpa*II-MSTD bands showed departure from the expected profile. In *S.* × *townsendii*, 12.0% *Ins2* *Hpa*II-MSTD bands, 12.2% *Cassandra* *Hpa*II-MSTD bands and 23.1% *Wis-like* *Hpa*II-MSTD bands showed departure from the expected profile. Methylation changes were not significantly different among TEs, in both hybrids (Fig. S1). When combined into a single dataset, 71 *Hpa*II-MSTD fragments out of 403 (17.6%) showed methylation changes in *S.* × *neyrautii* compared with the parents, with 23.9% of the bands that were demethylated (i.e. methylated in the parents but displayed as nonmethylated in the hybrid) and 76.1% that were nonmethylated in the parents and methylated in the hybrid. Similarly, 64 MSTD bands out of 407 (15.7%) showed methylation changes in *S.* × *townsendii* compared with the parents, with 29.7% of the bands that were demethylated at hybridization and 70.3% that were nonmethylated in the parents and methylated in the hybrid. Although the amount of methylated bands globally increased in both hybrids, the level of CpG methylation changes towards a nonmethylated vs a methylated state were not different from the parental levels of methylated (29.5%) vs nonmethylated (70.5%) bands (χ^2 tests: *S.* × *neyrautii*, $P = 0.386$ and *S.* × *townsendii*, $P = 0.923$). By contrast, random genomic sequences, as assessed here from *Hpa*II-MSAP profiles, showed little methylation changes after hybridization, with four MSAP bands (1.2%) altered from a nonmethylated to methylated state. This is significantly lower than MSTD for both *S.* × *neyrautii* (χ^2 53.13, $P < 0.001$) and *S.* × *townsendii* (χ^2 45.95, $P < 0.001$).

Methylation changes in *Hpa*II-MSTD bands were dependent on the parental origin (Fig. 6b, Table 1). In *S.* ×

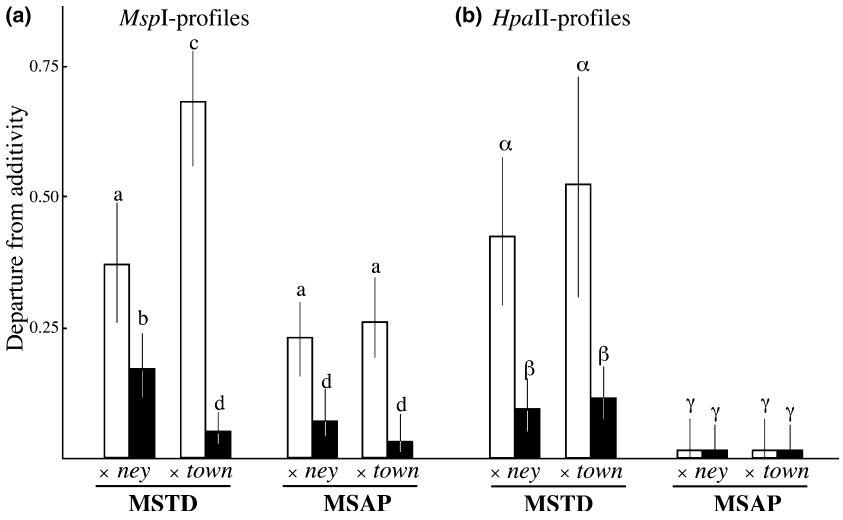


Fig. 6 Parent-specific structural (a) and CpG methylation changes (b) at hybridization in *Spartina,* as assessed following the parental origin (*Spartina alternifolia* or *Spartina maritima*) of bands showing departure from additivity in *Spartina* × *neyrautii* (× *ney*) and *Spartina* × *townsendii* (× *town*), for the three transposable elements (TEs) combined (*Msp*I- and *Hpa*II-methyl-sensitive transposon display (MSTD), respectively) and for genome-wide sequences (*Msp*I- and *Hpa*II-methyl-sensitive transposon display (MSAP), respectively); *alterniflora*-specific, open bars; *maritima*-specific, closed bars. Error bars indicate 95% confidence intervals. Proportions with distinct lower case letters are significantly different according to multiple chi-square tests.

neyrautii, 18 out of 42 (42.9%) MSTD bands of maternal (*S. alterniflora*) origin showed methylation changes, which is significantly more than 12 out of 125 (9.65%) for bands of paternal (*S. maritima*) origin (χ^2 21.39, $P < 0.001$). In *S.* × *townsendii*, 11 out of 21 (52.4%) MSTD bands of maternal (*S. alterniflora*) origin showed methylation changes, which is significantly more than 17 out of 146 (11.6%) for bands of paternal (*S. maritima*) origin (χ^2 8.59, $P = 0.003$). By contrast, MSAP reported no parental bias of methylation changes. As a whole, both hybrids showed similar epigenetic alterations (χ^2 1.73, $P = 0.188$), but methylation changes affected mostly the surroundings of maternal TE insertions following hybridization.

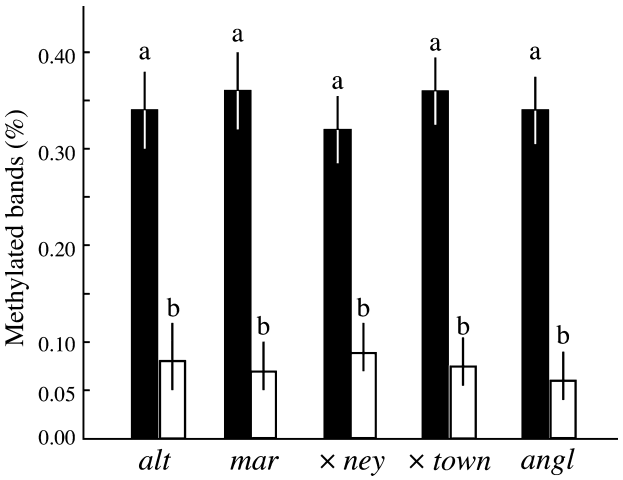


Fig. 7 Methylation levels of methyl-sensitive transposon display (MSTD, closed bars) bands (combined for the three transposable elements) and methyl-sensitive amplified polymorphism (MSAP, open bars) bands in the parental species *Spartina alterniflora* (*alt*) and *Spartina maritima* (*mar*), the homoploid F_1 hybrids *Spartina* × *neyrautii* (× *ney*) and *Spartina* × *townsendii* (× *town*), and the natural allopolyploid *Spartina anglica* (*angl*). Error bars indicate 95% confidence intervals. Proportions with distinct lower case letters are significantly different according to multiple χ^2 tests.

Effect of genome doubling on methylation levels

The CpG methylation changes near TE insertions after genome doubling were assessed among the *Msp*I-MSTD bands that were transmitted from *S.* × *townsendii* to *S. anglica* by recording departure from the expected *Hpa*II-MSTD profile (i.e. similarity to *S.* × *townsendii*). In the allopolyploid *S. anglica*, 11.6% *Ins2* bands, 4.2% *Cassandra* bands (4.2%) and 7.9% *Wis-like* bands showed departure from the expected *Hpa*II-MSTD profile (Fig. 3, Table 2). Among the 415 combined MSTD bands that were transmitted from *S.* × *townsendii* to *S. anglica*, 37 (8.9%) showed methylation changes (Fig. 5b). More specifically, 64.9% of the bands were demethylated vs 35.1% that exhibited neomethylation, which is significantly different from the amount of methylated (36.4%) vs nonmethylated (63.6%) bands in *S.* × *townsendii* (χ^2 11.26, $P < 0.001$). As a whole, departure from the expected *Hpa*II-MSTD patterns (i.e. methylation changes in the surrounding of TE insertions) was significantly higher at hybridization compared with genome doubling (χ^2 8.22, $P < 0.004$). By contrast, none of the 344 genome-wide MSAP bands that were transmitted to the polyploid showed departure from the expected profile, which is significantly lower than obtained by MSTD (χ^2 30.35, $P < 0.001$).

Together, these data show that substantial CpG methylation changes in the vicinity of TE insertions occurred during hybridization and to a lesser extent, genome doubling, compared with random loci. While apparently random in their direction, these epigenetic modifications predominantly affected the genome of maternal origin.

Discussion

In most studies involving allopolyploid systems, the response to hybridization is rarely distinguished from the effect of genome multiplication (Levy & Feldman, 2004; Comai, 2005; Otto, 2007; but see Hegarty *et al.*, 2006). In *Spartina*, all progenitor taxa (the parents *S. alterniflora* and *S. maritima*) as well as the homoploid F_1 hybrids (*S.* × *neyrautii* and *S.* × *townsendii*) are still established close to hybridization sites and the recent allopolyploid *S. anglica* thus represents an excellent model to contrast the early processes specifically affecting merged genomes compared with duplicated genomes in natural systems (Ainouche *et al.*, 2004a). Since low interindividual genetic variation was detected in all the taxa, relevant genome changes can be accurately tracked. By contrasting patterns of MSTD bands representing the TE component of the genome (i.e. sequences immediately flanking insertions) with MSAP bands that represent genome-wide random sequences, this study allowed, for the first time, to characterize the response of different genome fractions during the two-step formation of an allopolyploid species.

Polymorphic MSTD bands for the three TEs studied (*Ins2*, *Cassandra* and *Wis-like*) were mostly shared between the parental *S. maritima* and *S. alterniflora* species (Figs 2, 4), suggesting a relatively low transposition rate of the TEs studied in the recent past. However, in contrast to random sequences surveyed by MSAP, most parent-specific MSTD fragments were observed in *S. maritima* rather than *S. alterniflora*. This pattern likely suggests that the paternal species accumulated more TE insertions than the maternal species after their speciation, although a specific distribution of nonCpG methylation around TE insertions cannot be excluded. Random population processes may be responsible for a differential amplification of TEs as the small size of *S. maritima* fragmented populations (Yannic *et al.*, 2004) certainly increased the probability of fixing insertions.

More structural changes after hybridization than after genome doubling

Allopolyploidy is considered as a genomic shock generating important evolutionary diversification (Rieseberg, 2001; Comai, 2005; Otto, 2007). However, fewer structural changes were reported in *S. anglica* than in several allopolyploid systems (Ainouche *et al.*, 2004b). Congruently, the MSTD profiles of the hybrids and polyploid species indicate slight restructuring following allopolyploidy (Fig. 5a): 83.9% of the parental MSTD bands were observed in the hybrid *S.* × *townsendii*, with 96.3% of these transmitted to the allopolyploid *S. anglica*, which is little less than MSAP. Overall, only 13 new MSTD bands out of 480 were detected in *S.* × *neyrautii*, *S.* × *townsendii* and *S. anglica*, indicating that neither hybridization nor genome doubling induced a transposition burst for these elements. Indeed, structural changes accompanying allopolyploidy were predominantly MSTD band losses. The nature of structural changes around TE insertions may remain ambiguous since missing MSTD fragments may reflect segregating heterozygosity in the parents, CpCpG methylation change or sequence rearrangements encompassing TE insertions. Since MSTD band losses specifically affected *S. maritima*, a species showing very low genetic variation (Yannic *et al.*, 2004), structural rearrangements may be prevalent here. Whether this is restricted to the particular TEs investigated or represents a general trend is an open question. However, in the three different TE types analysed here, structural changes near TEs were predominantly observed after the parental genome merger and resulted in at least a five-fold increase in restructuring compared with genome doubling. Changes detected in randomly assayed sequences (e.g. MSAP; see Ainouche *et al.*, 2004a) exhibited a similar pattern and hybridization therefore appears as a major shock stimulating foremost structural changes compared with genome doubling.

As shock-induced restructuring was observed in the two independently formed F_1 hybrids, our data are congruent with Mc Clintock's hypothesis of unmasked incompatibilities (McClintock, 1984) and suggest that merging differentiated genomes into a single nucleus required immediate genomic changes to produce viable lineages. Although both structural changes near TEs and random loci affected chiefly the maternal genome, the TE component assessed here revealed contrasted rearrangements in the two hybrids (Fig. 6a). Unlike maternal and paternal random loci that exhibit similar levels of rearrangements in both hybrids, TE insertions from the maternal genome were more severely rearranged in *S.* × *townsendii* than in *S.* × *neyrautii*, while paternal ones showed the reverse tendency. Although paternal reorganization of other TE families cannot be excluded, it is interesting that the dynamics of MSTD bands are similar for the three TE families investigated and parallel morphological changes in *Spartina*. While deriving from very similar genotypes of the same maternal and paternal species and presenting similar MSAP patterns (Salmon *et al.*, 2005), the two independently formed hybrids display conspicuous morphological differences, *S.* × *neyrautii* being more similar to the maternal parent *S. alterniflora*. The particular changes induced by hybridization on genomic regions encompassing TEs may thus be associated with phenotypic specificities, suggesting that mechanisms targeting TE insertions have wide-ranging effects (Comai *et al.*, 2003).

Hybridization-induced methylation changes near TEs

Our results, focused on the CpG fraction of the genome and thus probably excluding CpNpG-rich heterochromatic regions, showed that sequences flanking TE insertions are

significantly more CpG methylated than random (MSAP) sequences in *Spartina* (Fig. 7). In contrast to our prediction that short TEs (here, *Ins2* and *Cassandra*) would be associated with the high-density gene fraction and low CpG methylation, while long TEs, such as full-length retrotransposons (here, *Wis*-like), would have accumulated in gene-poor regions and show high CpG methylation (Brookfield, 2005), insertions of the three TEs surveyed did not significantly differ in their CpG methylation status in the parental *Spartina* species (Fig. S2). This suggests that the CpG methylation status around TE insertions may thus be only loosely linked to the TE features. Accordingly, CpG methylation patterns around insertions of various TE families across the rice genome were correlated with neither the size of TE nor the number of inserted copies (Kashkush & Khasdan, 2007; Takata *et al.*, 2007).

Our results reveal that hybridization triggers CpG methylation changes that occur five times more frequently than after genome doubling (Fig. 5b). The instability associated with the shock of hybridization is thus inducing important epigenetic alterations at CpG sites in the surroundings of both TE insertions and random loci. MSTD bands however showed far more CpG methylation changes than MSAP bands, suggesting that TE insertions trigger epigenetic alterations in particular. Both CpG and nonCpG methylation contribute to the immobilization of TEs (Kato *et al.*, 2003) and may regulate the expression of developmental genes (Huettel *et al.*, 2006). However, CpG methylation seems of critical importance for evolutionary processes as it coordinates the stable inheritance of epigenetic marks, guiding *de novo* nonCpG methylation and chromatin remodeling (Mathieu *et al.*, 2007); it is also of foremost importance in maintaining TE silencing (Slotkin & Martienssen, 2007; Teixeira *et al.*, 2009). Accordingly, our results from MSTD are focused on the less heavily methylated fraction of the genome and provide here valuable insights into the interplay between TEs and epigenetic changes leading to substantial reorganization in genome regions prone to foster adaptation in plant populations.

To our knowledge, few studies have reported extensive CpG methylation changes specifically affecting the surrounding of TEs in response to stress (but see Long *et al.*, 2006). We are far from a complete understanding of the epigenetic regulation of TEs and flanking sequences, especially in Poaceae such as *Spartina*, which may have evolved specific epigenetic pathways (Zhang, 2008). Several non-mutually exclusive hypotheses may account for TE-specific CpG methylation changes (reviewed in Weil & Martienssen, 2008; Teixeira *et al.*, 2009). For example, since CpG methylation status around TE insertions is most likely controlled by specific enzymatic machinery, mechanisms preferentially targeting TEs, which are supposedly affecting non-CpG methylation to a large extent, may be intimately connected to those controlling CpG methylation across the genome (Kato *et al.*, 2003; Mathieu *et al.*, 2007). Testing such a hypothesis is beyond the scope of the present survey. However, hybridization seems to induce stochastic (i.e. nondirectional) CpG methylation repatterning around TE insertions in the non-heavily methylated fraction of the genome surveyed here. Indeed, the proportion of MSTD fragments changing towards a methylated vs a nonmethylated state was not significantly different from the parental frequencies of nonmethylated vs methylated bands, respectively. The methylation changes we observed were, however, non-random as they specifically affected MSTD bands from *S. alterniflora*, while random sequences showed an undiscriminating pattern. It remains difficult to firmly assess any TE type-specific epigenetic dynamics in response to genomic shock since the analysis of each TE separately reduced our statistical power. However, *Cassandra* and *Wis-like* (i.e. retrotransposons) did not show significantly more methylation changes than *Ins2* (i.e. class II TE) after hybridization (Figs S1, S2). We do not exclude the possibility that other TE types could present singular patterns, but our results suggest common CpG methylation changes affecting various members of the TE component during allopolyploid speciation in *Spartina*.

Genetic and epigenetic changes in the TE component during allopolyploid speciation

Our results indicate that the critical aspect of genome evolution in *Spartina* seems to be the specific CpG methylation changes around TE insertions. By decoupling the steps of allopolyploid speciation, this study showed that merging differentiated genomes mostly resulted in structural and epigenetic changes, which specifically affected the TE component of maternal origin in the present study. Accordingly, hybridization more than genome doubling is expected to induce immediate alterations to circumvent genetic incompatibility and produce a viable system (McClintock, 1984; Rieseberg, 2001). Among the putative underlying mechanisms, nucleocytoplasmic incompatibilities may result in asymmetrical contributions between the maternal and the paternal genomes in hybrids (Tiffin *et al.*, 2001), but are unlikely to be resolved by the breakdown of the maternal genome, as observed in this study. Since loci from the *S. alterniflora* genome prevail when reciprocally hybridized with other *Spartina* species (Anttila *et al.*, 2000), this preferential breakdown is apparently not associated with *S. alternifolia*-specific characteristic. Our results suggest that nuclear–nuclear incompatibilities were at work in the TE-specific alterations of the maternal genome during hybridization in *Spartina*. Genetic maternal effects may indeed result in asymmetrical incompatibilities between nuclear genomes (Turelli & Moyle, 2007). For example, dosage-dependent induction models are attractive to explain asymmetries affecting hybrid genomes with different parental TE load (Comai *et al.*, 2003;

Michalak, 2009). Here, the preferential alteration of the laden maternal TE component following two independent hybridization events may have been required to resolve genetic incompatibilities and produce viable hybrids of *Spartina*. By contrast, genome duplication induced few genomic modifications, but complementing the chromosome set and thus restoring fertility, allowed the allopolyploid *S. anglica* to emerge as a rapidly expanding species (Ainouche *et al.*, 2009). To what extent the genome dynamics associated with TEs revealed by the present study affects gene expression and adaptation remains an open question. In particular, the impact of TE insertions on gene products as well as genome-wide nonCpG methylation changes and chromatin remodeling deserve further investigation to broaden our understanding of genome evolution during the early steps of allopolyploidization.

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References

Ainouche ML, Baumel A, Salmon A. 2004a. *Spartina anglica* C.E. Hubbard: a natural model system for analysing early evolutionary changes that affect allopolyploid genomes. *Biological Journal of the Linnean Society* **82**: 475–484.

Ainouche ML, Baumel A, Salmon A, Yannic G. 2004b. Hybridization, polyploidy and speciation in *Spartina* (Poaceae). *New Phytologist* **161**: 165–172.

Ainouche M, Fortune M, Salmon A, Parisod C, Grandbastien M-A, Fukunaga M, Ricou M, Misset M-T. 2009. Hybridization, polyploidy and invasion: lessons from *Spartina* (Poaceae). *Biological Invasions* **11**: 1159–1173.

Anttila CK, King RA, Ferris C, Ayres DR, Strong DR. 2000. Reciprocal hybrid formation of *Spartina* in San Francisco Bay. *Molecular Ecology* **9**: 765–770.

Baumel A, Ainouche ML, Levasseur JE. 2001. Molecular investigations in populations of *Spartina anglica* C.E. Hubbard (Poaceae) invading coastal Brittany (France). *Molecular Ecology* **10**: 1689–1701.

Baumel A, Ainouche M, Kalendar R, Schulman AH. 2002. Retrotransposons and genomic stability in populations of the young allopolyploid species *Spartina anglica* C.E. Hubbard (Poaceae). *Molecular Biology and Evolution* **19**: 1218–1227.

Baumel A, Ainouche ML, Misset M-T, Gourret JP, Bayer RJ. 2003. Genetic evidence for hybridization between the native *Spartina maritima* and the introduced *Spartina alterniflora* (Poaceae) in South-West France: *Spartina* × *neyrautii* re-examined. *Plant Systematics and Evolution* **237**: 87–97.

Brookfield JFY. 2005. The ecology of the genome – mobile DNA elements and their hosts. *Nature Reviews Genetics* **6**: 128–136.

Cervera MT, Ruiz-Garcia L, Martinez-Zapater JM. 2002. Analysis of DNA methylation in *Arabidopsis thaliana* based on methylation-sensitive AFLP markers. *Molecular Genetics and Genomics* **268**: 543–552.

Chen ZJ. 2007. Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. *Annual Review of Plant Biology* **58**: 377–406.

Chen ZJ, Ha M, Soltis D. 2007. Polyploidy: genome obesity and its consequences. *New Phytologist* **174**: 717–720.

Comai L. 2005. The advantages and disadvantages of being polyploid. *Nature Reviews Genetics* **6**: 836–846.

Comai L, Madlung A, Josefsson C, Tyagi A. 2003. Do the different parental 'heteromes' cause genomic shock in newly formed allopolyploids? *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences* **358**: 1149–1155.

Gaut BS, Ross-Ibarra J. 2008. Selection on major components of Angiosperm genomes. *Science* **320**: 484–486.

Grandbastien M-A, Audeon C, Bonnivard E, Casacuberta JM, Chalhoub B, Costa APP, Le QH, Melayah D, Petit M, Poncet C *et al.* 2005. Stress activation and genomic impact of Tnt1 retrotransposons in Solanaceae. *Cytogenetic and Genome Research* **110**: 229–241.

Hegarty MJ, Barker GL, Wilson ID, Abbott RJ, Edwards KJ, Hiscock SJ. 2006. Transcriptome shock after interspecific hybridization in *Senecio* is ameliorated by genome duplication. *Current Biology* **16**: 1652–1659.

Huettel B, Kanno T, Daxinger L, Aufsatz W, Matzke AJM, Matzke M. 2006. Endogenous targets of RNA-directed DNA methylation and Pol IV in *Arabidopsis*. *EMBO Journal* **25**: 2828–2836.

Kalendar R, Tanskanen J, Immonen S, Nevo E, Schulman AH. 2000. Genome evolution of wild barley (*Hordeum spontaneum*) by Bare-1 retrotransposon dynamics in response to sharp microclimatic divergence. *Proceedings of the National Academy of Sciences, USA* **97**: 6603–6607.

Kalendar R, Tanskanen J, Chang W, Antonius K, Sela H, Peleg O, Schulman AH. 2008. Cassandra retrotransposons carry independently transcribed 5s RNA. *Proceedings of the National Academy of Sciences, USA* **105**: 5833–5838.

Kashkush K, Khasdan V. 2007. Large-scale survey of cytosine methylation of retrotransposons and the impact of readout transcription from long terminal repeats on expression of adjacent rice genes. *Genetics* **177**: 1975–1985.

Kato M, Miura A, Bender J, Jacobsen SE, Kakutani T. 2003. Role of CG and non-CG methylation in immobilization of transposons in *Arabidopsis*. *Current Biology* **13**: 421–426.

Leitch AR, Leitch IJ. 2008. Genomic plasticity and the diversity of polyploid plants. *Science* **320**: 481–483.

Levy AA, Feldman M. 2004. Genetic and epigenetic reprogramming of the wheat genome upon allopolyploidization. *Biological Journal of the Linnean Society* **82**: 607–613.

Lim KY, Kovarik A, Matyasek R, Chase MW, Clarkson JJ, Grandbastien M-A, Leitch AR. 2007. Sequence of events leading to near-complete genome turnover in allopolyploid *Nicotiana* within five million years. *New Phytologist* **175**: 756–763.

Lippman Z, May B, Yordan C, Singer T, Martienssen R. 2003. Distinct mechanisms determine transposon inheritance and methylation via small interfering RNA and histone modification. *PLoS Biology* **1**: E67.

Liu B, Wendel JF. 2003. Epigenetic phenomena and the evolution of plant allopolyploids. *Molecular Phylogenetics and Evolution* **29**: 365–379.

Long LK, Lin XY, Zhai JZ, Kou HP, Yang W, Liu B. 2006. Heritable alteration in DNA methylation pattern occurred specifically at mobile elements in rice plants following hydrostatic pressurization. *Biochemical and Biophysical Research Communications* **340**: 369–376.

Lynch M. 2007. *The origins of genome architecture*. Sunderland, MA, USA: Sinauer Associates, Inc.

Madlung A, Comai L. 2004. The effect of stress on genome regulation and structure. *Annals of Botany* **94**: 481–495.

Mallet J. 2007. Hybrid speciation. *Nature* **446**: 279–283.

Mathieu O, Reinders J, Caikovski M, Smathajitt C, Paszkowski J. 2007. Transgenerational stability of the *Arabidopsis* epigenome is coordinated by CG methylation. *Cell* **130**: 851–862.

McClintock B. 1984. The significance of responses of the genome to challenge. *Science* **16**: 792–801.

Melayah D, Lim KY, Bonnivard E, Chalhoub B, Dorlhac de Borne F, Mhiri C, Leitch AR, Grandbastien M-A. 2004. Distribution of the Tnt1 retrotransposon family in the amphidiploid tobacco (*Nicotiana tabacum*) and its wild *Nicotiana* relatives. *Biological Journal of the Linnean Society* **82**: 639–649.

Michalak P. 2009. Epigenetic, transposon and small RNA determinants of hybrid dysfunctions. *Heredity* **102**: 45–50.

Newcombe RG. 1998. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Statistics in Medicine* **17**: 857–872.

Otto SP. 2007. The evolutionary consequences of polyploidy. *Cell* **131**: 452–462.

Petit M, Lim KY, Julio E, Poncet C, Dorlhac de Borne F, Kovarik A, Leitch AR, Grandbastien M-A, Mhiri C. 2007. Differential impact of retrotransposon populations on the genome of allotetraploid tobacco (*Nicotiana tabacum*). *Molecular Genetics and Genomics* **278**: 1–15.

Ralston EJ, English JJ, Dooner HK. 1988. Sequence of three *bronze* alleles of maize and correlation with the genetic fine-structure. *Genetics* **119**: 185–197.

Rapp RA, Wendel JF. 2005. Epigenetics and plant evolution. *New Phytologist* **168**: 81–91.

Rice WR. 1989. Analysing tables of statistical tests. *Evolution* **43**: 223–225.

Rieseberg LH. 2001. Polyploid evolution: keeping the peace at genomic reunions. *Current Biology* **11**: R925–R928.

Rieseberg LH, Willis JH. 2007. Plant speciation. *Science* **317**: 910–914.

Salmon A, Ainouche ML, Wendel JF. 2005. Genetic and epigenetic consequences of recent hybridization and polyploidy in *Spartina* (Poaceae). *Molecular Ecology* **14**: 1163–1175.

Schranz ME, Osborn TC. 2004. *De novo* variation in life-history traits and responses to growth conditions of resynthesized polyploid *Brassica napus* (Brassicaceae). *American Journal of Botany* **91**: 174–183.

Shaked H, Kashkush K, Ozkan H, Feldman M, Levy AA. 2001. Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. *Plant Cell* **13**: 1749–1759.

Slotkin RK, Martienssen R. 2007. Transposable elements and the epigenetic regulation of the genome. *Nature Reviews Genetics* **8**: 272–285.

Soltis PS. 2005. Ancient and recent polyploidy in angiosperms. *New Phytologist* **166**: 5–8.

Takata M, Kiyohara A, Takasu A, Kishima Y, Ohtsubo H, Sano Y. 2007. Rice transposable elements are characterized by various methylation environments in the genome. *BMC Genomics* **8**: 469.

Teixeira FK, Heredia F, Sarazin A, Roudier F, Boccara M, Ciaudo C, Cruaud C, Poulain J, Berdasco M, Fraga MF *et al.* 2009. A role for RNAi in the selective correction of DNA methylation defects. *Science* **323**: 1600–1604.

Thompson JD, McNeilly T, Gray AJ. 1991. Population variation in *Spartina anglica* C.E. Hubbard. II. Reciprocal transplant among three successional populations. *New Phytologist* **117**: 129–139.

Tiffin P, Olson MS, Moyle LC. 2001. Asymmetrical crossing barriers in angiosperms. *Proceedings of the Royal Society of London Series B: Biological Sciences* **268**: 861–867.

Turelli M, Moyle LC. 2007. Asymmetric postmating isolation: Darwin's corollary to Haldane's rule. *Genetics* **176**: 1059–1088.

Waugh R, McLean K, Flavell AJ, Pearce SR, Kumar A, Thomas BBT, Powell W. 1997. Genetic distribution of Bare-1-like retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphisms (S-SAP). *Molecular & General Genetics* **253**: 687–694.

Weil C, Martienssen R. 2008. Epigenetic interactions between transposons and genes: lessons from plants. *Current Opinion in Plant Biology* **18**: 182–188.

Yannic G, Baumel A, Ainouche M. 2004. Uniformity of the nuclear and chloroplast genomes of *Spartina maritima* (Poaceae), a salt-marsh species in decline along the Western European Coast. *Heredity* **93**: 182–188.

Zhang X. 2008. The epigenetic landscape of plants. *Science* **320**: 489–492.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Structural and CpG methylation changes during allopolyploid speciation in *Spartina*.

Fig. S2 Methylation levels of methyl-sensitive transposon display (MSTD) bands for each of the three TEs and methyl-sensitive amplified polymorphism (MSAP) bands in the parental species, *Spartina alterniflora* and *Spartina maritima*, the independent homoploid F_1 hybrids *Spartina* × *neyrautii* and *Spartina* × *townsendii*, and the natural allopolyploid *Spartina anglica*.

Notes S1 Detailed protocol of methyl-sensitive transposon display (MSTD).

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