Biological Journal of the Linnean Society, 2004, **82**, 475–484. With 1 figure

Biological relevance of polyploidy: ecology to genomics
Edited by A. R. Leitch, D. E. Soltis, P. S. Soltis, I. J. Leitch and J. C. Pires

Spartina anglica C. E. Hubbard: a natural model system for analysing early evolutionary changes that affect allopolyploid genomes

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Received 16 June 2003; accepted for publication 5 January 2004

Spartina anglica arose during the end of the 19th century in England by hybridization between the indigenous *Spartina maritima* and the introduced East American *Spartina alterniflora* and following genome duplication of the hybrid (*S.* × *townsendii*). This system allows investigations of the early evolutionary changes that accompany stabilization of a new allopolyploid species in natural populations. Various molecular data indicate that *S. anglica* has resulted from a unique parental genotype. This young species contains two distinctly divergent homoeologous genomes that have not undergone extensive change since their reunion. No burst of retroelements has been encountered in the F_1 hybrid or in the allopolyploid, suggesting a 'structural genomic stasis' rather than 'rapid genomic changes'. However, modifications of the methylation patterns in the genomes of *S.* × *townsendii* and *S. anglica* indicate that in this system, epigenetic changes have followed both hybridization and polyploidization.

ADDITIONAL KEYWORDS: allopolyploidy – genome evolution – methylation-sensitive AFLP – multilocus markers – speciation.

INTRODUCTION

Recent studies on polyploidy have shown that genetic and epigenetic instabilities can affect recently duplicated genomes (reviewed in Comai, 2000; Wendel, 2000; Liu & Wendel, 2002, 2003; see also Chen *et al*., 2004; Kovarik *et al*., 2004; Lukens *et al*., 2004; Pires *et al*., 2004 – all this issue).

The immediate genetic redundancy resulting from allopolyploidy may be of critical importance for the evolutionary success of the polyploid (e.g. Brochmann *et al*., 2004 – this issue)

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Recent molecular studies on natural polyploid populations have shown that allopolyploid lineages can have multiple origins, increasing the level of genetic diversity available to the newly formed species (Soltis & Soltis, 1999; Doyle *et al*., 2004; Soltis *et al*., 2004 – both this issue). Moreover, allopolyploid genomes can be particularly dynamic at both the genome structure and the genome expression levels over the long- and short-term evolutionary time-scales (reviewed in Wendel, 2000; Liu & Wendel, 2002; Osborn *et al*., 2003).

Significant advances in revealing the occurrence and nature of the early evolutionary changes that occur in polyploid genomes have been made possible because of the availability of experimentally resynthesized allopolyploids involving well-known polyploid

model systems such as *Brassica* (Lukens *et al*., 2004; Pires *et al*., 2004), *Arabidopsis* (Chen *et al*., 2004), *Gossypium* (Adams & Wendel, 2004 – this issue), *Triticum–Aegilops* (Levy & Feldman, 2004 – this issue) or *Nicotiana* (Kovarik *et al*., 2004). Among the few well-documented natural allopolyploids of recent origin (i.e. at the human observation time-scale) is the classic example of alloploid speciation resulting in the formation of *Spartina anglica* C. E. Hubb. in western Europe at the end of the 19th century (Huskin, 1930; Guénégou, Citharel & Levasseur, 1988; Gray, Benham & Raybould, 1990). This species originated in England from hybridization between the native *Spartina maritima* (Curtis) Fernald and the introduced Eastern American *Spartina alterniflora* Loiseleur. The sterile first-generation hybrid *Spartina* × *townsendii* H. Groves & J. Groves gave rise to a fertile and successful allopolyploid, *S. anglica*, which has rapidly expanded in distribution and now occurs on several continents. As these species are perennial rhizomatous plants, both the sterile F_1 hybrid (*S.* × *townsendii*) and the initial allopolyploid populations of *S. anglica* are still growing in the site of hybridization, and may be compared with their parental species. This system is unique in that it allows investigation of the immediate consequences of hybridization on the one hand (in *S.* × *townsendii*), and genome duplication (in *S. anglica*) on the other. This enables evaluation of the fitness conferred by the duplication of a hybrid genome in the process of species establishment in natural populations. The *Spartina* model offers a rare opportunity to explore in natural conditions the 'revolutionary phase' (Levy & Feldman, 2002) following hybridization and genome duplication, in which various genetic and epigenetic adjustments must take place for the stabilization of the new species.

The purpose of this paper is to demonstrate the potential contribution of *S. anglica* research to a better understanding of the early evolutionary changes influencing allopolyploid genomes in natural populations. The following are presented: (1) the historical background of *S. anglica*, (2) an assessment of the evidence showing single vs. multiple origins of the allopolyploid and (3) a test of the 'rapid genome change' hypothesis in natural populations.

SPARTINA ANGLICA: HISTORY AND BACKGROUND

Spartina belongs to subfamily Chloridoideae of Poaceae. It comprises perennial plants that generally colonize salt marshes. A phylogenetic scheme of the *Spartina* species involved in the formation of *S.* × *townsendii* and *S. anglica* has been inferred from nuclear (*ITS* and *Waxy*) and chloroplast (*trn*L–*trn*L spacer) DNA data (Baumel *et al*., 2002a). The reticulate origin of *S. anglica* is presented in Figure 1, in which the parental species *S. maritima* and *S. alterniflora* belong to sister lineages of a strongly supported clade within the genus. Most *Spartina* species (13) are native to the New World (Mobberley, 1956) and before the 19th century, only one species (*S. maritima*) was known in the Old World. *Spartina alterniflora*, a salt marsh species native to the eastern coast of North America, was first observed in 1829 in Southampton Bay (southern England) where it was accidentally introduced via shipping ballast. Here, it hybridized with the native (West Euro-African) *Spartina maritima*. The resulting sterile hybrid was first collected in 1870 at the locality of Hythe and named *S.* × *townsendii* (Groves & Groves, 1880). Twenty-two years later (1892), a new vigorous and fertile form was recorded near Hythe. Although Huskin (1930) showed the polyploid nature of the latter, the two forms were maintained for 70 years under the same taxonomic designation (*S.* × *townsendii s.l.*) until Hubbard (1968) proposed *Spartina anglica* as a distinct species for the polyploid. Detailed morphological and chromosome studies (Marchant, 1963, 1967, 1968) established the chromosome numbers of the parental species ($2n = 62$ and $2n = 60$ for *S. alterniflora* and *S. maritima*, respectively), the hybrid *S.* × *townsendii* ($2n = 62$) and the polyploid *S. anglica* ($2n = 122$). Chromosome number variation, attributed to aneusomaty (Marchant, 1968), was observed in *S. anglica* where plants with $2n = 120$ or $2n = 124$ were also encountered, although at lower frequency than $2n = 122$. Chromosome pairing behaviour at meiosis (mostly bivalents with very few multivalents) strongly suggested amphiploidy for

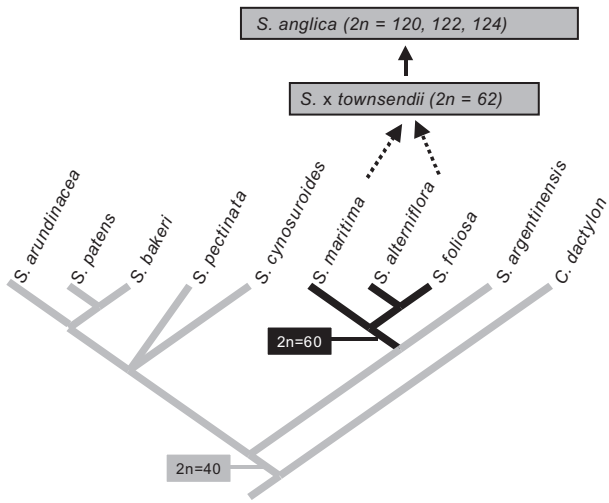

Figure 1. Origin of *S. anglica* and *S.* × *townsendii*. The phylogenetic relationships among *Spartina* species are redrawn from Baumel *et al*. (2002a). Chromosome numbers are from Marchant (1968).

S. anglica. According to the base chromosome number of the subfamily Chloridoideae ($x = 10$), the parental species are hexaploid.

The first molecular evidence of the allopolyploid origin of *S. anglica* was from isozyme studies (Guénégou *et al*., 1988; Raybould *et al*., 1991a) that revealed additivity of the allozyme markers present in *S. maritima* and *S. alterniflora* with fixed heterozygosity. Later, Ferris, King & Gray (1997) found that both *S.* × *townsendii* and *S. anglica* samples (from Hythe and Eling in Hampshire) displayed the same chloroplast DNA sequence (*trn*L intron) as *S. alterniflora*, which is therefore the likely female parent.

Although *S. anglica* has been found to be genetically depauperate for allozyme segregation variation (Raybould *et al*., 1991b), significant morphological variation is encountered within and among populations (Thompson, 1990a). Populations from ecologically similar situations are more similar than adjacent populations, with smaller inflorescence size and vegetative characters present in pioneer populations than in mature ones (Thompson, 1991). A series of experiments, involving common garden cultivation (Hill, 1990; Thompson, McNeilly & Gray, 1991a), reciprocal transplanting (Thompson, McNeilly & Gray, 1991b) and fitness component estimates in glasshouse experiments (Thompson, McNeilly & Gray, 1991c), elegantly showed that most variation was due to phenotypic plasticity, rather than to genetic plasticity. This plasticity to environmental change is interpreted as playing a central role in the fitness of the populations. Morphological plasticity was, however, less in successionally mature clonal populations, indicating age-related variation. Moreover, two distinct morphs ('graceful' and 'stocky') that are not related to variation in environmental conditions were encountered, suggesting different genotypes (Thompson *et al*., 1991c). The 'graceful' form resembles the sterile hybrid *S.* × *townsendii* with tall culms, long narrow leaves and small number of spikes, whereas the 'stocky' form is characterized by short plants with more spikes and short but wide leaves (Thompson, 1990a). These two forms occur at the extreme range of distinct phenotypes found in *S. anglica* in most estuaries (Thompson *et al*., 1991c).

Spartina anglica displays a wide ecological range in salt marshes, from the low-lying, intertidal mudflat to the upper marsh zone. Plants can tolerate submersion in salt water for up to 6 h per tide, enabling the species to colonize much further down the shore than other vascular plants and to occupy a vacant niche. Both significant seed production and extensive lateral clonal growth characterize this rhizomatous species. Dispersal may be accomplished through seeds or rhizome fragments dispersed by sea currents or by birds. Since its formation, *S. anglica* has spread all along the coast of the British Isles (Charman, 1990; Gray & Raybould, 1997). By contrast, the parental species remain confined to a few sites. *Spartina alterniflora* is restricted to one site, 3 miles from the initial hybridization site, and *S. maritima* has declined severely along the south-eastern British coast, where only a few clones may be surviving on Haling Island and the Isle of Wight (Raybould, Gray & Hornby, 2000). The wider ecological amplitude of the allopolyploid compared with its parental species illustrates greater invasive potential. *Spartina anglica* was naturally introduced in France at the beginning of the 20th century (Corbière, 1926). Since then, the species has progressed all along the western coast, causing dramatic ecological changes in some sites as a consequence of continuous elevation of the substrate (Baumel, Ainouche & Levasseur, 2001, and references therein). The ability of this species to accumulate large volumes of tidal sediments as a pioneer species has led to its deliberate introduction in several parts of the world (Northern Europe, Australia, New Zealand, China) for land reclamation. As a consequence of both artificial and natural propagation, *S. anglica* now has a large geographical distribution. The ecological consequence resulting from the spread of introduced plants that rapidly form dense monospecific swards have led to various local policies designed to control the species, including attempts at eradication by chemical or physical means (e.g. Hedge, Kriwoken & Ritar, 1997; Shaw & Gosling, 1997). Older British colonized sites began to display 'die-back' of *S. anglica* by the 1920s (e.g. in Poole Harbour, Gray & Raybould, 1997) that is probably related to the poor soil drainage in mature populations and to age-related decline in vigour (Thompson *et al*., 1991b). Thus, after expansion, it might be predicted that *S. anglica* would undergo a natural retreat, after having 'paved the way of its own destruction' (Gray, Marshall & Raybould, 1991). But the availability of potentially favourable sites enhanced by the global rise in sea-level due to global warming make this a prediction that is some way off (Thompson, 1990b). For evolutionary ecologists, the success of *S. anglica* remains a fascinating question that is now possible to explore using genomic approaches.

THE GENETIC 'CAPITAL DÉPART' OF *SPARTINA ANGLICA*

The East American *S. alterniflora* and the Euro-African *S. maritima* are sister species in a strongly supported palaeo-hexaploid lineage (Baumel *et al*., 2002a; Ainouche *et al*., 2004). However, pairwise nucleotide comparisons between *S. alternifora* and *S. maritima* reveal consistently divergent nuclear and chloroplast genomes (48 nucleotide changes, Table 1)

Table 1. Pairwise distances between 11 *Spartina* species: total character differences are based on nuclear *Waxy*, *ITS* and chloroplast *trn*T–*trn*L spacer sequences (1981 aligned bp)

		1	2	3	4	5	6	7	8	9	10	11
1	*S. arundinacea*	–										
2	*S. ciliata*	7	–									
3	*S. cynosuroides*	27	29	–								
4	*S. densiflora*	6	5	29	–							
5	*S. patens*	17	21	27	20	–						
6	*S. bakeri*	19	23	28	22	2	–					
7	*S. pectinata*	20	24	26	23	19	20	–				
8	*S. alterniflora*	56	57	61	55	52	53	49	–			
9	***S. maritima***	57	58	55	58	54	55	47	**48**	–		
10	*S. foliosa*	56	57	61	55	52	53	47	2	48	–	
11	*S. argentinensis*	33	38	42	35	34	35	31	39	51	39	

that might explain the sterility of the first-generation hybrid *S.* × *townsendii*.

As expected from founder effects in introduced clonal plants, the populations of *S. alterniflora* that were introduced in Western Europe lack molecular variation. Although native populations of *S. alterniflora* in North America display chloroplast sequence diversity, the chloroplast haplotype encountered in the populations of *S. alterniflora* from England and France (Baumel *et al*., 2003) is identical to that recorded by Antilla *et al*. (2000) from Massachusetts. Minimal genetic variation is also encountered in European populations for nuclear markers, although a few RAPD (randomly amplified polymorphic DNA) markers differentiate populations of southwestern France from those of Brittany and southern England. However, the data do suggest that in Western Europe, *S. alterniflora* populations have resulted from at least two different introductions (Baumel *et al*., 2003). More genetic diversity was expected from populations of the indigenous *S. maritima.* However, an extensive screening of the populations from Western Europe revealed a lack of molecular variation in both the nuclear and the chloroplast genomes (Yannic, Baumel & Ainouche, 2004), confirming the early isozyme studies performed on the British populations (Raybould *et al*., 1991b). Thus, both parental species lack much genetic variation in the region of hybridization.

The allopolyploid populations of *Spartina anglica* have been analysed in both its native range in western Europe and in a more recently colonized area in Australia (Baumel *et al*., 2001; A. Baumel, M. L. Ainouche, R. J. Bayer & M. T. Misset, unpubl. data) using various multilocus markers and sequences from the nuclear and chloroplast genomes. These analyses have revealed that the populations are composed of the same multilocus genotype that is rapidly expanding around the world. Interestingly, this major genotype is identical to that of *S.* × *townsendii*, the first-generation hybrid from Southampton Bay. Losses of a few markers from this initial genotype are observed but are rare and restricted to a few populations. Only one population from Brittany, on the Aven River, is fixed for the absence of one RAPD (Baumel *et al*., 2001) and one ISSR (inter simple sequence repeats, A. Baumel, unpubl. data) marker from the 'major genotype'. It is likely that these two markers, both of high molecular weight (1300 bp RAPD, 1200 bp ISSR), disappeared from an individual that subsequently founded the population. Moreover, all populations of *S. anglica* examined in the European populations display the same chloroplast DNA sequences as *S. alterniflora*, the maternal genome donor (Ferris *et al*., 1997; Baumel *et al*., 2001; A. Baumel & M. Ainouche, unpubl. data).

A potential source of genetic variation might result from backcrosses between *S. anglica* and its parental species *S. maritima* in the sites where they co-occur on the southern Brittany coast. In Saint-Armel (Morbihan), mixed populations are encountered with individuals displaying intermediate morphology and are suspected to be hybrids (J.-E. Levasseur & M.-C. Guenegou, pers. comm.). These individuals have been examined using isozyme (M.-T. Misset, unpubl. data), nuclear and chloroplast DNA markers (Baumel *et al*., 2001), and belong to the typical 'major genotype' of *S. anglica*, which displays a remarkable morphological plasticity. Distribution of the genotypes along the French and Spanish coast (Baumel *et al*., 2001; Yannic *et al*., 2004) provides no evidence of genetic exchange between *S. anglica* and its parental species. This is a different situation to that observed in California, where the eastern American *S. alterniflora* has also been introduced and has hybridized with the native

and closely related *S. foliosa*, and where numerous reciprocal hybrids backcross with the native parental species (Antilla *et al*., 2000).

Together, these results support the hypothesis of a unique allopolyploid genome produced in Southampton Bay, following either a unique hybridization event or multiple crosses involving similar parental genotypes. Investigations of DNA variation in *S. maritima* and *S. alterniflora* in Europe (Baumel *et al*., 2003; Yannic *et al*., 2004) detected few polymorphic markers and showed no correlation between variation in the allopolyploid and parental molecular diversity. Therefore, the most probable hypothesis is that populations of *S. anglica* are composed of the initial genotype, and that this species underwent a strong genetic bottleneck at the time of its formation. The few and infrequent mutations encountered in the populations of the allopolyploid do not extensively affect the allopolyploid genome as a whole, but do lead to some genetic individualization of clones.

Rather different modes of origin have been reported in other comparable recent allopolyploids that have formed during the last 100 years. The American allotetraploid *Tragopogon* species, *T. mirus* and *T. miscellus*, formed in the Palouse region (eastern Washington and Idaho, USA) have resulted from recurrent and reciprocal hybridization between the introduced *T. dubius*, *T. pratensis* and *T. porrifolius* (Soltis & Soltis, 1991; Cook *et al*., 1998; Soltis *et al*., 2004). This was also the case for the recent allohexaploid *Senecio cambrensis* that has formed after 1910 in England, where at least two independent origins have been reported in Wales and Scotland (Ashton & Abbott, 1992; Harris & Ingram, 1992; Abbott & Lowe, 2004 – this issue). The allotetraploid *Brassica napus* has also formed multiple times, as it contains, alternatively, the chloroplast genome of its diploid progenitors *B. oleracea* and *B. campestris* (Erickson, Strauss & Beversdorf, 1983). By contrast, a unique allopolyploidization event seems to have resulted in the formation of the allotetraploid cotton lineage during the Pleistocene (Wendel *et al*., 1999), and no evidence of multiple origins has been found in tetraploid and hexaploid wheat (Levy & Feldman, 2002), suggesting that even rare (i.e. unique) events have important evolutionary consequences.

SPARTINA ANGLICA, BETWEEN 'RAPID GENOMIC CHANGES' AND 'GENOMIC STASIS'

Recent investigations on experimentally resynthesized polyploids have revealed that genomic instability may arise in allopolyploids because they do not display parental genome additivity (Wendel, 2000; Levy & Feldman, 2002; Liu & Wendel, 2002; Osborn *et al*., 2003, and references therein; Kovarik *et al*., 2004). In *Spartina anglica*, most populations are composed of a 'major genotype', which represents strict additivity of all the parental DNA fragments generated by various multilocus fingerprinting methods based on ISSR or RAPD and PCR-RFLP markers (Baumel *et al*., 2001). Both parental nuclear sequences are present in the young allopolyploid for single copy genes such as *Waxy* (Baumel *et al*., 2002a) and repetitive rRNA genes that are not homogenized by concerted evolution (Baumel *et al*., 2001).

To examine whether retroelements have been activated in the young allopolyploid, *S. anglica* has been investigated using a transposon display method based on IRAP (inter-retrotransposon amplified polymorphisms) and REMAP (retrotransposon – microsatellite amplified polymorphisms) markers adapted from Kalendar *et al*. (1999). The screening of insertional polymorphisms in the populations of *S. anglica* by IRAP and REMAP revealed again that the most frequent multilocus genotype was identical to the first-generation hybrid *S.* × *townsendii* (Baumel *et al*., 2002b). IRAP and REMAP generated profiles of 296 markers, of which 13 were polymorphic in *S. anglica* and which constituted 14 multilocus genotypes. 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. Of particular interest are two new IRAP bands detected in the population from Keyhaven (UK). These new bands correspond to integrations of a retrotransposon, which are likely to have occurred since the origin of the allopolyploid. However, they represent only 0.68% of 296 retrotransposon insertions scored in *S. anglica*, indicating that no burst of retroelement activation is occurring in the young allopolyploid genome. Table 2 summarizes the multilocus patterns generated by various fingerprint methods that allow comparison of the major genotype of *S. anglica* (identical to *S.* × *townsendii*) to the parental species *S. maritima* and *S. alterniflora*. A marker is either present or absent in each species, and most frequent combinations correspond to parental additivity in *S. anglica* (93.5% of 1581 surveyed DNA fragments). The major genotype does not exhibit any marker specific to *S. anglica* (i.e. – – + combination, Table 2) for RAPD, ISSR, IRAP or REMAP fragments. Only two AFLP fragments (out of 984 generated by various primer combinations) are restricted to *S. anglica* (Table 2). Few markers are present in only one parental species (and absent in *S. anglica*). These parental fragment losses cannot be interpreted as new elimination in the allopolyploid, as the same patterns are also observed in *S.* × *townsendii*, but they may be related to an immediate result of hybridization; moreover, considering the dominant nature of the markers used in these

Table 2. Multilocus comparison between the 'major' genotype of *S. anglica* (Ang) and the parental species *S. maritima* (Mar) and *S. alterniflora* (Alt). + = presence, – = absence of amplified fragments using five PCR-based fingerprint methods (ISSR, RAPD, IRAP, REMAP, AFLP)

	Alt	+	+	–	+	–	–	+	
	Mar	+	–	+	–	+	–	+	
	Ang	+	+	+	–	–	+	–	
									Total
ISSR		17	39	42	5	5	0	0	108
RAPD		33	56	57	13	6	0	0	165
IRAP		31	78	97	8	10	0	0	224
REMAP		15	33	41	8	3	0	0	100
AFLP		447	246	247	37	3	2	2	984
Total		543	452	484	71	27	2	2	1581
(%)		(34.3)	(28.6)	(30.6)	(4.5)	(1.8)	(0.1)	(0.1)	
			93.5%						

analyses, the possibility of heterozygosity in the hexaploid parents *S. alterniflora* and *S. maritima* cannot be excluded. Lack of structural changes in *S. anglica* might be related to the substantial genome differentiation between the homoeologous subgenomes mentioned above, leading to preferential chromosome pairing and immediate stabilization of the newly formed allopolyploid. Although few multivalents were observed in meiosis (Marchant, 1968), no evidence for allelic segregation was recorded from either allozyme (Raybould *et al*., 1991a) or nucleotide (Baumel *et al*., 2001, 2002b) data. This absence of correlation between occasional multivalent formation and homoeologous recombination has also been reported in synthetic allopolyploid cotton (Liu *et al*., 2001). Absence of parental marker recombination and high ploidal level (12*x*) in *S. anglica* also suggest the possibility of apomixis, as a result of intergenomic conflict in a species of hybrid origin (Carman, 1997), and this hypothesis needs to be explored further.

Our results contrast with the rapid and non-random structural changes, including sequence elimination, that have been reported in wheat (Liu *et al*., 1998a, b; Ozkan, Levy & Feldman, 2001; Levy & Feldman, 2004) and *Brassica* (Song *et al*., 1995) allopolyploids. Furthermore, natural (1–2 million years old) allopolyploid *Gossypium* species display non-independent evolution of homoeologous rRNA genes (Wendel, Schnabel & Seelanan, 1995) and retroelements (Zhao *et al*., 1998), but parental additivity and independent evolution of low- and single-copy genes (Cronn, Small & Wendel, 1999; Adams & Wendel, 2004). Genomic additivity was, however, encountered in newly synthesized allotetraploid and allohexaploid cottons (Liu *et al*., 2001).

To address the question of whether epigenetic changes occur in the F_1 hybrid and/or the allopolyploid, *S.* × *townsendii* and *S. anglica* were compared with the parental species using methylation-sensitive AFLP, with the isoschizomers *Msp*I/*Hpa*II combined to *Eco*RI. *Msp*I and *Hpa*II cut at the 5′-CCGG recognition site with different sensitivity to cytosine methylation (Xiong *et al*., 1999). The results are summarized in Table 3: *Spartina maritima* and *S. alterniflora* display 8.9% and 9.0% methylated sites, respectively, whereas only 6.6% and 6.8% sites were methylated in the hybrid and the allopolyploid. Among the 17 methylated sites encountered in *S.* × *townsendii*, 13 were inherited from the parental species (*S. maritima* and *S. alterniflora*). Four parental fragments exhibited a different methylation pattern and six parental fragments were lost in the hybrid. *Spartina anglica* inherited from *S.* × *townsendii* both the 13 parental fragments, and two (of four) fragments that displayed a modified methylation pattern. The other two altered parental fragments were specific to *S. anglica*. Additionally, the allopolyploid displayed one new methylated site that was absent in both the parental species and the F_1 hybrid. Six parental fragments (4 + 2, Table 3) were lost in *S. anglica* and *S.* × *townsendii*: four fragments were absent in both species, whereas two others were species-specific losses. Altogether, these data indicate that methylation changes do occur in *Spartina* following both hybridization and genome duplication; the significance of these changes needs to be explored further by estimating intraspecific methylation variation in the parental species, the hybrid and the allopolyploid.

Methylation changes in young (experimentally resynthesized) allopolyploids have been previously analysed using methylation-sensitive AFLP in *Triticum* (Shaked *et al*., 2001; Levy & Feldman, 2004), *Gossypium* (Liu *et al*., 2001; Adams & Wendel, 2004), *Arabidopsis* (Madlung *et al*., 2002; Chen *et al*., 2004) and

Table 3. Methylation-sensitive AFLP of genomic DNA from *S. maritima*, *S. alterniflora*, *S.* × *townsendii* and *S. anglica* (selective (+4) primer combinations: *Hpa*II/*Msp*I + TCAA – *EcoR*I + ACG; *Hpa*II/*Msp*I ± TCAA – *Eco*RI + AGC; *Hpa*II/*Msp*I + TCAC – *EcoR*I + AGC; *Hpa*II/MspI + TCAC – *EcoR*I + AAC)

	S. maritima	*S. alterniflora*	*S.* × *townsendii*	*S. anglica*
Methylated fragments	17/192	18/200	17/259	18/264
(%)	(8.9)	(9.0)	(6.6)	(6.8)
Methylated sites inherited from parents	–	–	13	13
(%)			(76.5)	(68.4)
Alteration of methylated parental fragments	–	–	4	4
			(11.7)	(21.0)
New (species specific) methylated sites	–	–	–	1
			(11.7)	(10.5)
Loss of parental methylated sites	–	–	6 = 4 + 2*	6 = 4 + 2*

*Species-specific fragment losses.

Brassica (Lukens *et al*., 2004). In *Triticum*, both F_1 hybrids and allopolyploids displayed about 7% altered methylated sites, and 4.4% changes were restricted to the allopolyploids (Shaked *et al*., 2001). Wide genomic changes in cytosine methylation have been reported in synthetic *Arabidopsis* allotetraploids, including hypermethylation and demethylation in the allopolyploids compared with parental species. Demethylation was most frequent, and was interpreted as a genomic response to hypermethylation that causes phenotypic instability in the newly formed allopolyploids (Comai *et al*., 2000; Madlung *et al*., 2002). By contrast, no significant methylation alteration was encountered in synthetic *Gossypium* allopolyploids, suggesting 'genomic stasis' in this system (Liu *et al*., 2001). However, biased expression and epigenetically induced gene silencing have been recently demonstrated in both natural and synthetic allotetraploid *Gossypium* species (Adams *et al*., 2003; Adams & Wendel, 2004). According to the observed patterns of expression in natural and synthetic allopolyploids, some of these alterations are believed to have arisen early after polyploid formation, and maintained in modern allopolyploid cotton species. Various alterations of expression have been also reported in other synthetic allopolyploids (Osborn *et al*., 2003, and references therein; Kashkush, Feldman & Levy, 2002, 2003; He *et al*., 2003). These approaches are providing new insights into understanding, at the functional level, the mechanisms that may promote the adaptive success of duplicated genomes.

In conclusion, the vigorous invasive *S. anglica* represents a particular model in which there is no evidence of multiple origins and in which genetic diversity is restricted at the intra-individual, intergenomic level, as this young dodecaploid species contains two well-differentiated hexaploid genomes in the same nucleus. How these parental genomes interact at the gene expression level remains an open question that will be explored in the near future. No extensive genetic changes have been encountered, but epigenetic modifications do occur. These are consistent with the important morphological plasticity encountered in natural populations. When comparing our results with those obtained for other young allopolyploids, it appears that each system may respond uniquely to hybridization and genome duplication. The differences elucidate the need for co-ordinating investigations on various groups through international research networks.

ACKNOWLEDGEMENTS

This work has benefited from the financial support of the UMR CNRS 6553, University of Rennes 1 (France), from CNRS–CSIRO (PICS 932), and CNRS–NSF (12578) grants. Jonathan Wendel (Iowa State University) is thanked for stimulating exchanges and helpful discussions on *Spartina*. Andrew Leitch, Doug Soltis and one anonymous reviewer are thanked for constructive comments and corrections on a previous version of this manuscript.

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