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ORIGINAL PAPER

Hybridization, polyploidy and invasion: lessons from *Spartina* (Poaceae)

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Abstract In this paper, we examine how the *Spartina* system has helped our understanding of the genomic aspects of allopolyploid speciation in the context of biological invasion. More specifically the respective roles of hybridization and genome duplication in the success of newly formed allopolyploid species are explored. Hybridization appears to have triggered genetic and epigenetic changes in the two recently formed European homoploid hybrids *S.* × *townsendii* and *S.* × *neyrautii*. Deviation from parental structural additivity is observed in both hybrids, with different patterns when considering transposable element insertions or AFLP and methylation alteration. No important changes are observed in the invasive allopolyploid *Spartina anglica* that inherited the identical genome to *S.* × *townsendii*. The repeated rRNA genes are not homogenized in the allopolyploid, and both parental repeats are expressed in the populations examined. Transcriptomic changes suggest possible gene silencing in both hybrids and allopolyploid. In the long-term of evolutionary time, older hexaploid *Spartina* species (*Spartina alterniflora*, *Spartina maritima* and *Spartina foliosa*) appear to have selectively retained differential homeologous copies of nuclear genes. *Waxy* gene genealogies suggest a hybrid (allopolyploid) origin of this hexaploid lineage of *Spartina*. Finally, nuclear and chloroplast DNA data indicate a reticulate origin (alloheptaploid) of the invasive *Spartina densiflora*. All together these studies stress hybridization as a primary stimulus in the invasive success of polyploid *Spartina* species.

Keywords *Spartina* · Allopolyploidy · Hybridization · Invasion · Genome evolution · Phylogeny

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Introduction

Invasive species are of primary interest in evolutionary ecology not only because they have important

impacts on ecosystems and biodiversity in our modern human-dominated environments, but because they illustrate a fundamental biological process, that is, the establishment and expansion of new populations in a short period of evolutionary time (Levine 2003; Sax et al. 2007). Invasive species of recent origin are ideal model systems to investigate the early evolutionary mechanisms associated with their ecological success by comparisons with closely related species.

In plants, newly formed polyploids and particularly those of hybrid origin (allopolyploids), are frequently invasive species (Brown and Marshall 1981; Barrett and Richardson 1986; Pandit et al. 2006). Neopolyploids provide the unique opportunity to compare a new species to its parents as the parent species are easier to identify than for older polyploids where the progenitors may have become extinct. Notable examples of new allopolyploid species that spread rapidly and exhibit larger ecological amplitude than the parents were formed during the last century, such as the allopolyploids *Tragopogon mirus* and *T. miscellus* (goatsbeard) in North America (Novak et al. 1991; Soltis et al. 2004), *Senecio cambrensis* (Welsh ragwort) and *S. eboracencis* in UK (Abbott and Lowe 2004), and *Cardamine schulzii* (bittercress) in central Europe (Urbanska et al. 1997). But one of the most striking examples is represented by the salt marsh grass *Spartina anglica* a model of recent allopolyploid speciation (Ainouche et al. 2004a), which has resulted in the spectacular expansion of a new polyploid species that has invaded several continents (Thompson 1991). It has been suggested that hybridization combined with polyploidy confers an immediate ecological aptitude to invade new habitats (Barrett and Richardson 1986), and recent insights from evolutionary genetics have greatly helped our understanding of the evolutionary processes affecting invasive plant species (Schierenbeck and Ainouche 2006).

Allopolyploidy results from the combination of two important evolutionary events: interspecific hybridization, which is the merger of two divergent (homeologous) nuclear genomes, and polyploidization where whole genomes are duplicated. The recent awareness that genome duplication is a much more widespread phenomenon than previously thought in Eukaryotes (e.g. Wolfe 2001; McLysaght et al. 2002; Cui et al. 2006), has generated a growing interest in the genomic consequences of polyploidy. During the last decade, various studies have revealed that these events have critical impact at the molecular, cellular and (organismal) phenotypic levels (reviewed in Comai 2000; Wendel 2000; Osborn et al. 2003; Riddle and Birchler 2003; Adams and Wendel 2005; Albertin et al. 2006; Chen 2007).

In this paper, we examine how the *Spartina* system has helped our understanding of the genomic aspects of allopolyploid speciation in the context of biological invasion, with particular focus on *S. anglica* as a particularly appropriate model (Ainouche et al. 2004a). More specifically the respective roles of hybridization and genome duplication in the success of newly formed allopolyploid species will be explored.

Spartina: a history of recurrent hybridization and polyploidization

Genus *Spartina* is comprised of about 13–15 species that are all perennial, and colonize coastal or inland salt marshes. Most species are native to the New-World, and until the nineteenth century, *Spartina maritima* was the only native species known in the Old-World, on the Euro-African Atlantic coast.

After some debate in the literature (e.g. Huskins 1930; Marchant 1963) concerning chromosome numbers of *Spartina*, Marchant (1968) was the first to establish that the basic chromosome number in *Spartina* is $x = 10$, as in most other Chloridoideae. The extant *Spartina* species either are tetraploid ($2n = 40$), hexaploid ($2n = 60$–62), or dodecaploid ($2n = 122, 124$), with possible aneuploidy (Marchant 1968). Polyploidy has played an important role in the genus as all species analysed to date are polyploids with no known diploid species. The numerous small chromosomes make accurate counts particularly difficult in *Spartina*, and most taxa need additional cytological investigations at the population level. The earliest comprehensive taxonomic study of the genus was performed by Mobberley (1956) who detected several cases of putative hybrid origin of species on the basis of morphology (e.g. *Spartina* × *caespitosa*, intermediate between the North-East American species *Spartina patens* and *Spartina pectinata*, or the South-East American *Spartina longispica* suspected to be hybrid between the East-American *Spartina alterniflora* and the South-American *Spartina densiflora*. But the most spectacular cases of hybridization

resulted from recent events following introduction of *Spartina* species outside their native range.

The hexaploid *S. alterniflora* ($2n = 62$) was introduced in the mid-1970s in the San Francisco Bay of California where it now co-occurs with native *S. foliosa* ($2n = 60$). *Spartina alterniflora* plants spread rapidly with greater tolerance of tidal submersion (Daehler and Strong 1997). Hybridization between the native and the introduced species has been shown to occur in both directions (Antilla et al. 2000), with *S. alterniflora* exhibiting greater male fitness (Antilla et al. 1998). Recurrent back-crosses and pollen swamping have resulted in hybrid swarms that display most frequently the chloroplast genome of *S. foliosa* and up to 90% nuclear markers specific to *S. alterniflora* (Ayres et al. 1999; Antilla et al. 2000). The fitness superiority (Ayres et al. 2007) and spread of the hybrids in California marshes (Ayres et al. 2004) is considered as a threat to the native *S. foliosa* populations (Ayres et al. 2003), and recent surveys have confirmed the great ecological impact of these hybrids is in the tidal marshes of the San Francisco Bay in California (Ayres et al. 2004). Other interspecific hybridization events have been further recorded: the austral cordgrass *S. densiflora*, native to South America, was also introduced to California, probably from Chile, during the late eighteenth century via solid ballast (Spicher and Josselyn 1985; Bortolus 2006). It is now well-established in Humboldt Bay where it is the dominant salt marsh plant (Kittelson and Boyd 1997). *Spartina densiflora*, first thought to be a variant of the native *S. foliosa*, was transplanted during the late 1970s to the San Francisco Bay for marsh restoration (Faber 2000) where it is now spreading (Ayres et al. 2004). Recently, hybrids between introduced *S. densiflora* and native *S. foliosa* individuals have been recorded in San Francisco Bay and this again may have important evolutionary impact in the Californian salt marshes (Ayres and Lee 2004; Ayres et al. 2008).

In Europe, *S. alterniflora* was accidentally introduced during the nineteenth century in southern England and western France, where it hybridized with the native *S. maritima* ($2n = 60$). In England, hybridization recorded in 1870 gave rise to *S.* × *townsendii*, a perennial sterile hybrid (Groves and Groves 1880). Another sterile hybrid between *S. alterniflora* and *S. maritima* was discovered in 1892 in southwest-France in the Bidassoa estuary (Foucaud 1897), and named *Spartina* × *neyrautii* (Jovet 1941). According to their different morphology, some authors suggested that *S.* × *neyrautii* and *S.* × *townsendii* might result from reciprocal crosses; however, molecular data revealed that both hybrids share the same chloroplast genome of *S. alterniflora* that was then the maternal parent to both hybrids (Baumel et al. 2003).

After 1890 in England, fertile plants were recorded that appeared to have resulted from chromosome doubling in *S.* × *townsendii* (Marchant 1963), thus leading to the formation of a new allopolyploid species named *S. anglica* (Hubbard 1968). The vigorous plants have rapidly colonized the British (Raybould et al. 1991a; Thompson 1991) and French (Guénégou and Levasseur 1993) salt marshes and estuaries, via natural dispersal of seeds and rhizomes by tidal flow, currents and sea birds. *Spartina anglica* has a large ecological amplitude along the successional marsh gradient, tolerating several hours of immersion at high tides, and thus is able to occupy a vacant niche as a pioneer species in the low tide zone. The robust shoots, rhizomes and root system enable this species able to accumulate large volumes of tidal sediments. This causes a rise of the sediments and makes the habitat more terrestrial, allowing colonization by other salt marsh plant species. By modifying the physical structure of intertidal coastal zones, and altering salt-marsh dynamics, *S. anglica* is considered an "ecosystem-engineer" (sensu, Jones et al. 1994; Boumat et al. 2005). After a variable period of rapid expansion, *S. anglica* populations experience "dieback" in older colonized sites, which is probably caused by age-related decline of vigour or by competition with other species after niche elevation (Gray 2004; An et al. 2004).

Spartina anglica was deliberately introduced in several parts of the world (North Europe, Australia, New Zealand, China, North America) for land reclamation and marsh restoration purposes. Over 175,000 plant fragments were brought by ship from Poole harbour around the world between 1924 and 1936 (Hubbard 1965). *Spartina anglica* has rapidly expanded in its introduced range, and has now a worldwide distribution. The rapid spread of the introduced populations has lead to various attempts to control or eradicate the species via either physical removal or chemical treatments (Cornette et al. 2001; Hacker et al. 2001; Hammond and Cooper 2002;

Hedge et al. 2004; Cottet et al. 2007), and *Spartina anglica* is now listed among the 100 "World's worst" invaders (IUCN 2000). Its expansion has increasingly fascinated the ecological research community, as illustrated at the three international invasive *Spartina* conferences held in 1990 (Seattle, WA, USA), 1997 (Olympia, WA, USA) and 2004 (San-Francisco CA, USA). In the abundant literature on the dramatic ecological changes brought about by *S. anglica*, however, there is no clear consensus about the respective beneficial or negative impacts of this new species (Doody 1990). *Spartina anglica* is considered a threat to salt marsh conservation by displacing native species and by changing water circulation patterns after sediment accretion (Gray et al. 1997). It may have negative economic impact by modifying oyster fishery sites and tourist activity zones. In contrast, positive effects include coastal protection and stabilization, formation of nesting area for various seabird species, increased diversity of epibenthic species (Doody 1990), sediment oxygenation and detoxification, and in phytoremediation (Lee 2003).

Explanations for the invasive success of *S. anglica* have been long debated (e.g. Thompson 1991), and this question is of particular interest in the context of global climatic change, especially in the northern boundary of its geographical range (Loebl et al. 2006). Phenotypic plasticity in this species is considered as a feature that may have significantly influenced spread and persistence of the populations in the varying environment of salt-marsh succession (Thompson et al. 1991a, b). Physiological traits of *S. anglica* include a C4-type metabolism with unusual rates of photosynthesis at relatively low temperatures compared to other C4 species, allowing both tolerance to salinity and colonization of cool temperate climatic regions (Long et al. 1975). Survival of *S. anglica* in anoxic sediments is facilitated by its particular ability to develop aerenchyma systems that supply the submerged plants with athmospheric oxygen and efficiently transport oxygen to the roots (Maricle and Lee 2002). *Spartina anglica* displays enhanced mechanisms to transport O_2 and exhibits five time higher H_2S removal than its progenitor species *S. alterniflora* (Lee 2003). The young allopolyploid has tolerance to highly reducing and sulfidic sediments conditions, which may explain its ability to colonize successfully low-marsh zones (Maricle et al. 2006).

The various biological (morpho-anatomical, physiological) traits that make *S. anglica* so successful have, with no doubt, evolved with the allopolyploidy event, which has focused our attention on the genomic determinants of invasiveness in a context of hybridization and genome duplication. It should be stressed that the consequences of these important mechanisms may vary in nature and intensity at different evolutionary timescales during the history of a species (Wendel 2000). By its recurrent history of hybridization and polyploidization (Ainouche et al. 2004b), the genus *Spartina* offers particular opportunities to explore the fate of allopolyploid genomes over short and long-term of the evolutionary time.

A window into the birth of an invasive hybrid species: the "Genome shock" hypothesis in nascent allopolyploid *Spartina*

The hybrids and new allopolyploid that have formed in western Europe provide a unique opportunity to analyze the immediate consequences of hybridization and polyploidy in natural populations and to compare the new species to its extant progenitors. The available records concerning the timing of these events and the progression of the invasive populations along the coast of various continents provide an invaluable historical context for evolutionary genetic studies. The first molecular evidence for the hybrid origin of *S. anglica* came from the analysis of isozyme loci that exhibited additive allelic composition of the patterns observed for *S. maritima* and *S. alterniflora* and fixed heterozygosity (Guénégou et al. 1988; Gray et al. 1990). Narrow allozyme diversity at the sampled British sites in both the parental species and the allopolyploid was also encountered (Raybould et al. 1991a, b). Various DNA marker analyses revealed that the allopolyploid displays the same multilocus genotype as the British F1 hybrid *S.* × *townsendii*, and lacks interindividual genetic diversity. The same "major genotype" (Baumel 2001; Baumel et al. 2001, 2002a; Ainouche et al. 2004c) was predominantly encountered in British, French and Australian populations, although some variants were recorded by Ayres and Strong (2001), that resulted from "maritima-type" RAPD or ISSR fragment loss. All the individuals examined in the populations of *S. anglica* from England (Ferris et al. 1997) and France (Baumel et al. 2001, 2003)

exhibited the same chloroplast sequences as the F1 hybrids *S.* × *townsendii* and *S.* × *neyrautii* and as *S. alterniflora*, which was therefore deduced to be the maternal genome donor in both hybridization events (England and France). The presence of only one chlorotype (from *S. alterniflora*) together with the lack of interindividual variation in *S. anglica* reinforce the "unique origin" hypothesis of the allopolyploid; as the parental species *S. maritima* and *S. alterniflora* lack variation in the region where they hybridized (Raybould et al. 1991b; Baumel et al. 2003; Yannic et al. 2004), any recurrent hybridization event would have involved very similar parental genotype. In any case, *S. anglica* seems to have experienced a strong genetic bottleneck at the time of its formation in southern England (Ainouche et al. 2004a), which contrasts with the multiple origins reported in several recent allopolyploids (e.g. *Tragopogon*) where recurrent and bidirectional hybridisation events involving different parental genotypes resulted in increased genetic variation of the new species (Soltis and Soltis 1999). Consequently, genetic diversity in *S. anglica* relies primarily on the subsequent dynamics of its hybrid genome.

Although the parental species *S. maritima* and *S. alterniflora* derive from a common hexaploid ancestor, their nuclear sequences display consistent nucleotide changes (Baumel et al. 2002b). Thus, two divergent genomes are reunited in the same nucleus of the sterile hybrids *S.* × *neyrautii*, *S.* × *townsendii* and the fertile allopolyploid *S. anglica.* The merger of differentiated genomes after interspecific hybridization was early described as a "genomic shock" (McClintock 1984) entailing profound instabilities and restructuring of the genomes; these have recently attracted much attention in the context of allopolyploidy (Comai et al. 2003; Riddle and Birchler 2003; Adams and Wendel 2005; Chen and Ni 2006). Experimentally resynthesized polyploids have revealed that hybridization and genome duplication may be followed by immediate and extensive structural changes, evidenced in *Brassica* (Song et al. 1995), *Triticum* (Liu et al. 1998) and *Nicotiana* (Lim et al. 2007). Various mechanisms appeared to be involved, such as DNA fragment elimination associated with transposable element activation (Levy and Feldman 2004; Chantret et al. 2005), homeologous recombination (Nicolas et al. 2007), intergenomic translocations (Lim et al. 2004) or replacement of rDNA units with novel derivatives (Kovarik et al. 2004). Rapid epigenetic and expression alterations are expected in allopolyploids (Riddle and Birchler 2003; Osborn et al. 2003; Liu and Wendel 2003; Chen 2007). Preferential restructuring was observed in the paternal genome of synthetic *Nicotiana* allopolyploids, which was in accordance with changes observed during natural evolution of tobacco (Skalická et al. 2005). In contrast, structural "stasis" was found in synthetic allopolyploid *Gossypium* (Liu et al. 2001) and natural young *Tragopogon* allopolyploids (Pires et al. 2004), indicating that different systems may respond variously to allopolyploidy.

DNA fragment additivity and elimination

The genomes of the recent hybrids and allopolyploid of *Spartina* were found mostly additive (93.5%) with respect to parents when investigated using multilocus RAPD, ISSR, IRAP and REMAP markers (Baumel et al. 2001, 2002a; Ainouche et al. 2004a). In multigene families that may undergo rapid concerted evolution such as rDNA genes, both parental repeat types were found in most populations of *S. anglica* investigated to date in England and in France (Fig. 1a). Diagnostic restriction sites discriminate the internal transcribed spacer (ITS) regions of *S. maritima* and *S. alterniflora* sequences and allow rapid population screening and check for additive patterns in the allopolyploid. However, one individual sampled in Brittany displayed only a "maritima-type" repeat (Fig. 1b), suggesting that variable levels of concerted evolution may occur in some populations. This hypothesis should be explored further, together with the potential variation of the respective parental repeats as encountered in the young populations of the allopolyploids *T. mirus* and *T. miscellus* (Kovarik et al. 2005).

Genome-wide AFLP analyses were performed (Salmon et al. 2005) in order to explore the immediate genetic consequences of hybridization and polyploidisation in *S. alterniflora* and *S. maritima* derivative species (*S.* × *neyrautii*, *S.* × *townsendii* and *S. anglica*). The parental species displayed consistent interspecific polymorphism that allowed examination of departures from additivity at multiple loci in the hybrids and the allopolyploid. About 10% of parental AFLP fragments were absent in the F1

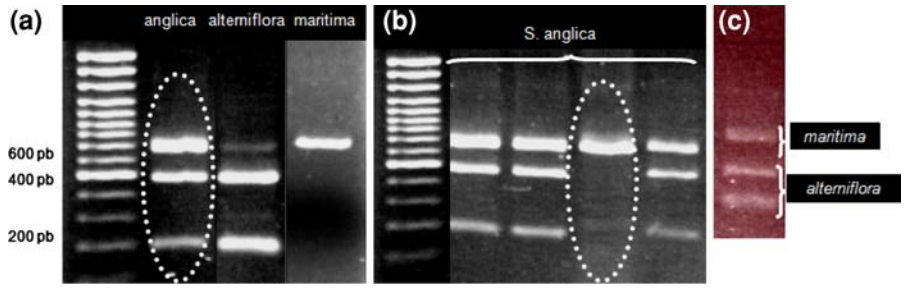

Fig. 1 Restriction patterns of the *ITS* region of nrDNA (digested with *Msp*I). **a** Additivity of the parental patterns in *S. anglica*. **b** Loss of *alterniflora*-type repeats in one individual of *S. anglica* sampled in Brittany (Anse de Poulguin, Finistère). **c** Restriction patterns on cDNA from leaves of *S. anglica*

hybrids, most originating from the maternal parent, *S. alterniflora.* Although the two hybrids exhibited some differences, most of the missing DNA fragments (60%) were common, which suggests that the same genetic changes occurred in the two independently formed hybrids from England and from France. Principal component analysis (PCA) of the AFLP data (Fig. 2a) illustrates the genomic divergence of the parental species, and departure from the parental additivity of the hybrids that display very similar changes. In contrast, genome doubling in *S. anglica* did not alter further the genome structure inherited from *S.* × *townsendii* (Fig. 2a).

Transposable element activation

As mentioned above, transposable elements have been shown to be involved in hybrid genome restructuring. The screening of insertional polymorphisms in the populations of *S. anglica* using IRAP and REMAP markers initially developed on barley (Kalendar et al. 1999) did not detect significant bursts of transposition (Baumel et al. 2002a). Sequence specific amplified polymorphism (SSAP) profiles of transposable elements have been generated in *Spartina*, according to a retrotransposon-anchored PCR strategy. This AFLP-derived method was successfully employed to detect multiple insertional events in allopolyploids (Petit et al. 2007). By using a primer designed in the retroelement Cassandra previously characterised in *S. alterniflora* (AY603377), insertional polymorphism was explored in *Spartina*. Consistent alterations were encountered between the parental species, the hybrids and the allopolyploid (Table 1). *Spartina maritima* presented a higher number of SSAP fragments than *S. alterniflora*, and most of the SSAP was observed between the two parental species. Interestingly, a higher amount of species-specific Cassandra insertions is detected in *S. maritima*, suggesting that this species could be more permissive than *S. alterniflora* to Cassandra amplification. However, no burst of transposition was detectable after hybridization or genome doubling as suggested by the few new SSAP bands encountered in the hybrids and the allopolyploid (Table 1). Most of the changes are band losses that have originated during the successful merging of differentiated genomes by hybridization. Several SSAP bands of *S. alterniflora* (maternal) origin were eliminated in both independently formed hybrids, albeit the loss was more pronounced in *S.* × *townsendii*. In contrast, loss of SSAP bands of *S. maritima* (paternal) origin was much more pronounced in *S.* × *neyrautii*, and as a result, SSAP patterns of *S.* × *neyrautii* and *S.* × *townsendii* were rather different, as illustrated by the PCA analysis of the SSAP fragments (Fig. 2b). *S. anglica* is very close to its progenitor *S.* × *townsendii*, indicating that genome doubling did not trigger major alteration of Cassandra's insertions.

SSAP and AFLP comparison

SSAP and AFLP patterns were compared by a Co-inertia analysis (Dolédec and Chessel 1994) here, significantly maximising the joint structure between PCA upon AFLPs and SSAP, respectively (Fig. 2c). Since the Co-inertia analysis between AFLP and SSAP data resulted in significant association, the dynamics of the transposable element Cassandra and the whole genome appear non-independent. Although

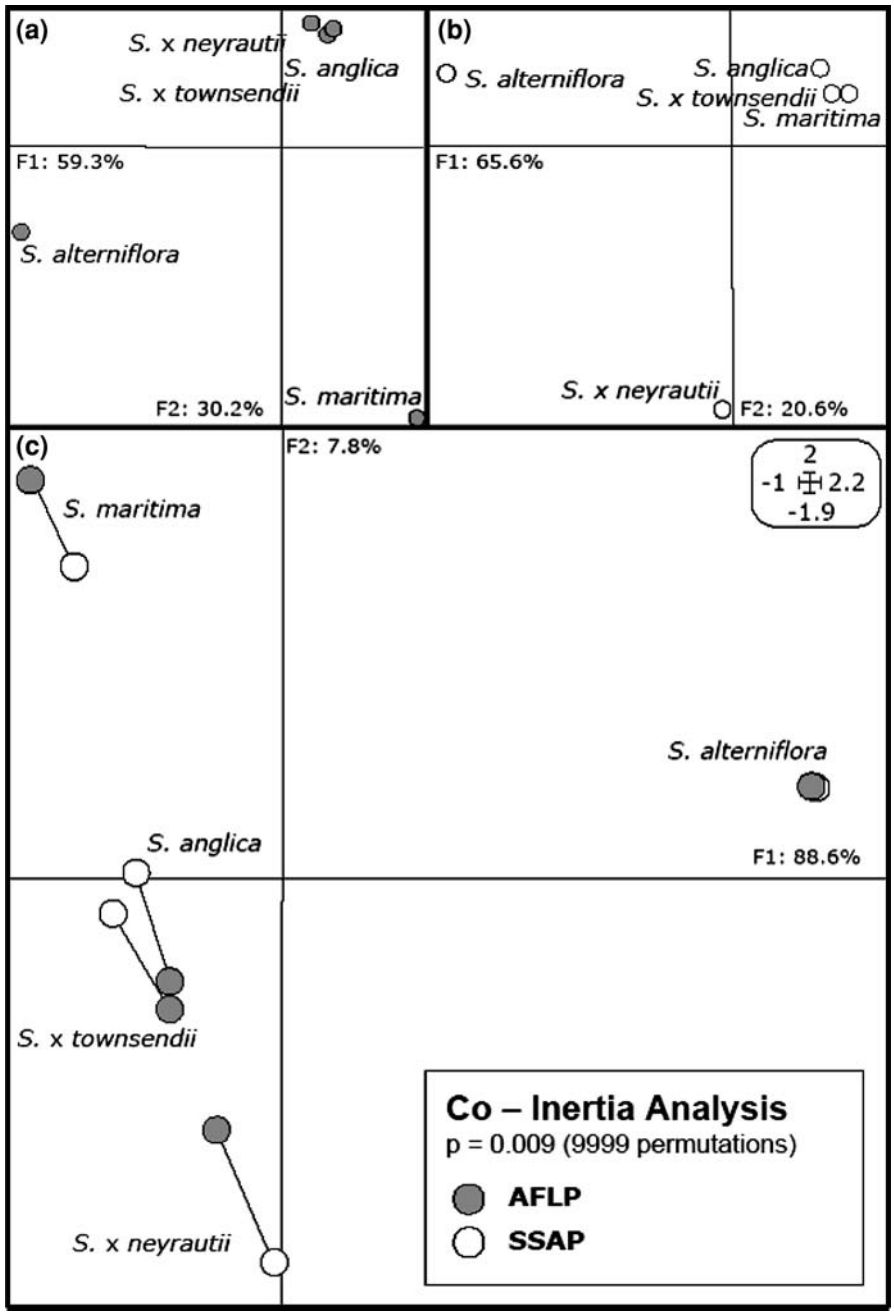

Fig. 2 Ordinations of the genome-wide markers characterised in the parental taxa of *S. anglica*. Amplified fragment length polymorphism (*AFLP*) profiles of the taxa are presented as *open circles* and sequence specific amplified polymorphism (SSA) profiles of the transposable element Cassandra are presented as *grey circles*. **a** Principal component analysis (PCA) on covariance matrix among the AFLP profiles. The first and the second axes of the ordination summarise 59.3 and 30.2% (respectively) of the variance contained in the dataset. **b** PCA on covariance matrix among the SSAP profiles of Cassandra. The first and the second axes of the ordination summarise 65.6 and 20.6% (respectively) of the variance contained in the dataset. **c** Co-inertia analysis maximising the joint structure of the two PCA presented in (**a**) and (**b**) in a *single reduced space*. Significance of the association has been tested by 9,999 Monte–Carlo permutations

AFLP showed that *S.* × *neyrautii*, *S.* × *townsendii* and *S. anglica* present globally similar genomes, SSAP data based on the Cassandra elements suggest that the repetitive sequences of *S.* × *townsendii* and the allopolyploid altered the genome in a way that mainly resembles the paternal parent (*S. maritima*). In contrast, repetitive sequences of *S.* × *neyrautii* were apparently not altered in this way, with significant losses from both parental genomes. This pattern indicates that Cassandra had different dynamics in these two hybrids that were derived independently from the same maternal and paternal species. Interestingly, this pattern agrees with the pronounced morphological differences between the hybrids. Given that most of the molecular changes affected the maternal genome, especially in *S.* × *townsendii*,

Table 1 Origin and dynamics of the SSAP bands (Cassandra) in the recent *Spartina* F1 hybrids and allopolyploid

Origin of the SSAP band	SSAP bands observed in				
	Parents	Hybrids			Allopolyploid
		Both hybrids	*S.* × *neyrautii*	*S.* × *townsendii*	*S. anglica*
S. alterniflora	34	8	13	2	9
S. maritima	78	59	3	16	75
Both parents	97	93	1	3	96
New band	–	3	1	1	0
Hybrid	–	–	–	–	3

massive loss of AFLP and SSAP loci is unlikely to be explained by nucleo–cytoplasmic interactions (Tiffin et al. 2001).

It is tempting to fit these observations into a model of differential genome dosage (Dilkes and Comai 2004; Josefsson et al. 2006). No clear transposition of Cassandra was detected in *Spartina* hybrids, however, it can be speculated that merging two parental genomes with rather different transpositional permissivities through hybridization would have created an unbalanced situation, in which potential amplifications would have led to deleterious genomic situations and survival of the hybrid genome would have been insured via restructurings reducing the potential activity of Cassandra elements, especially of *S. alterniflora* origin. This hypothesis could perhaps be further reinforced by the observation that the hybrid *S.* × *townsendii* (that exhibits more similar SSAP patterns to the paternal parent than *S.* × *neyrautii*) maintains a large number of vigorous individuals in England and produced a fertile allopolyploid derivative, while *S.* × *neyrautii*, which was unable to eliminate most of the *S. alterniflora* maternal elements, is declining in the Basque region of France after facing anthropogenic habitat modification (Hubbard et al. 1978; Baumel et al. 2003).

Epigenetic and expression alterations

Epigenetic changes were investigated using methylation-sensitive AFLP (MSAP) in *Spartina* (Salmon et al. 2005). Only 52% parental additivity was encountered in the hybrids, with more than 34% of methylation changes shared by *S.* × *neyrautii* and *S.* × *townsendii*, which suggested the reproducibility of the changes in the two natural hybridization events. Interestingly, most of the methylation changes that are observed in the allopolyploid *S. anglica* were also already present in the F1 hybrid *townsendii* (30%). About 10% novel methylation alterations that were initiated in the hybrid were also observed in the allopolyploid. Thus, epigenetic changes were triggered by hybridization rather than by genome duplication during the allopolyploidization process.

Epigenetic phenomena play important roles in the regulation of gene expression and have been shown to be involved in dramatic rapid phenotypic changes in synthetic allopolyploids (reviewed in Osborn et al. 2003). The important methylation alterations, contrasting with the relative structural additivity encountered in *S. anglica* could account for the important morphological plasticity reported for this species. Epigenetic changes that are potentially reversible provide a flexible way to respond to various environmental or genomic stresses (Chen 2007). Silencing of homoeologous rRNA genes, for example, led to the well-known "nucleolar dominance" phenomenon where epigenetic processes may interact with intergenomic homogenization in allopolyploids (Dadejová et al. 2007). In *S. anglica*, most of the individuals examined to date that exhibit both parental repeat-types of rRNA genes display also both parental transcripts from *S. maritima* and *S. alterniflora* (Fig. 1c). Joly et al. (2004) found preferential expression of rRNA homeologues in natural *Glycine* allopolyploids, but this bias was absent in synthetic polyploids and F1 hybrids. Global expression patterns are currently being examined in *Spartina*, and preliminary screening using cDNA AFLP indicate that both hybridization (in *S.* × *townsendii*) and genome duplication (in *S. anglica*) entail

transcriptional changes resulting from parental cDNA band loss (Ainouche et al. 2004c). Although these fragment losses could be partly related to genomic changes as found by Tate et al. (2006) in recent *Tragopogon* allopolyploids, they also may reveal occurrence of gene silencing in the *Spartina* hybrid and allopolyploid. Gene silencing, unequal homeologous gene expression, and subfunctionalization, where a gene acquires different expression patterns, were demonstrated in both synthetic and natural allopolyploid cotton, which indicates that such immediate functional plasticity may operate over long periods of evolutionary time (Adams et al. 2003).

Genome evolution in "older" polyploid *Spartina*: gene histories in a reticulate context

Polyploidization entails the immediate duplication of all genes, thus resulting in global functional redundancy that increases the structural and metabolic plasticity of the newly formed genome. As the extant species of *Spartina* present different ploidy levels, they offer the opportunity to explore the fate of duplicated genes (homeologues) in the long-term perspective. Homeologues may undergo various evolutionary fates (e.g. see Adams and Wendel 2004 for a review). At the structural level, the duplicated loci may be either retained or lost (Ohno 1970; Lynch and Force 2000; Lynch and Conery 2000). Highly repeated regions may undergo concerted evolution following interlocus homogenization towards one of the parental repeats (e.g. rDNA genes, Wendel et al. 1995). As this phenomenon is progressive, this homogenization can be partial in a given polyploid species (Alvarez and Wendel 2003; Rauscher et al. 2004). This dynamic is an important parameter to consider when reconstructing polyploid species history using phylogenetic approaches. Moreover, distinguishing orthologous copies (diverging after dichotomic speciation), homeologous copies (i.e. orthologues from different species reunited in the same nucleus after allopolyploidization) and paralogous copies (diverging after individual gene duplication) is of critical importance in nuclear genes (Small et al. 2004). In phylogeny, tree topologies may be used to infer gene history versus organismal (species) history in a given historical framework.

The first phylogenetic study in *Spartina* performed by Baumel et al. (2002b) was based on nuclear (*Waxy*, *ITS*) and chloroplast (*trnT–trnL*) genes. By comparing the topologies obtained with the different data sets this study revealed that genus *Spartina* was split in two major lineages. The first one included all the tetraploid species from the new world except *S. argentinensis* which was placed in the second lineage as sister to a sub-clade containing the three hexaploid species *S. maritima*, *S. foliosa* and *S. alterniflora*. The molecular divergence between these species was consistent with their hybridization patterns (Ainouche et al. 2004b), i.e. fertile hybrids and back-crossing followed hybridization between weakly divergent sister species (*S. foliosa* and *S. alterniflora*), and sterile F1 hybrids resulted from hybridization between more divergent species (*S. maritima* and *S. foliosa*).

These studies indicate that the hexaploid *Spartina* share a common hexaploid ancestor and provide a historical framework for further investigations. For instance, how many gene copies have the tetraploid and hexaploid *Spartina* species retained over their evolutionary history? For repetitive rDNA genes, no sequence heterogeneity was encountered in hexaploid and tetraploid *Spartina* species by Baumel et al. (2002a), suggesting that these species are old enough to have completed homogenization through concerted evolution, thus contrasting with the presence of both parental repeats in the recent allopolyploid *S. anglica.*

The evolutionary dynamics of the low-copy number gene *Waxy* was investigated in genus *Spartina* (Fortuné et al. 2007). Extensive molecular cloning and analyses of gene tree topologies revealed a more complicated situation than previously thought: the *Waxy* gene underwent additional gene duplication prior to formation of genus *Spartina*, leading to the existence of two paralogs. It is not clear yet whether these paralogs are a consequence of a paleoduplication in the Poaceae (Paterson et al. 2004) or instead resulted from independent individual gene duplication in the Chloridoideae subfamily. With respect to these paralogs, six duplicated *Waxy* copies are expected in a hexaploid species and four duplicated copies in a tetraploid species. However, from one to three copies and one to two copies were encountered in the hexaploids and tetraploids, respectively. The *Waxy* gene displayed differential pattern or gene retention and loss among the lineages (i.e. different copies were retained in the different species). Copy-number was not correlated with the ploidy level as

some tetraploid species such as *S. patens* displayed two copies of *Waxy* whereas only one copy was found in the hexaploid *S. maritima*. All the retained copies were selectively constrained as suggested by the relative rates of synonymous and non-synonymous substitutions in the coding portions of the gene (Fortuné et al. 2007). These patterns well illustrate the dynamics of nuclear gene duplication and loss, as observed for ADH genes in polyploid cotton (Small and Wendel 2002).

The *Waxy* gene trees also provided new insights on older, reticulate events that have contributed to the formation of polyploid *Spartina* species (Fortuné et al. 2007, 2008). The gene tree topologies allowed the identification of three divergent homoeologous copies that have contributed to the hexaploid lineage of *Spartina*, suggesting that the hexaploid species *S. foliosa*, *S. alterniflora* and *S. maritima* have a hybrid (allopolyploid) origin (Fig. 3). Another species, *S. densiflora*, displayed conflicting phylogenetic patterns among different nuclear and chloroplast data sets (Baumel et al. 2002b). This conflict was interpreted as resulting from either paralogous sampling in a polyploid species or from a possible consequence of reticulate events where the analysed sample of *S. densiflora* was collected (California). The possibility of hybridization between *S. densiflora* and hexaploid species was confirmed recently by the occurrence of hybrids recorded between native *S. foliosa* and introduced *S. densiflora* in California (Ayres and Lee 2004) as mentioned above. However, extensive molecular analyses (Fortuné et al. 2008) from various *S. densiflora* samples collected in both introduced populations from California and South-America (Chile and Argentina where the species is native) indicated that all the populations investigated exhibited almost identical chloroplast and *ITS* sequences which were very similar to those of the tetraploid *S. arundinacea* (native to subantarctic islands). Three different copies were encountered for the nuclear

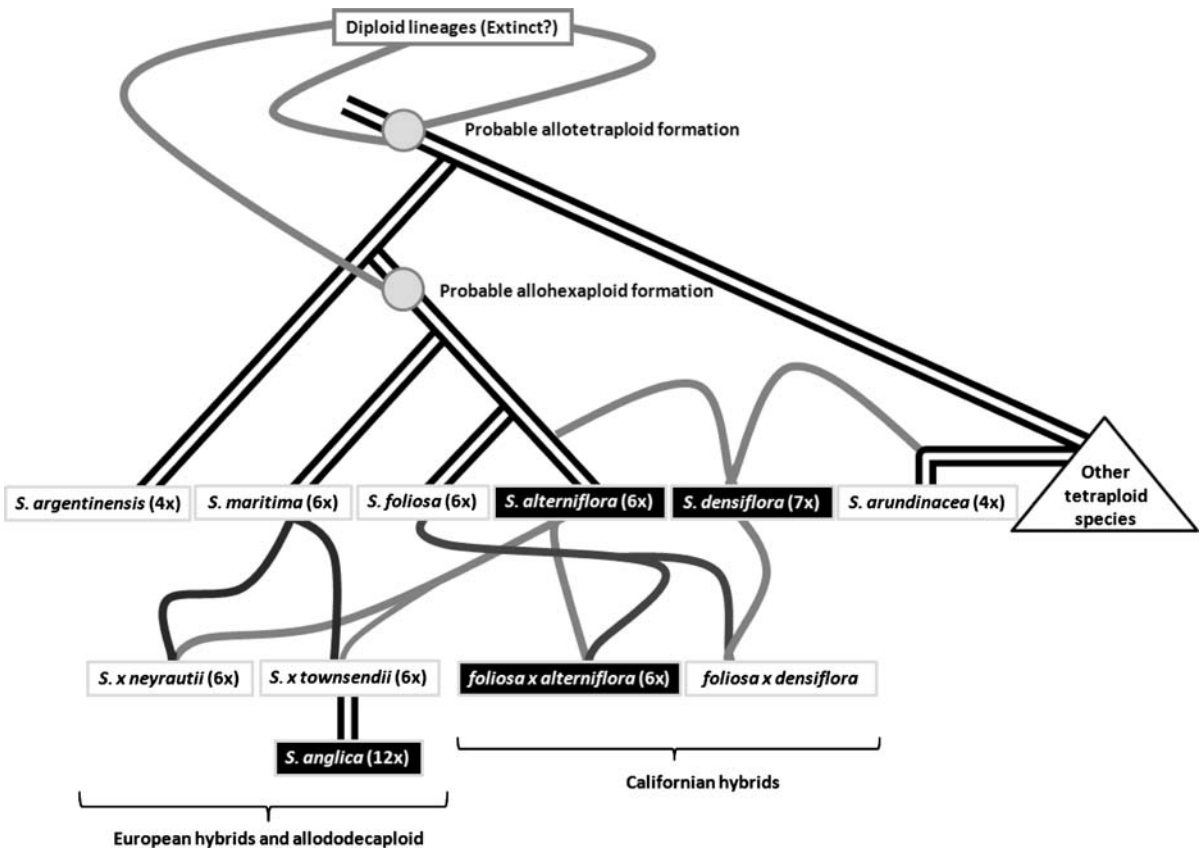

Fig. 3 Divergent (*straight lines*) and reticulate (*curved lines*) phylogenetic relationships in genus *Spartina*. *Filled boxes* indicate notorious invasive species

Waxy gene: one copy is sister to a copy found in *S. arundinacea* and the two others are sister to copies of the hexaploid species (*S. alterniflora*, *S. maritima*), confirming the hybrid origin between the two lineages. Chromosomes counts and genome size measurements using flow cytometry analyses (Ayres et al. 2008; Fortuné et al. 2008) revealed that this species has $2n = 70$ chromosomes in both native and introduced populations. Consequently, *S. densiflora* appears to be alloheptaploid resulting from hybridization between a tetraploid species related to *S. arundinacea* and a hexaploid species probably related to *S. alterniflora* that is native to the eastern coast and occurs in South-America (Fig. 3). *S. densiflora* is of particular interest as it is also known to be very invasive in the regions where it has been introduced (e.g. California and Spain), with a large ecological range (Mobberley 1956; Nieva 1996; Kittelson and Boyd 1997; Clifford 2002; Nieva et al. 2002; Vicari et al. 2002; Bortolus 2006). Hybridization in this case appears to have involved even distant lineages in *Spartina* (Fig. 3).

Summary and conclusions

In conclusion, what we have learned from the *Spartina* system is that this genus has a deeper reticulate history than expected. Hybridization represents the primary stimulus in the evolutionary dynamics of newly formed allopolyploid genomes. The most important changes have been found to be triggered by hybridization, rather than by genome duplication in the young invasive *S. anglica*. These changes entail (a) parental genomic fragment loss that may affect differentially various parts of the genome in the two independently formed F1 hybrids *S.* × *townsendii* and *S.* × *neyrautii* and, (b) consistent epigenetic alterations shared by the two F1 hybrids. Redundancy and gene dosage related effects are likely to affect gene expression in the neo-dodecaploid *S. anglica* that has duplicated the *S.* × *townsendii* hybrid genome. Current analyses are underway, aiming to explore the impact of allopolyploidization on ecologically relevant genes in invasive *Spartina*. Now that molecular tools and approaches allow exploring the deepest history of the *Spartina* lineages, it becomes even more evident that the recurrent merging of differentiated genomes through hybridization, combined with polyploidy had critical impact on the evolutionary success of invasive *Spartina* species.

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