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Hybridization, polyploidy and speciation in *Spartina* (Poaceae)

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Summary

Hybridization and polyploidy are well illustrated in the genus *Spartina*. This paper examines how recent molecular approaches have helped our understanding of the past and recent reticulate history of species, with special focus on allopolyploid speciation. *Spartina* species are tetraploid, hexaploid or dodecaploid perennials, most of them being native to the New World. The molecular phylogeny indicates an ancient split between the tetraploid and the hexaploid species, with *S. argentinensis* as sister to the hexaploid lineage. Recent hybridization and polyploidization events involved hexaploid species, resulting from introductions of the east-American *S. alterniflora*. In California, ongoing hybridizations with its sister species *S. foliosa* result in introgressant hybrid swarms. In Europe, hybridization with *S. maritima* resulted in *S.* × *neyrautii* (France) and *S.* × *townsendii* (England), with. *S. alterniflora* as the maternal parent. The allopolyploid *S. anglica* resulted from chromosome doubling of *S.* × *townsendii*. This young allopolyploid contains divergent homoeologous subgenomes that have not undergone significant changes since their reunion. Hybridization, rather than genome duplication, appears to have shaped the allopolyploid genome at both the structural and epigenetic levels.

Introduction

Hybridization and polyploidy have long been recognized as major phenomenons promoting genetic diversity (e.g. via introgression) and speciation (at the homoploid or allopolyploid levels) in plants (Stebbins, 1950; Grant, 1971; Rieseberg, 1997). One of the most conspicuous contributions of the recent development of molecular markers and genomic approaches to our understanding of the speciation process is the awareness that reticulate evolution is even more frequent than previously thought. The combined use of maternally inherited cytoplasmic markers with those biparentally inherited from the nuclear genome has allowed a more precise parentage identification in many plant groups, with the possibility to detect multiple and recurrent origins of species (Soltis *et al.*, 1992) and to get a more accurate picture of species formation via introgression (Rieseberg & Brunsfeld, 1992). Phylogenetic approaches and comparative analysis of gene trees have revealed the reticulate history of various species (Wendel & Doyle, 1998; Cronn *et al.*, 2003; Doyle *et al.*, 2004). Finally, recent approaches have contributed to explore the genomic consequences (at both the structural and

functional levels) of allopolyploid speciation (Wendel, 2000; Levy & Feldman, 2002; Liu & Wendel, 2002; Osborn *et al.*, 2003; Soltis & Soltis, 2004).

In this paper, we examine how these approaches have helped our understanding of speciation in the grass genus *Spartina* Schreb., where hybridization and polyploidy represent past and ongoing important evolutionary forces.

Evolutionary history of the genus *Spartina*

Ploidy levels, distribution and morphological affinities of the *Spartina* species

The genus *Spartina* represents a well-supported monophyletic lineage in the subfamily Chloridoideae (Hsiao *et al.*, 1999), with most species originating from the New World. Polyploidy has played an important role in the genus, as all species analysed to date are polyploids, with no known diploid species. After a period of uncertainty concerning chromosome numbers of *Spartina*, Marchant (1968) was the first to establish that the basic chomosome number in *Spartina* is × = 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). The numerous small chromosomes make accurate counts particularly difficult in *Spartina*, and most American taxa definitely need additional cytological investigations at the population level.

The *Spartina* genus is composed of perennial plants, which are C4 – photosynthesis species as most Chloridoids. This makes the plants able to tolerate a large range of climatic conditions in both hemispheres. Most species are salt-tolerant and colonize coastal or inland saltmarshes. The most comprehensive taxonomic study of the genus was performed almost 50 yr ago (Mobberley, 1956) and in spite of the limited number of recognized species (14 not including hybrids, see below) a number of taxonomic problems definitely need further exploration with modern approaches. Mobberley (1956) delineated three complexes of species on the basis of morphology. The first one is composed of *Spartina arundinacea*, *Spartina ciliata* and *Spartina spartinae* (syn. *Spartina argentinensis*) which are characterized by a smooth and cylindrical panicle with numerous short and closely imbricate spikelets and which display usually hard and slender culms. *Spartina arundinacea* occurs on two distant island groups in the South Atlantic and Indian Oceans (Tristan de Cunha, Saint Paul, Amsterdam islands). *Spartina ciliata* is distributed along the east coast of South America, from Brazil to Argentina). *Spartina spartinae* occurs in two disjunct areas in North – Central America and in South-America.

The second morphological complex contains species characterized by fleshy and succulent culms, spikelets less closely imbricate than those in the other complexes. This group includes the three known hexaploid species in the genus; *Spartina foliosa*, a species limited to the Pacific coast of North America; *Spartina maritima*, the only Old World species if we except recent taxa of hybrid origin (see below); and *Spartina alterniflora*, a variable species that is distributed along the east coast of North and South America. This species has been introduced into North-western United States, and Western Europe, where it hybridized with indigenous species (see below). Mobberley (1956) placed in the same morphological complex *Spartina longispica*, a taxon that is limited to the river Plate in both Argentina and Uruguay, and of putative hybrid origin between *S. alterniflora* and *Spartina densiflora* (third complex).

The third morphological complex includes all remaining (seven) species that display hard culms, spreading spikes with closely imbricate spikelets. *Spartina bakeri* and *Spartina patens* are two morphologically similar species. *Spartina bakeri* is found in Florida and Georgia. Its particular vegetative habit, lacking rhizomes, and its tolerance to fresh water usually distinguish it. *Spartina patens* (syn. *Spartina juncea*; *Spartina versicolor*) is a polymorphic species distributed along the east North-American coast, from Canada to the Caribbean and Central America. *Spartina pectinata* is another variable species that has a wide range in North America (Canada and United States) from the eastern coast to inland marshes of the middle–west, to Alberta. *Spartina* × *caespitosa* had a controversial status, displaying features that relate to both *S. patens* and *S. pectinata*. After careful analysis of the morphological variability and hybrid index calculations, Mobberley (1956) confirmed the hybrid status of this species. *Spartina cynusoroides* and *S. gracilis* are two uniform species. The former is distributed along the eastern North-American coast, from Massachusetts to the Gulf of Mexico. *Spartina gracilis* has a wide distribution from Mexico to Canada where it occurs in plains and mountains regions at altitudes from sea level to 7200 feet. *Spartina densiflora* is a variable species with large a distribution in southern America, on both the East coast of Brazil and Argentina and the West coast of Chile. It is also encountered in California where it was introduced during the 19th century. Mobberley (1956) noticed that in the northern portion of the east coast range, plants have fewer and longer spikes than those from the southern portion and from Chile.

Molecular phylogeny and reticulate events

A molecular phylogeny of the *Spartina* genus has been recently performed using nuclear (*Waxy* and *ITS*) and chloroplast (*Trn*L-*Trn*T spacer) DNA sequences (Baumel *et al.*, 2002a). This analysis has revealed that the *Spartina* genus evolved in two main lineages ('clade I' and 'clade II'). The first one is composed of all hexaploid species that belong to the morphological Complex 2 of Mobberley (1956): the Euro-African *S. maritima*, the East-American *S. alterniflora* and the West American *S. foliosa*. *Spartina alterniflora* appears as a closely related sister species to *S. foliosa*, with very few nucleotide differences (Baumel *et al.*, 2002a). These species

hybridise commonly in California where *S. alterniflora* has been introduced (see below), which raises the question of the specific status of these two morphologically similar and nonreproductively isolated taxa, occurring primarily in separate areas. *Spartina foliosa* is a morphologically uniform species (Mobberley, 1956) and differs from *S. alterniflora* by few phenotypic (e.g. size of the plants), ecological characters such as tolerance to tidal submersion (higher in *S. alterniflora*), and slight flowering precocity for *S. foliosa* (Daehler & Strong, 1997). Although limited information exists about the level of nuclear genetic diversity in *S. foliosa* populations over its entire range, only one chloroplast haplotype is recorded in this species (Anttila *et al.*, 2000), contrasting with the greater molecular diversity of *S. alterniflora* in both the chloroplast (Anttila *et al.*, 2000) and nuclear (Perkins *et al.*, 2002) genomes.

Spartina maritima is more genetically differentiated from *S. alterniflora*, probably as a consequence of a longer period of geographic separation on different continents. Although occupying a wide and discontinuous range from Western Europe to South Africa along the Atlantic coasts, this species displays a remarkable genetic uniformity. Interestingly, it rarely produces seeds in its northern range, and appears to reproduce asexually (Yannic, 2001; Yannic *et al.*, 2004). The tetraploid *S. spartinae* (syn. *S. argentinensis*) that was reunited in the same morphological complex (1) as *S. arundinacea* and *S. ciliata* by Mobberley (1956) is placed at the base of the hexaploid clade, which is well differentiated from the second major lineage of *Spartina* ('clade II'; Baumel *et al.*, 2002a) containing the remaining tetraploid species. This second clade includes all the remaining tetraploid species from the morphological complexes 1 and 3 defined by Mobberley (1956). *Spartina arundinacea* and *S. ciliata* belong to the same subclade, which agrees with their morphological similarity. The molecular data also confirm the morphological resemblance between *S. patens* and *S. bakeri* that appear closely related (Baumel *et al.*, 2002a). The position of *S. densiflora* is interesting to consider: a sample collected from California has been analysed by Baumel *et al.* (2002a). A significant incongruence was encountered between the *ITS* and the *Waxy* phylogenies. The ITS data place this sample in the same clade as *S. arundinacea* and *S. ciliata* in the 'clade II' (bootstrap support: 100%). Moreover, *S. arundinacea* and the analysed sample of *S. densiflora* were found to share a 426-bp deletion in the chloroplast spacer *trn*T-*trn*L. By contrast, the *Waxy* data place *S. densiflora* at the base of the 'clade I' (bootstrap support 99%) comprising *S. spartinae*, and the hexaploid lineage ('*alterniflora*/*foliosa*/*maritima*'). This incongruence has been interpreted as a possible reticulation event involving hybridization between introduced *S. densiflora* plants with other *Spartina* species that are present in the sampled area, namely the native *S. foliosa* or the introduced *S. alterniflora* that belong to the 'clade I'. This seems to be confirmed by our recent analysis of another sample of *S. densiflora* from Argentina (A. Baumel & M. Ainouche, unpublished). Interestingly, this sample displays an identical *Waxy* sequence to that of *S. arundinacea*, in accordance with the ITS and the chloroplast DNA data, then indicating that the phylogenetic incongruence is restricted to the Californian specimen. Indeed additional sampling in both native and recently colonized areas of *S. densiflora* and screening of sequence heterogeneity are needed for a better understanding of the history of the populations.

Recent hybridization and speciation events in *Spartina*

The intensification of human–caused intercontinental exchanges over the two last centuries has dramatically increased the dispersal of species and their introduction outside their native range, creating new opportunities for hybridization with native species. This is particularly well documented for the East-American *S. alterniflora* that has been introduced on the West-American Pacific coast on one hand and on the West-European Atlantic coast on the other hand.

Spartina alterniflora has been introduced in the mid-1970s in the San Francisco Bay of California where it now co-occurs with native *S. foliosa*. *Spartina alterniflora* plants spread rapidly with greater tolerance of tidal submersion (Daehler & Strong, 1997). Hybridization between these two outcrossing, wind-pollinated species occurs during overlapping of their flowering period. Hybridization has been shown to occur in both directions (Ayres *et al.*, 1999). The greater male fitness of *S. alterniflora* that produces more viable pollen than the native species and recurrent back-crosses have resulted in hybrid swarms that display most frequently the chloroplast haplotype of *S. foliosa* and up to 90% nuclear markers specific to *S. alterniflora* (Anttila *et al.*, 2000), according to the previously well-described 'chloroplast capture' process through pollen swamping (Rieseberg & Wendel, 1993), considered as a conservation threat to the native *S. foliosa* populations.

In Europe, *S. alterniflora* has been introduced accidentally by shipping ballast during the end of the 19th century in both southern England and western France. In England (Southampton Bay), hybridization with *S. maritima* resulted in a sterile hybrid *S.* × *townsendii* (Groves & Groves, 1880) that is still growing by vegetative means, forming a vigorous population near Southampton. Chromosome doubling in this hybrid gave rise to a new fertile allopolyploid species, *Spartina anglica* ($2n$ = 122–124), a vigorous and aggressive perennial plant that has been actively colonising British salt marshes since its formation (Hubbard, 1968; Raybould *et al.*, 1991a). *Spartina anglica* displays wider ecological amplitude than its parents across the successional sequence of salt marsh zones (Thompson, 1991). Contrasting with its introduced parental species *S. alterniflora* that remains localized on few sites, *S. anglica* has rapidly spread along the West-European coast (Baumel *et al.*, 2001). As this species is characterized by a particular ability to increase sediment accumulation, it has been

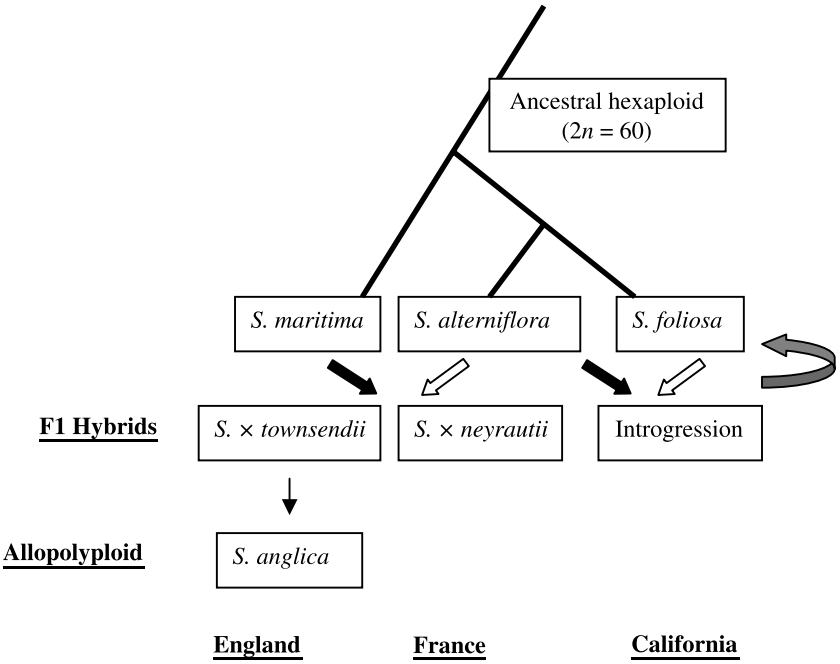

Fig. 1 Recent hybridization and allopolyploid speciation in the hexaploid lineage of *Spartina*, as a consequence of *S. alternaflora* introductions. Empty arrows represent maternal genome donors in the hybridization events.

introduced for land reclamation in several continents (China, Australia, New Zealand). However, rapid invasion and dramatic ecological changes in the colonised areas have led to the development of various local policies designed to control spread of the species. Contrasting with most allopolyploid species that have formed multiple times through recurrent hybridization (Soltis & Soltis, 1999), several lines of evidence indicate that *S. anglica* has undergone a severe genetic bottleneck at the time of its formation in England (Raybould *et al.*, 1991a; Ayres & Strong, 2001; Baumel *et al.*, 2001; Ainouche *et al.*, 2004) with *S. alterniflora* as the maternal genome donor (Ferris *et al.*, 1997; Baumel *et al.*, 2001). As the parental species were found to be genetically depauperate in Western Europe (Raybould *et al.*, 1991b; Yannic, 2001; Yannic *et al.*, 2004), *S. anglica* has resulted from either a unique hybridization event or from multiple events involving similar parental genotypes. No hybrids between *S. anglica* and its native parental species have been found in the sites where the two species co-occur (Baumel *et al.*, 2001; Yannic *et al.*, 2004).

In France, another sterile hybrid has been described on the Spanish border and has been called *Spartina* × *neyrautii* (Foucaud, 1897). After a period of uncertainty, this taxon has been recognized as deriving from hybridization between introduced *S. alterniflora* and native *S. maritima* plants. As this hybrid displayed a very different morphology than the British hybrid *S.* × *townsendii*, it was believed for a long time to result from the reciprocal cross, that is, *S. maritima* as maternal parent (Marchant, 1977). After the hybridization site has been severely disturbed by land reclamation and airport construction, the survival of *S.* × *neyrautii* was questioned in the 1970s (Hubbard *et al.*, 1978). In a recent survey of the *Spartina* populations in south-west France, Baumel *et al.* (2003) found near Hendaye a hybrid isolated clone that displays species-specific nuclear markers of both *S. maritima* and *S. alterniflora*. This hybrid displays the features that have been previously reported for *S.* × *neyrautii*, including morphological resemblance to *S. alternifora* and pollen sterility. A flow cytometry analysis (Baumel *et al.*, 2003) revealed that this hybrid has the same ploidy level as *S.* × *townsendii*, that is, half the DNA content of the allopolyploid *S. anglica*. None of the examined populations from this region displayed both hybrid pattern and dodecaploid genome size, indicating that allopolypoidisation did not occur in this region as it occurred in southern England. The maternally inherited chloroplast genome of *S.* × *neyrautii* is identical to that of *S. alterniflora* which is then the maternal parent, as for *S.* × *townsendii* and *S. anglica* (Baumel *et al.*, 2003). Therefore, *S.* × *neyrautii* is not the reciprocal hybrid as it was previously hypothesized. However, despite the low nuclear DNA variation recorded in the parental species *S. alterniflora* and *S. maritima* from Western Europe (Baumel *et al.*, 2001; Yannic *et al.*, 2004), few Randomly Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) markers allowed us to differentiate the genotypes of *S. alterniflora* and *S. maritima* that have been involved in the parentage of *S.* × *townsendii* on one hand and of *S.* × *neyrautii* on the other hand (Baumel *et al.*, 2003).

The introductions of *S. alterniflora* in the American Pacific coast and in Europe have rather different consequences (Fig. 1): in the former case, hybridization and recurrent backcrosses involving weakly divergent sister taxa result in successful hybrid swarms and new genotype combinations. In the other case, hybridization between more divergent species

resulted in sterile hybrids and in a new invasive allopolyploid species that is genetically isolated from its parents.

All together, these studies indicate that evolutionary history of the *Spartina* species has been accompanied by reticulation events deduced from either traditional morphological analyses (e.g. *S. longispica*, *S.* × *caespitosa*) or from incongruence between gene trees (*S. densiflora* from California). As presented above, this common occurrence of hybridization is also strongly supported by the most recent part of the *Spartina* history.

Speciation and rapid genome changes: hybridization vs duplication in *Spartina*

A number of studies have recently contributed to reinforce the idea that allopolyploid speciation may be accompanied by rapid genetic, epigenetic and genomic changes (Wendel, 2000; Liu & Wendel, 2003). The merger of two differentiated genomes in the same nucleus through hybridization, followed by duplication of the hybrid genome, entails various mechanisms (interactions between the homoeologous duplicated genomes via gene conversion and concerted evolution, reshuffling of the genome resulting from occasional meiotic interactions between homoeologous chromosomes, gene loss, gene silencing or new expression through epigenetic regulation, transposable element activation …) that are of critical importance for the evolutionary success of the new species.

The *Spartina* system is unique in that it allows analysis of the consequences of hybridization in two independent events (England for *S.* × *townsendii* and France for *S.* × *neyrautii*; Fig. 1) and the early effects of genome duplication in the young (less than 150 yr old) allopolyploid populations of *S. anglica* that are expanding around the world (Ainouche *et al.*, 2004). The parental species are identified and still extant, which allows comparison of the actual parental genomes to the hybrid and the allopolyploid species.

Baumel *et al.* (2001) have found that *S. anglica* populations of Western Europe are composed of one 'major' multilocus genotype that is identical to the first generation hybrid *S.* × *townsendii*, characterized by the additivity of RAPD and ISSR parental markers. Both parental nuclear sequences are present in the hybrid and the allopolyploid for single copy genes such as *Waxy* (Baumel *et al.*, 2002a) as well as repetitive rDNA genes that are not homogenised by concerted evolution (Baumel *et al.*, 2001). However, quantitative estimates of each parental rDNA type in the allopolyploid genome have not been performed, and we cannot exclude the possibility that different populations may exhibit various amounts of each parental type, as recently shown in the young allopolyploid Tragopogons (Soltis *et al.*, 2004) or in the neopolyploid Glycine (Doyle *et al.*, 2004). A retrotransposon display approach indicated no burst of retroelement activation in the allopolyploid *S. anglica* (Baumel *et al.*, 2002b).

All together, these investigations generated approx. 600 markers in the dodecaploid *S. anglica* genome (Ainouche *et al.*, 2004). In order to evaluate the consequences of hybridization and genome duplication at a larger number of loci, the two hybrids *S.* × *neyrautii* and *S.* × *townsendii*, and the allopolyploid, *S. anglica*, were compared to their parental species, *S. maritima* and *S. alterniflora*, using Amplified Fragment Length Polymorphism (AFLP). This fingerprinting technique involves the restriction of genomic DNA by two restriction enzymes (here, *Eco*RI – *Mse*I and *Pst*I – *Mse*I) followed by selective amplification of a subset of the restriction fragments, and has been used successfully for the screening of structural changes in polyploids (Liu *et al.*, 2001; Ozkan *et al.*, 2001). By using a combination of primers with different selective nucleotides, this method allows sampling loci from a large fraction of the genome. The molecular basis of the polymorphism (i.e. presence vs absence of a given amplified fragment) is usually sequence polymorphism affecting either the restriction site or nucleotides adjacent to the restriction site that prevent selective amplification. Deletions, insertions and rearrangements may affect the presence or the size of the restriction fragments. As most of the polymorphism results from presence/absence of a priming site, AFLP markers are usually considered as dominant. Polymorphic fragments (i.e. discriminant) between the parental species *S. maritima* and *S. alterniflora* were first screened, and deviation from additivity (i.e. loss of parental fragment, or appearance of new fragment) was examined in the hybrids and the allopolyploid. Two samples of *S. alterniflora* have been analysed, in order to represent as much as possible the actual genotypes involved in the parentage of the hybrids, in England (Southampton Bay) and in southwest France (Hendaye). *Spartina maritima* is now extinct in both hybridization sites, and this species has been recently found remarkably genetically depauperate in Western Europe (Yannic *et al.*, 2004). The sample introduced in the present study was collected from Noirmoutiers (France). The F1 hybrids were collected in Hythe, Southampton Bay (*S.* × *townsendii*) and Hendaye (*S.* × *neyrautii*). The allopolyploid *Spartina anglica* were analysed in two populations: Keyhaven (England) and Baie des Veys (France). A combination of 11 selective primers generated 982 DNA fragments of which 534 were found to discriminate *S. alterniflora* from *S. maritima*. Both hybrids inherited 89.7% of these discriminant fragments (Table 1). *Spartina* × *neyrautii* and *S.* × *townsendii* lack 9.0% and 7.3% of the discriminant parental fragments, respectively. The absence of these fragments concern mostly those from the maternal parent *S. alterniflora* (Table 1), as they represent 87.5% of the parental fragments absent in *S.* × *neyrautii* (chi-square = 27, df = 1, $P < 0.001$) and 89.7% of the parental fragments absent in *S.* × *townsendii* (chi-square = 24.6, df = 1, $P < 0.001$). Deviation from strict additivity (i.e. loss of parental fragment or appearance of new fragments) was observed: 34 lost fragments and 7 new fragments (6.4% changes) were shared by the two hybrids. Ten (1.3%) and 27 (3.2%) changes were unique to *S.* × *townsendii* and *S.* × *neyrautii*, respectively. Interestingly, most changes are

Table 1 Fate of parental amplified fragment lenght polymorphism (AFLP) fragments in the hybrids *Spartina* × *neyrautii* and *S.* × *townsendii* generated by *Eco*RI – *Mse*I and *Pst*I – *Mse*I digestions (11 selective primer combinations)

	Parental fragments	Inherited fragments in both hybrids	Absent in S. × neyrautii	Absent in *S.* × *townsendii*
Common parental fragments	448	445 (99.3%)	3 (0.7%)	2 (0.4%)
Discriminant fragments				
S. maritima	250	242 (96.8%)	6 (2.4%)	4 (1.6%)
S. alterniflora	284	237 (83.4%)	42 (14.8%)	35 (12.3%)
Total discriminant fragments between parents	534	479 (89.7%)	48 (9.0%)	39 (7.3%)

Table 2 Fate of the parental methylated (MSAP) fragments in *Spartina* × *neyrautii* (N) and *S.* × *townsendii* (T). *Eco*RI-*Hpa*II/*Msp*I digestions (8 selective primer combinations)

	Identical patterns in the two hybrids			Different patterns in the two hybrids		
Parental methylation patterns	Additivity	Fragment loss	Methylation alteration	Fragment loss in N	Methylation alteration in N	Loss in N, Methylation alteration in T
Monomorphic fragments (4)	3	0	0	1	0	0
Polymorphic fragments between parents (28)	16 (57.1%) 24 (85.7%)	5 (17.9%)	3 (10.7%)	1 (3.6%)	2 (7.1%) 4 (14.3%)	1 (3.6%)

repeatable in both hybrids. The allopolyploid *S. anglica* inherited 492 (99.4%) of the fragments from *S.* × *townsendii*, and displayed only two new DNA fragments, with three additional '*S. alterniflora* – fragment' losses.

The epigenetic alterations that might be encountered in the hybrids and the allopolyploid have been explored using Methylation Sensitive AFLP (MSAP; Reyna-Lopez *et al.*, 1997). This procedure involves the use of two isoschizomers (*Mse*I and *Hpa*II) in parallel reactions. These enzymes recognize the same sequence (5′-CCGG) but differ in their sensitivity to DNA methylation at the inner cytosine. A difference in band patterns indicates a methylation change (Xiong *et al.*, 1999), and the additivity of the parental patterns has been examined in the hybrids and the allopolyploid as mentioned above with AFLP. Less parental additivity of the methylation patterns (57.1%) is encountered in the hybrids (Table 2) than structural additivity reported above with AFLP (89.7%, Table 1). Most of the methylation changes (28.6%) are common to *S.* × *neyrautii* and *S.* × *townsendii*, which indicates the reproducibility of the changes in the two different hybridization events. Only four restriction sites (14.3%) exhibited differential patterns between these taxa. The methylation changes encountered in *S. anglica* (not shown) were already present or initiated (i.e. different methylation state of the restriction site in the parents, the hybrid and the allopolyploid) in *S.* × *townsendii*; only one methylation change (over 34) was specific to *S. anglica*, indicating that epigenetic changes are triggered by hybridization rather than by genome duplication.

Both AFLP and MSAP reveal that hybridization, rather than polyploidization, is the main process that has shaped the structure of the allopolyploid genome of *S. anglica*. The AFLP analysis indicates that parental genome additivity, rather than structural instability is encountered in this system, as previously found with other markers (Baumel *et al.*, 2001, 2002b). The structural changes affected mostly absence of DNA fragments from the maternal parent *S. alterniflora*; as AFLP are dominant markers, these changes may result from either modifications of the restriction site or from parental heterozygosity. A 'genomic stasis' has been reported in the allopolyploid *Gossypium* species in both short-term (Liu *et al.*, 2001) and long-term (Senchina *et al.*, 2003) of the evolutionary time scale. These findings contrast with those reporting rapid and dramatic structural changes in *Brassica* (Song *et al.*, 1995) or *Triticum* – *Aegilops* (Levy & Feldman, 2002) allopolyploids. Changes in the hybrid – allopolyploid *Spartina* system appear mostly of epigenetic nature. As the homoploid F1 hybrid has limited fertility and reproduces mostly vegetatively, it is the polyploid nature of *S. anglica* that has enabled demographic establishment and ecological spread. This suggests that there may be additional fitness advantages to polyploidy not obviously related to structural genomic changes.

Open questions and future directions

Diversification of the *Spartina* genus raises interesting questions regarding biogeography, history and evolution of species. The phylogenetic framework emerging from the recent approaches indicates that this genus displays various examples of disjunct distributions at different levels of the phylogeny. Considering the extremely weak nuclear and chloroplast DNA sequence divergence between the sister *S. foliosa* and *S. alterniflora* (Baumel *et al.*, 2002a) and the absence of reproductive barriers (Daehler & Strong, 1997), one could not exclude the hypothesis that the West American *S. foliosa* might actually result from an ancient dispersal of the East-American *S. alterniflora* on the Pacific coast, followed by independent evolution of geographically separated populations. The history of the Euro-African *S. maritima* that is basal in the hexaploid lineage is an interesting and still open question: Yannic *et al.*, 2003) hypothesized that current populations of *S. maritima* could actually represent a relic from a wider distribution of an ancestral hexaploid species, or alternatively, that *S. maritima* was introduced from North America to the Eastern Atlantic coast and subsequently vanished from the New World. Comparative analyses of various gene histories in this clade should help exploring this question and estimating the divergence time between *S. maritima* and its sister lineage *S. alterniflora*/*S. foliosa*.

The tetraploid clade (Baumel *et al.*, 2002a) is composed of five closely related species belonging to two sister groups. The first one is composed of three species native from South America or Austral islands (*S. ciliata*, *S. densiflora*, *S. arundinaceae*). The second one contains two species native from the East American coast (*S. patens* and *S. bakeri*). Understanding the past ecological events explaining the distribution of these five species should provide especially interesting data on the historical biogeography of the American flora. As outlined above, of particular interest is the history of *S. densiflora* that has a disjunct distribution on the Eastern and Western South American coasts.

Origin of the *Spartina* genus is another exciting question. Most species are American, suggesting an American origin to *Spartina*, but the presence of the Euro-African *S. maritima* may be congruent with an alternative hypothesis considering American species as remnant of a worldwide ancestor related to the *Sporobolus* genus that has been found sister to *Spartina* (Hisao *et al.*, 1999). The split between the hexaploid and the tetraploid lineages seems very ancient (Baumel *et al.*, 2002a) and has been followed by the disappearance of the diploid ancestors. Careful estimations of the divergence time between species from different ploidy levels should provide critical explanations to such issues.

The recent hybridization and polyploidization events in *Spartina* allow exploring the immediate genetic and genomic consequences of alloploid speciation at the structural and epigenetic levels. No extensive structural changes have affected the divergent homoeologous subgenomes that are duplicated in the young *S. anglica*, although the preferential absence of *S. alterniflora* – specific AFLP fragments in both hybrids, has to be explored further. Methylation changes do occur, and seem enhanced first by hybridization. The extent of the methylation polymorphism in different populations of the allopolyploid, the identification of the sequences that are affected by epigenetic changes, as well understanding the relative contributions of the homoelogous subgenomes to the transcriptome are important questions regarding the adaptive success of this new invasive species.

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