

Facilitated invasion by hybridization of *Sarcocornia* species in a salt-marsh succession

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Summary

1 We examined salt marsh development over 17 years (1984–2001) in a rapidly accreting, well-drained lagoon of a macrotidal marsh in south-west Spain. Scattered, elevated tussocks of colonizing *Spartina maritima* expanded radially until they either coalesced or were separated only by narrow drainage channels. We recorded changes in elevation of the tussocks and investigated the cover and biomass of successive species invading them.

2 Sediment accretion produced a mean annual increase in tussock elevation of 3.5 cm. *Sarcocornia perennis* had begun to invade the raised centres of the tussocks by 1984, subsequently displacing *Spartina maritima* radially to become dominant by 1990. A hybrid form of *Sarcocornia*, which appeared on some of the tussocks occupied by *S. perennis* in 1997, also expanded radially and had achieved dominance on many of the invaded tussocks by 2001, by virtue of its more erect growth form and rapid accumulation of high above- and below-ground biomass.

3 Tussocks not yet invaded by the hybrid remain dominated by *S. perennis* and have maintained sediment accretion rates and redox potentials similar to those that have been invaded.

4 Genetic analysis, using random amplified DNA (RAPD) markers, indicated that the hybrid was a cross between the diploid *Sarcocornia perennis* ($2n = 18$) and the octaploid *S. fruticosa* ($2n = 72$), a high-marsh species, and suggested that each hybrid individual may have resulted from a separate pollination of an indigenous *S. perennis* plant.

5 Invasion by the new hybrid thus probably occurred as a result of pollen flow from high-marsh *S. fruticosa*, some 1 km distant, to the stigmas of the established dominant *S. perennis*. Succession might therefore be facilitated genetically rather than simply by the enhanced sediment accretion, which ameliorated the effects of submersion and low sediment redox potentials that presumably exclude *S. fruticosa* from lower parts of the marsh.

Key-words: genetic facilitation, marsh elevation, positive interaction, RAPD marker, redox potential, sediment accretion, tidal wetland

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Introduction

The importance of both positive and negative interactions between species in the plant communities of coastal wetlands is now clearly established (e.g. Callaway 1994; Bertness & Leonard 1997; Callaway & Walker 1997;

Costa *et al.* 2003). Amongst these are interactions involved in the successional replacement of species as tidal salt marshes develop (Bertness 1991). There have been rather few long-term studies of salt marsh succession (e.g. Roozen & Westhoff 1985; Olff *et al.* 1997; Willis 2000), although salt marsh systems can be an excellent model for investigating the mechanisms underlying successional processes. Colonization

of low-lying mud flats by species that are relatively tolerant of prolonged submersion and low sediment redox potentials (Castellanos *et al.* 1998; Castillo *et al.* 2000; Davy *et al.* 2001) can promote sediment accretion sufficiently to ameliorate these conditions and allow invasion by less tolerant species. Castellanos *et al.* (1994) examined the colonization of bare intertidal sediments at Odiel marshes on the Atlantic coast of south-west Spain by *Spartina maritima* (Curtis) Fern. and its facilitation of invasion by *Sarcocornia perennis* (Miller) A.J. Scott (*Arthrocnemum perenne* (Miller) Moss). *Spartina* established tussocks that accreted vertically and expanded radially; evidence from tussocks with a wide range of elevation and diameter showed that *Sarcocornia* subsequently invaded their centres, where the sediments were less reducing, and became increasingly dominant as accretion progressed, until *Spartina* had been virtually eliminated from all but the expanding edges.

Although such three-dimensional spatial data are good evidence for successional change in salt marsh vegetation, they are at best a surrogate for the direct analysis of changes with time (Davy & Costa 1992; Davy 2000). Marsh development at Odiel was initiated by physiographic changes associated with the construction in 1977 of a dike to protect a navigable channel. In such a young system, long-term studies would be expected to reveal further species invasions, concomitant changes in abundance, and their underlying mechanisms. A prominent recent colonist has been a fertile, apparently hybrid form of *Sarcocornia* with more upright growth than *S. perennis*; it is morphologically distinct from *S. perennis* but has certain characteristics of *S. fruticosa* (L.) A.J. Scott, a species normally found in a different environment at higher elevations of Mediterranean marshes (Alvarez *et al.* 2000). Such hybrids have been reported previously (Castroviejo & Coello 1980; Castroviejo 1990; Castroviejo & Lago 1992). This led to the hypothesis that a hybrid might have arisen locally that was able to colonize and dominate a developing habitat intermediate in elevation between those usually occupied by its parent species. As such, it could represent a novel mechanism of successional invasion and species replacement, in addition to playing an important role in the structuring of these salt marsh communities. Hybridization is well documented in salt marsh dominants of the genus *Spartina*, both in Europe (Raybould *et al.* 1991) and North America (Daehler & Strong 1997; Ayres *et al.* 1999; Anttila *et al.* 2000) and the best known example, *Spartina anglica*, has proved extremely invasive (e.g. Gray *et al.* 1991). However, all of these hybrids have resulted from an inter- or trans-continental alien introduction, rather than being a part of an indigenous succession.

The aim of the work described in this paper was to elucidate further the mechanisms of salt marsh succession. Our particular objectives were: (i) to examine changes in tussock size, surface elevation and species representation over a 17-year period; (ii) to characterize

the new coloniser at Odiel Marshes genetically, in terms of DNA (RAPD) markers; (iii) to test the hypothesis that it is a hybrid between *Sarcocornia perennis* and *S. fruticosa*; and (iv) to investigate its mode of invasion and its significance for successional development of the salt marsh.

Materials and methods

FIELD SITE

Work was carried out in an accreting, well-drained lagoon (the 'Laguna de Don Claudio' of Castellanos *et al.* 1994) at Odiel Marshes, in the joint estuary of the Odiel and Tinto rivers on the Atlantic coast of south-west Spain (37°08' to 37°20' N, 6°45' to 7°02' W). Odiel is one of the largest salt marshes in the Iberian Peninsula and is protected as a 'Natural Reserve of the Biosphere'. The semidiurnal tides have a mean range of 2.10 m and a mean spring tidal range of 2.97 m, representing 0.40–3.37 m above Spanish Hydrographic Zero (SHZ). Mean sea level is +1.85 m relative to SHZ. The physiography, climate and vegetation have been described by Castellanos *et al.* (1994, 1998).

GENETIC ANALYSIS OF *SARCOCORNIA*

Random amplified polymorphic DNA (RAPD) markers were developed as tools for the characterization of *Sarcocornia* species and hence non-destructive identification of their hybrids.

Collection of plant material and extraction of genomic DNA

Eleven morphologically unequivocal samples of *Sarcocornia perennis* were taken at random from nine tussocks in the well-drained successional lagoon under study and two others were taken from different tussocks in a neighbouring lagoon with impeded (ponded) drainage ('Laguna de Ludovico' of Castellanos *et al.* 1994). Likewise, five *S. fruticosa* samples were gathered from separate tussocks chosen randomly from two populations located c. 1 km from the lagoons (two from one population and three from the other). Six samples of the putative *Sarcocornia* hybrid were taken, each from the centre of a different tussock from which *S. perennis* had also been sampled in the well-drained lagoon.

Samples of the succulent stem segments (including inflorescences) were kept at –20 °C until DNA extraction. Plant material (0.1 g) was ground in liquid nitrogen with a mortar and pestle and DNA was extracted using Dneasy Plant Mini Kits (QIAGEN, Hilden, Germany).

DNA amplification (RAPD)

The PCR conditions for RAPD followed the protocols of Luque *et al.* (1995). RAPD reactions were performed

Table 1 Numbers of amplification products (bands) obtained per primer in RAPD analysis of *Sarcocornia* taxa from Odiel Marshes, south-west Spain

Primer	Sequence	Total	Monomorphic	Polymorphic
1	5'-TACGCATAGCT	1	1	0
2	5'-GTTGCGCGAC	1	1	0
3	5'-TGCTGCAGGT	16	4	12
4	5'-GGTGATCAGG	26	8	18
5	5'-ACCCGGTCAC	14	5	9
6	5'-CAGCTCACGA	20	7	13
7	5'-ACGCGCATGT	18	8	10
8	5'-CGGCCACTGT	17	10	7
9	5'-CAGTCCCTGG	10	9	1

in a 50- μ L reaction volume, containing 20 ng μ L⁻¹ of genomic DNA, dATP, dCTP, dGTP, dTTP (each at 200 μ M final concentration), 2.0 μ M primer, 4.0 mM MgCl₂, 1 \times Stoffel buffer (10 mM tris HCl, 10 mM KCl, pH = 8.3) and 5 units of AmpliTaq DNA polymerase (AmpliTaq Stoffel fragment, Applied Biosystems, Foster City, USA). Nine arbitrary primers (Amersham-Pharmacia Biotech, Ceranyola, Spain and Cultek, Uherstal, Belgium) were used to characterize *Sarcocornia* types (Table 1).

Amplification was performed in a Perkin Elmer 2400 Thermal Cycler; DNA was first denatured at 95 °C for 5 min followed by 45 cycles of 1 min at 95 °C, 1 min at 37 °C, 2 min at 72 °C, and ending with 10 min at 72 °C. Amplification products were analysed by electrophoresis in 2.0% agarose gels and visualized by ethidium bromide staining and illumination with UV light. For band size determination, a 123-bp DNA ladder (Sigma-Aldrich, Saint Louis, USA) was run in each gel. All amplifications were repeated at least twice to check the stability of amplification products.

RAPD fragment homology test

The homology of comigrating RAPD bands was tested by treatment with restriction endonucleases (Rieseberg 1996). Amplification products were isolated from gels and purified using GFX PCR DNA and gel purification kit (Amersham Biosciences, Piscataway, USA). Purified fragments were then digested with two restriction endonucleases that have four-base pair recognition sequences: *Hae*III (GG/CC) and *Hinf*I (G/ANTC). Digestion products were compared electrophoretically, as described above.

Data analysis

Bands were scored as present (1) or absent (0) (Tansley & Brown 2000) and the coefficient of Nei & Li (1979) was used to calculate a similarity matrix (NTSYS-pc computer package, version 1.8). UPGMA (unweighted pair-group method, arithmetic average) clustering, as described by Sneath & Sokal (1973), and the SHAN (sequential, hierarchical, and nested clustering) were used to construct a phenogram showing genetic similarity between populations. The cophenetic value

matrix based on phenogram distances was compared with the original similarity matrix by cophenetic correlation to test its reliability (Mantel 1967). The matrix correlation *r*-value is based on cophenetic correlation analysis, which produces a normalized Mantel *Z* statistic, where *r* > 0.90 is considered a very good fit with the original distance matrix.

ANALYSIS OF VEGETATION IN THE FIELD

Eight raised tussocks of colonizing *Spartina maritima* were treated as permanent plots and the areas of all species present on them were recorded in October 1984, 1990, 1997 and 2001. An additional eight tussocks were analysed in 2001. As invasions by subsequent species were essentially centrifugal, the concentric areas of the species present were derived from measurements of either minimum and maximum diameters or circumference (depending on shape) at the bases of their canopies, taking care to avoid obviously coalesced tussocks. Relative cover of each species was calculated as a percentage of the total area of the raised tussock.

Above-ground plant biomass was harvested from transects across representative permanently marked colonizing tussocks in March 1997 and October 2001, to compare tussocks that had been invaded by the hybrid *Sarcocornia* with those that had not. Each transect was a belt of contiguous quadrats (15 cm radially, 25 cm wide) across the radius of a tussock. Dry mass was determined after drying at 75 °C for 48 h.

The vertical distribution of below-ground biomass of each species was determined in October 2001, by taking sediment cores (10.5 cm diameter, 20 cm deep) under the main canopy area of each species from five replicate tussocks that had been invaded by the hybrid *Sarcocornia* and a further five that had not. Cores were divided into 5-cm layers, and roots and rhizomes were washed carefully from them, before being dried and weighed. Roots of *S. perennis* and the hybrid were distinguished by their colour, size and shape: hybrid roots and rhizomes were thicker, lighter and had more marked nodes than those of *S. perennis*. We handled the samples carefully in order to keep the smaller roots attached to the larger roots and rhizomes, which were easier to recognize.

ENVIRONMENTAL MEASUREMENTS

Topography

Elevations, relative to Spanish Hydrographic Zero (SHZ), were surveyed to a resolution of 2 cm with a Leica NA 820 theodolite at the centre and the edge of 16 tussocks in October 1984, October 1990, March 1997 and October 2001; reference points were determined in relation to measurements of tidal extremes (Ranwell *et al.* 1964).

Redox potential

In October 2001 sediment redox potential was recorded in five replicate tussocks that had been invaded by the hybrid *Sarcocornia*, and five that had not. Redox potential was measured using a Crison pH/mV meter and redox electrode system. Determinations were made under the main canopy area of each *Sarcocornia* type, lower in the tidal frame and in peripheral bare sediment, all at known surface elevations. Redox potentials were obtained from two sediment depths in the rooting zone (0–10 and 11–20 cm).

STATISTICAL ANALYSIS

Analysis was carried out using 'Statistica' release 5.1 (Statsoft Inc.). Pearson correlation coefficients were calculated between environmental variables. Environmental measurements were compared between species zones by one-way analysis of variance. Means were compared using the Tukey's honestly significant differences test only if an *F*-test was significant at the 0.05 level of probability. Data were tested for homogeneity of variance with the Levene test ($P > 0.05$).

Results

ANALYSIS OF RAPD MARKERS

Nine primers yielded a total of 123 DNA fragments with an average of 13.7 bands per primer (Table 1). Overall, the fraction of monomorphic bands was 43.1% and that of polymorphic bands 56.9%. Primer 4 had the highest percentage of polymorphic fragments between samples (69.2%), whereas primers 1 and 2 did not produce any polymorphic fragments. The putative hybrid contained DNA fragments from each parent as well as one novel fragment (Table 2). Of the bands in the hybrid, 16 were derived from *S. fruticosa* but, apparently, only two from *S. perennis*. In order to confirm a contribution from *S. perennis* to the putative hybrid, the larger (492 bp) of their two pairs of comigrating fragments (Fig. 1a) was tested for homology. Congruent profiles of digestion products of this fragment from the two taxa (Fig. 1b) indicated that they were homologous.

Co-phenetic correlation analysis strongly supported the reliability of the phenogram based on the original

distance matrix ($r = 0.989$). The first phenogram division (Fig. 2) grouped all *S. perennis* samples (including those from the well-drained and poorly drained marshes) with 88% similarity; the other group included all *S. fruticosa* samples (from the high marsh) and all of the samples of *S. perennis* × *fruticosa* (from the well-drained marsh) with 92% of similarity. The hybrid *Sarcocornia* samples had 95% similarity between them and formed a coherent subgroup that was distinct from the *S. fruticosa* subgroup. *S. perennis* samples from the well-drained marsh showed a 92% similarity and they were clearly segregated from the two conspecific samples in the adjacent poorly drained lagoon. *S. perennis* samples 2 and 3, gathered from the same tussock, were genetically distinct, as were samples 8 and 9 from another tussock; this indicates that large tussocks have been colonized by more than one individual of *S. perennis*.

STRUCTURE OF COLONIZING TUSSOCKS

The centres of the colonizing tussocks accreted vertically at a nearly constant rate of 3.5 cm year⁻¹ between 1984 and 2001 (from 2.15 to 2.76 m above SHZ) in the well-drained lagoon; accretion at the edges of the tussocks was much slower until 1997 (*c.* 0.9 cm year⁻¹) but then accelerated rapidly as the tussocks began to meet and coalesce (Fig. 3a). The mean area of the tussocks increased approximately linearly over the whole period (Fig. 3b). *Spartina maritima* and *Sarcocornia perennis* were the only species present from 1984 to 1990. The *Sarcocornia* hybrid (subsequently identified as *S. perennis* × *fruticosa*) and *Atriplex portulacoides* both began to colonize this marsh between 1990 and 1997 (Fig. 3c). By 2001, there were no *Spartina* tussocks that had not been colonized by *Sarcocornia perennis* and it had become the most abundant species, with a cover of *c.* 70% of the tussock area. The *Sarcocornia* hybrid had colonized 13% of the tussocks by 1997 and 82% by 2001, when it had become the third most abundant species, with a cover of 13.2%. The expansion in cover of both *Sarcocornia* taxa was at the expense of that of the founding *Spartina maritima*, which suffered a net loss of 58%.

The changes from 1990 to 2001 in distribution of above-ground biomass within what had originally been *Spartina* tussocks reflected the centrifugal growth of invading species. In tussocks that were not invaded by the hybrid *Sarcocornia*, radial transects showed the expansion of *Sarcocornia perennis* from just the central area in 1990 to a dense, sprawling canopy with virtually complete cover by 2001; only a low density of *Spartina maritima* tillers persisted around the periphery (Fig. 4a). In contrast, in tussocks where the hybrid *Sarcocornia* became established, *S. perennis* had not expanded much beyond the central area by 1997 and had mostly been displaced by the hybrid, which eliminated it from all but the edges by 2001; the substantial outer ring of *Spartina maritima* tillers still present in 1997 had almost disappeared after a further 4 years (Fig. 4b).

Table 2 Genetic characterization from RAPD analysis of *Sarcocornia fruticosa* ($n = 5$), *S. perennis* ($n = 13$) and the hybrid *S. perennis* \times *fruticosa* ($n = 6$) from Odiel Marshes, south-west Spain. The presence (+) or absence (-) of all fragments (bands) that were polymorphic between taxa and monomorphic within them is shown. Fragments that were polymorphic within taxa are not shown

	Fragments (bp)	<i>S. fruticosa</i>	<i>S. perennis</i>	<i>S. perennis</i> \times <i>fruticosa</i>
Primer 3	134.3	+	-	+
	178.1	+	-	+
	196.5	+	-	+
	207.8	+	-	+
	227.6	+	-	+
	351.1	-	+	-
	368.5	-	+	-
	441.5	-	+	-
Primer 4	168.2	-	+	-
	176.7	+	-	+
	182.4	-	+	-
	217.7	+	-	+
	268.2	+	-	+
	332.7	-	+	-
	340.7	+	-	+
	411.9	+	-	+
	451.9	+	-	-
	497.7	+	-	+
	512.5	+	-	-
565.8	-	+	-	
Primer 5	198.2	+	-	+
	286.3	-	+	+
Primer 6	246	+	+	-
Primer 7	214.2	-	+	-
	222.2	+	-	+
	301.9	+	-	+
	351.1	+	-	+
	629.2	-	+	-
Primer 8	293.7	-	-	+
	324	+	-	-
	439.8	+	-	+
Primer 9	492	-	+	+

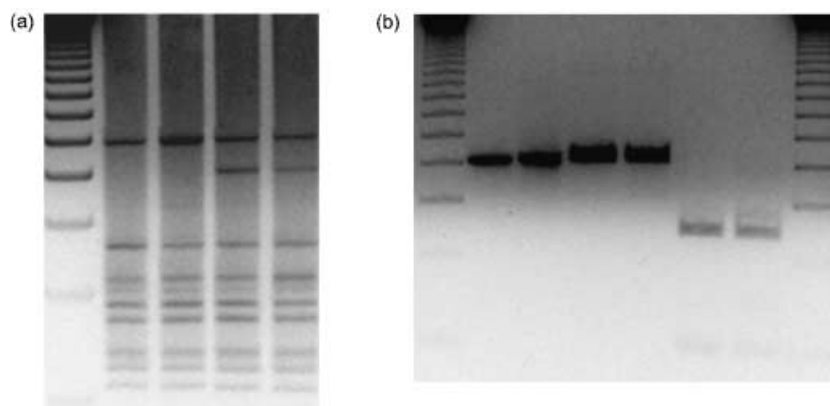


Fig. 1 Agarose gels showing RAPD products that have been visualized with ethidium bromide staining and UV illumination. (a) Amplification products obtained with primer 9, showing the comigrating 492 bp fragments: lane 1, 123 bp ladder; lanes 2 and 3, *Sarcocornia fruticosa*; lane 4, *Sarcocornia perennis*; lane 5, the putative *Sarcocornia* hybrid. (b) Restriction-enzyme homology test of comigrating fragments (492 bp) obtained with primer 9: lanes 1 and 8, 123 bp ladders; lane 2, undigested gel-isolated fragment from *S. perennis*; lane 3, undigested gel-isolated fragment from the *Sarcocornia* hybrid; lane 4, gel-isolated fragment from *S. perennis* digested with *Hae*III; lane 5, gel-isolated fragment from the *Sarcocornia* hybrid digested with *Hae*III; lane 6, gel-isolated fragment from *S. perennis* digested with *Hae*III and *Hinf*I; lane 7, gel-isolated fragment from the *Sarcocornia* hybrid digested with *Hae*III and *Hinf*I.

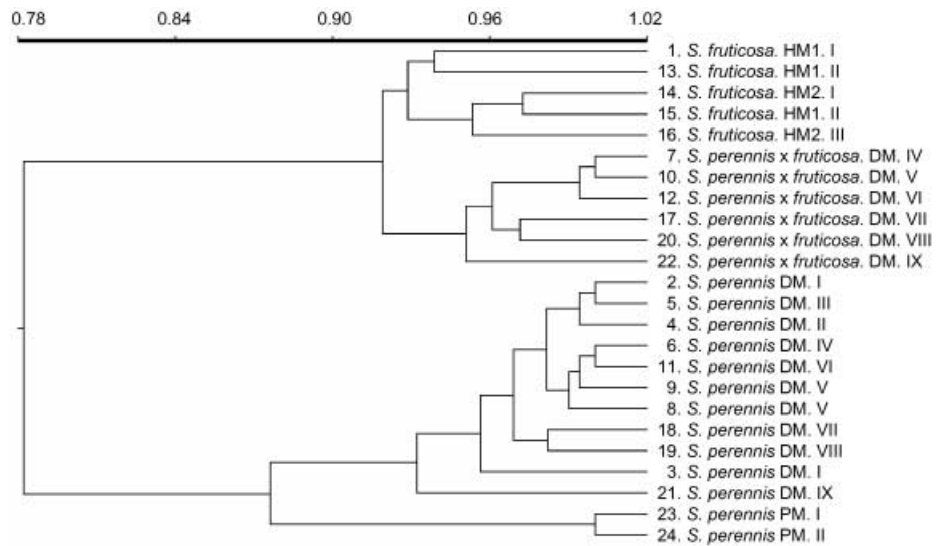


Fig. 2 Phenogram of 24 clones of *Sarcocornia* taxa from Odiel Marshes, south-west Spain based on similarities generated by UPGMA clustering of RAPD band scores. Thirteen clones of *S. perennis*, five of *S. fruticosa* and six of the hybrid *S. perennis* × *fruticosa* are represented. DM, well-drained marsh; PM, ponded (poorly drained) marsh; HM, high marsh. Roman numerals represent individual tussocks of origin.

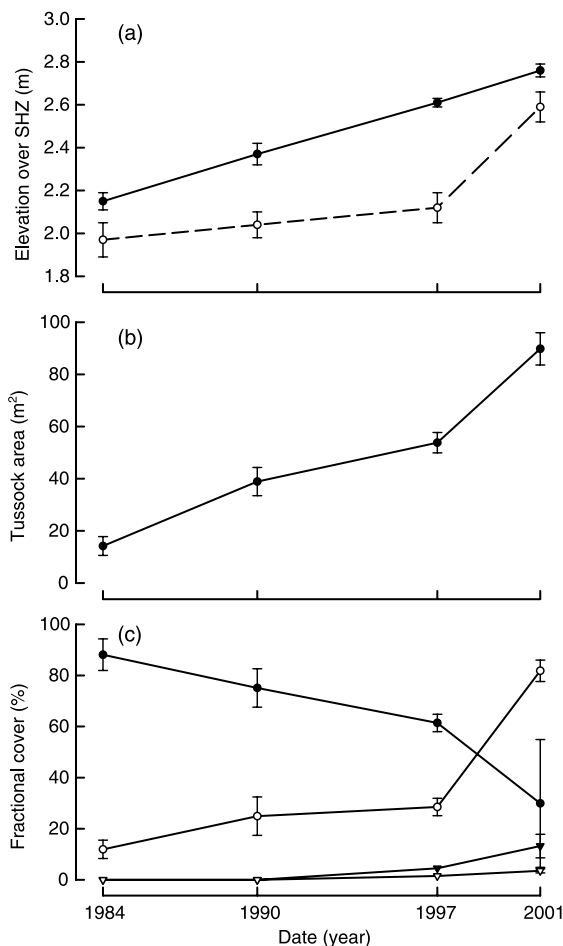


Fig. 3 Successional development of marsh in the well-drained lagoon at Odiel marshes, south-west Spain between 1984 and 2001. (a) Changes in elevation at the centre (●) and edge (○) of expanding tussocks. (b) Change in area of tussocks. (c) Changes in the fractions of the tussock area occupied (%) by halophyte species: *Spartina maritima* (●); *Sarcocornia perennis* (○); *S. perennis* × *fruticosa* (▼); *Atriplex portulacoides* (▽). Vertical bars represent ± SE.

Determination of below-ground biomass in 2001 showed that rhizomes and roots of *Spartina* persisted in tussocks that had not been invaded by hybrid *Sarcocornia*. Such roots and rhizomes were mainly below the more superficial roots of *Sarcocornia perennis* (below 10 cm depth) in areas where *Spartina* tillers persisted and only below 15 cm depth in areas under the *Sarcocornia perennis* canopy (Fig. 5a). In tussocks that it had invaded, hybrid *Sarcocornia* was as dominant underground as above, with a much higher biomass than determined anywhere for the other species (Fig. 5b). A low biomass of *Sarcocornia perennis* roots persisted deeper in the sediment (below 15 cm) under the hybrid canopy, and a substantial biomass was maintained, particularly near the surface, in the peripheral areas still occupied by itself and *Spartina maritima*. Very little biomass of *Spartina* rhizomes and roots survived at the edges of these tussocks.

SEDIMENT REDOX POTENTIAL

Redox potential was highly correlated with topographic elevation on and around the tussocks in October 2001 (Fig. 6). Both surface (0–10 cm) and deeper (11–20 cm) samples showed a linear increase in redox potential with elevation from highly negative values in the intervening channels with bare sediment to positive ones on the vegetated tussocks. There were no significant differences in redox potential between the zones predominantly occupied by *Sarcocornia perennis* ($+124 \pm 5$ mV at 0–10 cm; $+118 \pm 12$ mV at 10–20 cm) and its hybrid ($+134 \pm 9$ mV at 0–10 cm; 123 ± 16 mV at 10–20 cm) (Tukey's Test, $P < 0.05$). Neither were there significant differences in elevation between the centres of tussocks invaded by the hybrid and centres of those still dominated by *S. perennis*.

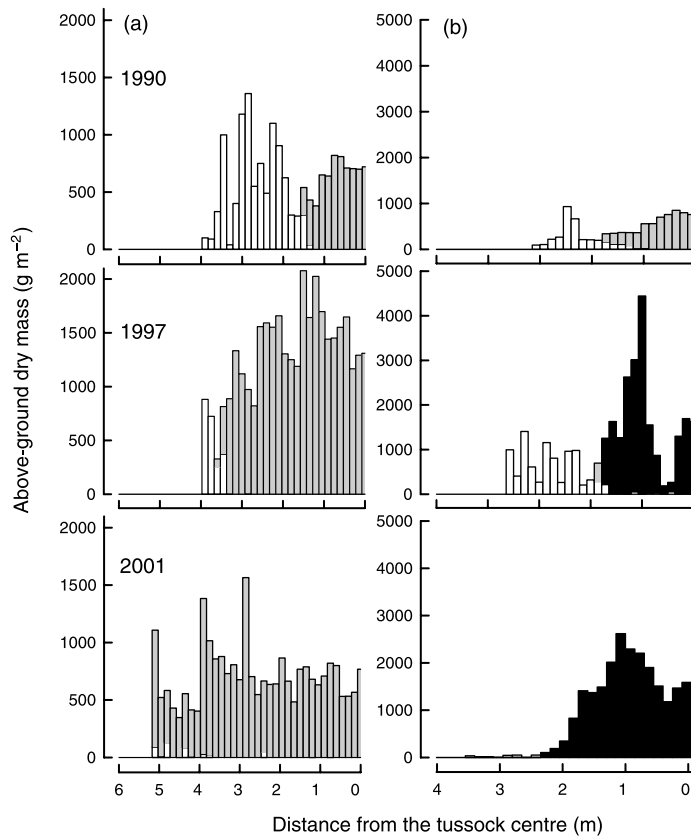


Fig. 4 Distribution of dry mass in radial transects of two representative, permanently marked tussocks in the well-drained lagoon at Odiel marshes, south-west Spain, in 1990, 1997 and 2001. (a) Tussock not invaded by *Sarcocornia perennis* × *fruticosa*. (b) Tussock invaded by *Sarcocornia perennis* × *fruticosa*. Species: *Spartina maritima* (□); *Sarcocornia perennis* (▣); *Sarcocornia perennis* × *fruticosa* (■). These tussocks were included in those sampled for below-ground dry mass (Fig. 5).

Discussion

The appearance and rise to local dominance of the hybrid *Sarcocornia* adds a remarkable new dimension to the successional processes already documented for these rapidly accreting, well-drained salt marsh systems (Castellanos *et al.* 1994). The hybrid has not appeared on any tussock that was not first colonized by *Sarcocornia perennis* and such dependence on prior colonization could therefore be a striking and individual form of facilitation ('genetic facilitation') that does not appear to have been reported previously. The resulting hybrid seedlings, which were apparently able to become established on the highest parts of the tussocks, then developed into plants with a more upright growth form and greater biomass that could out-compete the more prostrate, sprawling maternal species. The hybrid attained dominance in only a few years on many of the tussocks that it was able to invade, both in terms of canopy and root biomass. Comparison with the continued dominance of *S. perennis* on those tussocks that were not invaded leaves little doubt as to the relative competitive abilities of the two types at the higher marsh elevations characteristic of the tussocks.

The long-term data show that *S. perennis* had already begun to colonize the higher tussocks in 1984.

By 1990 it was evident that its ability to displace the primary colonist, *Spartina maritima*, was dependent on continuing sediment accretion that locally increased elevation, reduced submergence and created a less hypoxic and thus more favourable root environment (see Castellanos *et al.* 1994). The hybrid *Sarcocornia* reported here was not recorded until 1997. In the intervening 13 years, the centres of the tussocks experienced average vertical accretion of 46 cm. It is very likely that the hybrid is less tolerant of reducing conditions than *S. perennis*, because its other parent is restricted to the higher margins of the marsh that are rarely inundated with seawater. If so, its eventual establishment would have been facilitated also by the ability of *S. perennis* to trap and stabilize sediment over this period, as accretion was much slower around the edges of the tussocks. Although the redox potential of the surface sediment of *Sarcocornia*-dominated tussocks in 2001 was very similar to that in 1990, the deeper layer (10–20 cm) had changed from highly reducing (−168 mV) to strongly oxidizing (*c.* +120 mV) over this interval (cf. Castellanos *et al.* 1994). Several studies have now described facilitation by amelioration of anoxia in lower parts of salt marshes (Bertness & Leonard 1997). However, tussocks not invaded by the hybrid have continued to accrete at a similar rate to those not invaded; consequently, the small differences in elevation and sediment

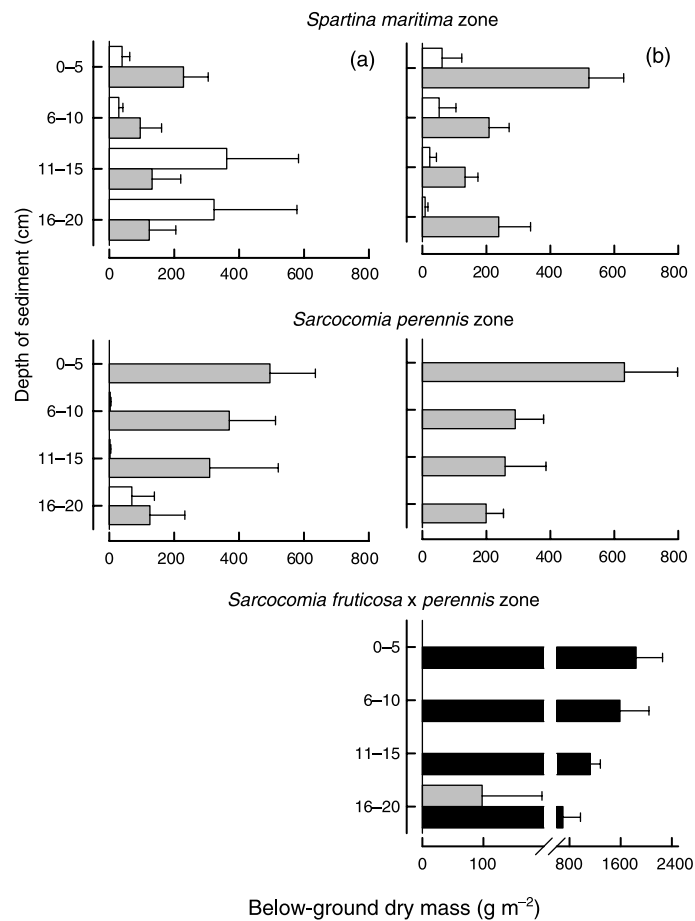


Fig. 5 Depth profiles of below-ground dry mass in different vegetational zones of tussocks in the well-drained lagoon at Odiel marshes, south-west Spain, in October 2001. Values are means (+ SE) of five tussocks. (a) Tussocks not invaded by *Sarcocornia perennis* × *fruticosa*. (b) Tussocks invaded by *Sarcocornia perennis* × *fruticosa*. Species: *Spartina maritima* (□); *Sarcocornia perennis* (▣); *Sarcocornia perennis* × *fruticosa* (■).

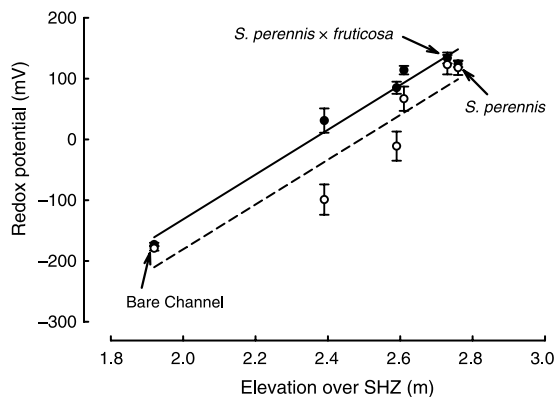


Fig. 6 Relationships between sediment redox potential at two depths and surface elevation above Spanish Hydrographic Zero (SHZ) of representative tussocks in the well-drained lagoon at Odiel marshes, south-west Spain, in October 2001. Depths: 0–10 cm (●), $y = -868 + 368x$, $r^2 = 0.98$, $P < 0.001$; 10–20 cm (○) $y = -917 + 368x$, $r^2 = 0.87$, $P < 0.01$. Elevations representing areas occupied by *Sarcocornia perennis* and *Sarcocornia perennis* × *fruticosa*, and the bare channels between tussocks are marked. $n = 10$ (five tussocks invaded by the hybrid and five not invaded by the hybrid).

redox potential between areas occupied by the hybrid and *S. perennis* in 2001 were not significant.

As one parent (*Sarcocornia perennis*) was already established as a dominant in the central areas of the raised tussocks and the other (*S. fruticosa*) was mainly distributed on high marshes some 1 km distant, it is more than likely that the hybridization occurred *in situ* on the tussocks. It is unlikely that the hybridization occurred on the high marsh and that hybrid seeds were transported to the tussocks in the lagoon. Hence the 'invasion' would probably have been through wind-borne pollen from *S. fruticosa* being deposited on the receptive stigmas of *S. perennis*. The flowering period of *S. perennis* (September to November) starts about 1 month earlier than that of *S. fruticosa* (October to December) in southern Spain and flowers of both species are protandrous (Valdés *et al.* 1987); hence outbreeding is likely and ample pollen of *S. fruticosa* could be available to *S. perennis* stigmas in October and November. A contribution to the delay in invasion may have been simply the time taken for the coincidence of suitable conditions for cross-pollination, as many of the tussocks have still not been invaded. The genetic

and environmental facilitation of the hybrid by *S. perennis* and its subsequent superior competitive ability over its progenitor provide further evidence of the complexity of successional processes; facilitation mechanisms may dominate under more extreme environmental conditions and inhibition by competition may play a greater role in structuring communities when abiotic stress has been reduced (Connell & Slatyer 1977; Miles 1987; Callaway *et al.* 2002). Consequently, some species that begin their lives as the beneficiaries of nurse plants become competitors with their former benefactors (Callaway & Walker 1997).

Although intermediate between its parents, the hybrid was morphologically more similar to the more geographically distant *S. fruticosa* than to the indigenous *S. perennis*. This may be explained by the relative genomic contributions of the parents. Previous reports suggest that *S. perennis* is typically diploid ($2n = 18$), whereas *S. fruticosa* is typically octaploid ($2n = 72$) and hybrids may be $2n = 45, 54$ or 72 (Castroviejo & Coello 1980; Castroviejo 1990; Castroviejo & Lago 1992). Recently, Luque *et al.* (unpublished observations) have confirmed counts of 18, 72 and 45, respectively, in material of the three types from Odiel and therefore *S. fruticosa* would have contributed four times as many chromosomes to the progeny as *S. perennis*. The genetic analysis using RAPDs confirmed the substantially greater affinity of the hybrid forms with *S. fruticosa* than with *S. perennis*. RAPD markers have become established as a valuable and virtually non-destructive tool for characterization of hybrids (Elisiário *et al.* 1999; Lim *et al.* 1999). Unlike morphological markers they are independent of stage of development and environmentally induced variation.

It remains to be seen how long the hybrid will persist in this successional sequence and whether it will prove invasive elsewhere. Only sporadic hybrids between these species have been reported previously (Castroviejo & Lago 1992). This stage in the succession could represent a temporary hybrid zone that can only exist between certain elevational limits because of the colonizing opportunities provided by an intermediate and disturbed habitat (Rieseberg & Carney 1998). *Atriplex portulacoides* also appeared in 1997 in small amounts but may have the potential to become a dominant. Likewise the contribution of hybrid vigour to the competitive ability of the hybrid is not known (Rieseberg & Carney 1998). Alternatively, the RAPD evidence is compatible with the early stages of a genic view of speciation (Wu 2001). Hybridization has been a force in shaping salt-marsh communities, particularly in the genus *Spartina*. The best-known example is *S. anglica*, an allopolyploid species that arose after the N. American *S. alterniflora* was accidentally introduced to Britain and crossed with native *S. maritima*; it was able to colonize mudflats lower in the tidal frame than its native parent and proved to be an invasive dominant in such habitats for many decades, although many extensive stands have undergone 'die-back' subsequently (see Gray *et al.* 1991). Genetic analysis points to a bottleneck at the

time of speciation and the chloroplast DNA has revealed that *S. alterniflora* was the maternal parent (Ferris *et al.* 1997; Baumel *et al.* 2001). RAPD markers, however, suggest that there may have been several hybridization events with different pollen parents (Ayres & Strong 2001).

The *Sarcocornia* hybrid in the Odiel successional series may show similarities to the *Spartina* one: only one species is likely to have been the maternal parent (*S. perennis*) and variability in RAPD markers was consistent with the likelihood (from their spatial distribution on the tussocks) that each individual resulted from a different pollination. Despite the dominant effects of the *S. fruticosa* genome on RAPD variation in the hybrid, there were some indications of genetic correspondence between particular hybrids and putative mothers on the same tussock: the hybrid on tussock IX was the most dissimilar to all other hybrids examined and, likewise, *S. perennis* on that tussock was the most dissimilar to all its conspecifics; conversely, the hybrids on tussocks IV, V and VI were all very similar, as were the individuals of *S. perennis* on the same tussocks; the individuals examined on tussocks VII and VIII were each other's closest relatives for both taxa. In another recent example, introduction of *Spartina alterniflora* from the east to the west coasts of the USA has allowed hybridization with the native *S. foliosa* in San Francisco Bay. Pollen swamping and superior siring ability by the alien threatens the genetic integrity and even existence of *S. foliosa* locally (Anttila *et al.* 1998). In this case hybrid formation has been reciprocal (Anttila *et al.* 2000) and nine different categories of hybrid, indicating backcrossing and introgression, have been detected by RAPD analysis (Ayres *et al.* 1999).

The changes in surface elevation and the composition and structure of the vegetation that have occurred over the 17 years of this study provide direct evidence of the mechanisms of salt-marsh succession. The findings support and considerably extend conclusions based largely on evidence from spatial distribution (Castellanos *et al.* 1994). Elsewhere, genetic differences between populations of a single foundation species (*sensu* Dayton 1975) such as *Spartina alterniflora* have been shown to affect considerably the functional properties of salt-marsh ecosystems (Seliskar *et al.* 2002). Although hybridization has long been recognized as a major force in invasions by alien salt-marsh dominants of the genus *Spartina*, this study of *Sarcocornia* has demonstrated successional development directly involving invasion through hybridization with an existing dominant. Such 'genetic facilitation' may have a wider role in salt marshes, or in succession generally, and may be more readily detected with the increasing use of molecular markers.

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References

- Alvarez, J., Alcaraz, F. & Ortiz, R. (2000) Soil salinity and moisture gradients and plant zonation in Mediterranean salt marshes of southeast Spain. *Wetlands*, **20**, 357–372.
- Anttila, C.K., Daehler, C.C., Rank, N.E. & Strong, D.R. (1998) Greater male fitness of a rare invader (*Spartina alterniflora*, Poaceae) threatens a common native (*Spartina foliosa*) with hybridization. *American Journal of Botany*, **85**, 1597–1601.
- Anttila, C.K., King, R.A., Ferris, C., Ayres, D.R. & Strong, D.R. (2000) Reciprocal hybrid formation of *Spartina*. San Francisco Bay. *Molecular Ecology*, **9**, 765–770.
- Ayres, D.R., Garcia-Rossi, D., Davis, H.G. & Strong, D.R. (1999) Extent and degree of hybridization between exotic (*Spartina alterniflora*) and native (*S. foliosa*) cordgrass (Poaceae) in California, USA determined by random amplified polymorphic DNA (RAPDs). *Molecular Ecology*, **8**, 1179–1186.
- Ayres, D.R. & Strong, D.R. (2001) Origin and genetic diversity of *Spartina anglica* (Poaceae) using nuclear DNA markers. *American Journal of Botany*, **88**, 1863–1867.
- Baumel, A., Ainouche, M.L. & Levasseur, J.E. (2001) Molecular investigations in populations of *Spartina anglica* C.E. Hubbard (Poaceae) invading coastal Brittany (France). *Molecular Ecology*, **10**, 1689–1701.
- Bertness, M.D. (1991) Interspecific interactions among high marsh perennials in a New England salt marsh. *Ecology*, **72**, 125–137.
- Bertness, M.D. & Leonard, G.H. (1997) The role of positive interactions in communities: lessons from intertidal habitats. *Ecology*, **78**, 1976–1989.
- Callaway, R.M. (1994) Facilitative and interfering effects of *Arthrocnemum subterminale* on winter annuals. *Ecology*, **75**, 681–686.
- Callaway, R.M., Brooker, R.W., Choler, P., Kikvidze, Z., Lortie, C.J., Michalet, R. *et al.* (2002) Positive interactions among alpine plants increase with stress. *Nature*, **417**, 844–848.
- Callaway, R.M. & Walker, L.R. (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology*, **78**, 1958–1965.
- Castellanos, E.M., Figueroa, M.E. & Davy, A.J. (1994) Nucleation and facilitation in saltmarsh succession: interactions between *Spartina maritima* and *Arthrocnemum perenne*. *Journal of Ecology*, **82**, 239–248.
- Castellanos, E.M., Heredia, C., Figueroa, M.E. & Davy, A.J. (1998) Tiller dynamics in *Spartina maritima*. Successional and non-successional Mediterranean salt marsh. *Plant Ecology*, **137**, 213–225.
- Castillo, J.M., Fernández-Baco, L., Castellanos, E.M., Luque, C.J., Figueroa, M.E. & Davy, A.J. (2000) Lower limits of *Spartina densiflora* and *S. maritima* in a Mediterranean salt marsh determined by different ecophysiological tolerances. *Journal of Ecology*, **88**, 801–812.
- Castroviejo, S. (1990) *Sarcocornia*. *Flora Iberica: Plantas Vasculares de la Península Ibérica e Islas Baleares*, Vol. 2. *Platanaceae–Plumbaginaceae (partim)* (eds S. Castroviejo, M. Lainz, G. López González, P. Montserrat, F. Muñoz Garmendia, J. Paiva & L. Villar), pp. 526–531. Real Jardín Botánico, C.S.I.C., Madrid.
- Castroviejo, S. & Coello, P. (1980) Datos cariológicos y taxonómicos sobre los Salicorniinae A.J. Scott Ibéricas. *Anales Jardín Botánico de Madrid*, **37**, 41–73.
- Castroviejo, S. & Lago, E. (1992) Datos acerca de la hibridación en el género *Sarcocornia* (Chenopodiaceae). *Anales Jardín Botánico de Madrid*, **50**, 163–170.
- Connell, J.H. & Slatyer, R.O. (1977) Mechanisms of succession in natural communities and their role in community stability and organization. *American Naturalist*, **111**, 1119–1144.
- Costa, C.S.B., Marangoni, J.C. & Azevedo, A.M.G. (2003) Plant zonation in irregularly flooded salt marshes: relative importance of stress tolerance and biological interactions. *Journal of Ecology*, **91**, in press.
- Daehler, C.C. & Strong, D.R. (1997) Hybridization between introduced smooth cordgrass (*Spartina alterniflora*; Poaceae) and native California cordgrass (*Spartina foliosa*) in San Francisco Bay, California, USA. *American Journal of Botany*, **84**, 607–611.
- Davy, A.J. (2000) Development and structure of salt marshes: community patterns in time and space. *Concepts and Controversies in Tidal Marsh Ecology* (eds M.P. Weinstein & D.A. Kreeger), pp. 137–156. Kluwer Publishing, Dordrecht, The Netherlands.
- Davy, A.J., Bishop, G.F. & Costa, C.S.B. (2001) Biological flora of the British Isles: *Salicornia* L. (*Salicornia pusilla* J. Woods, *S. ramosissima* J. Woods, *S. europaea* L., *S. obscura* P.W. Ball & Tutin, *S. nitens* P.W. Ball & Tutin, *S. fragilis* P.W. Ball & Tutin and *S. dolichostachya* Moss). *Journal of Ecology*, **89**, 681–707.
- Davy, A.J. & Costa, C.S.B. (1992) Development and organization of saltmarsh communities. *Coastal Plant Communities of Latin America* (ed. U. Seeliger), pp. 157–178. Academic Press, San Diego.
- Dayton, P.K. (1975) Experimental evaluation of ecological dominance in a rocky intertidal algal community. *Ecological Monographs*, **45**, 137–159.
- Elisiário, P.J., Justo, E.M. & Leitão, J.M. (1999) Identification of mandarin hybrids by isozyme and RAPD analysis. *Scientia Horticulturae*, **81**, 287–299.
- Ferris, C., King, R.A. & Gray, A.J. (1997) Molecular evidence for the maternal parentage in the hybrid origin of *Spartina anglica* C.E. Hubbard. *Molecular Ecology*, **6**, 185–187.
- Gray, A.J., Marshall, D.F. & Raybould, A.F. (1991) A century of evolution in *Spartina anglica*. *Advances in Ecological Research*, **21**, 1–62.
- Lim, S., Teng, P.C., Lee, Y. & Goh, C. (1999) RAPD analysis of some species in the genus *Vanda* (Orchidaceae). *Annals of Botany*, **83**, 193–196.
- Luque, T., Ruiz, C., Avalos, J., Calderón, I.L. & Figueroa, M.E. (1995) Detection and analysis of genetic variation in *Salicornieae* (Chenopodiaceae) using random amplified polymorphic DNA (RAPD) markers. *Taxon*, **44**, 53–63.
- Mantel, N.A. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.
- Miles, J. (1987) Vegetation succession: past and present perceptions. *Colonization, Succession and Stability* (eds A.J. Gray, M.J. Crawley, P.J. Edwards), pp. 1–29. Blackwell Scientific, Oxford.
- Nei, M. & Li, W.H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the USA*, **76**, 5269–5273.
- Oloff, H., de Leeuw, J., Bakker, J.P., Platerink, R.J., van Wijnens, H.J. & de Munck, W. (1997) Vegetation succession and herbivory in a salt marsh: changes induced by sea level rise and silt deposition along an elevational gradient. *Journal of Ecology*, **85**, 799–814.
- Ranwell, D.S., Bird, E.C.F., Hubbard, J.C.R. & Stebbings, R.E. (1964) *Spartina* salt marshes in Southern England. V. Tidal submergence and chlorinity in Poole Harbour. *Journal of Ecology*, **52**, 627–641.
- Raybould, A.F., Gray, A.J., Lawrence, M.J. & Marshall, D.F. (1991) The evolution of *Spartina anglica* C.E. Hubbard (Gramineae): the origin and genetic variability. *Biological Journal of the Linnean Society*, **43**, 111–126.
- Rieseberg, L.H. (1996) Homology among RAPD fragments in interspecific comparisons. *Molecular Ecology*, **5**, 99–105.
- Rieseberg, L.H. & Carney, S.E. (1998) Tansley Review, 102: Plant hybridization. *New Phytologist*, **140**, 599–624.

- Roozen, A.J.M. & Westhoff, V. (1985) A study on long-term salt-marsh succession using permanent plots. *Vegetatio*, **61**, 23–32.
- Seliskar, D.M., Gallagher, J.L., Burdick, D.M. & Mutz, L.A. (2002) The regulation of ecosystem functions by ecotypic variation in the dominant plant: a *Spartina alterniflora* salt-marsh case study. *Journal of Ecology*, **90**, 1–11.
- Sneath, P.H.A. & Sokal, R.R. (1973) *Numerical Taxonomy*. M. Freeman, San Francisco.
- Tansley, S.A. & Brown, C.R. (2000) RAPD variation in the rare and endangered *Leucadendron elimense* (Proteaceae): implications for their conservation. *Biological Conservation*, **95**, 39–48.
- Valdés, B., Aparicio, A., Blanca, G., Cabezudo, B., Clemente, M., Cubas, P. *et al.* (1987) *Flora Vascular de Andalucía Occidental*. Ketres Editora, Barcelona, Spain.
- Willis, A.J. (2000) The changing structure and vegetational history of the 85-year-old saltmarsh at Berrow, North Somerset. *British Saltmarshes* (eds B.R. Sherwood, B.G. Gardiner & T. Harris), pp. 65–80. Linnean Society/Forrest Text, Ceredigion.
- Wu, C.-I. (2001) The genic view of the process of speciation. *Journal of Evolutionary Biology*, **14**, 851–865.

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