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A seascape genetic analysis reveals strong biogeographical structuring driven by contrasting processes in the polyploid saltmarsh species *Puccinellia maritima* and *Triglochin maritima*

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Abstract

Little is known about the processes shaping population structure in saltmarshes. It is expected that the sea should act as a powerful agent of dispersal. Yet, in contrast, import of external propagules into a saltmarsh is thought to be small. To determine the level of connectivity between saltmarsh ecosystems at a macro-geographical scale, we characterized and compared the population structure of two polyploid saltmarsh species, *Puccinellia maritima* and *Triglochin maritima* based on a seascape genetics approach. A discriminant analysis of principal components highlighted a genetic structure for both species arranged according to a regional pattern. Subsequent analysis based on isolation-by-distance and isolation-by-resistance frameworks indicated a strong role of coastal sediment transport processes in delimiting regional structure in *P. maritima*, while additional overland propagule dispersal was indicated for *T. maritima*. The identification and comparison of regional genetic structure and likely determining factors presented here allows us to understand the biogeographical units along the UK coast, between which barriers to connectivity occur not only at the species level but at the ecosystem scale. This information is valuable in plant conservation and community ecology and in the management and restoration of saltmarsh ecosystems.

Keywords: discriminant analysis of principal components, isolation by resistance, polyploidy, *Puccinellia maritima*, saltmarsh, *Triglochin maritima*

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Introduction

Saltmarshes are an excellent example of ecotones as they lie at the transition between terrestrial and marine ecosystems. The influence of the tides on this environment creates a range of extreme conditions (e.g. disturbance, salinity, inundation) leading to a species poor ecosystem, typically of salt tolerant plants that form vegetation communities strongly stratified along an elevation gradient. Saltmarshes provide a broad range of ecosystem services, such as preventing land erosion, providing nitrogen and carbon storage, and forming a refuge for economically important species of fish or crustacean species (Gedan *et al.* 2009). However, the extent of this ecosystem has been dramatically reduced by historical land reclamation and is under further threat, both due to continuing anthropogenic pressures and saltmarshes being trapped between rising sea levels and fixed sea walls, the phenomenon dubbed 'coastal squeeze' (Gedan *et al.* 2009). To counteract the loss, restoration programmes relying on natural recolonization of the plant community have been implemented in the UK (Wolters *et al.* 2005). Understanding the mechanisms shaping the connectivity between saltmarsh ecosystems is therefore of primary importance to design efficient management and restoration policies.

The halophytes composing the north-western Europe saltmarsh vegetation are known to be morphologically variable across their range. They were therefore

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extensively used in classical morphogenetic studies. For example, the early work done on morphological variation in *Plantago maritima* was highly important in the identification and definition of plant 'ecotypes' (Gregor 1938). I *Aster tripolium*, the production of ray florets varies widely both within and between populations and this characteristic was shown to be at least partially genetically inherited (Clapham *et al.* 1942; Duvigneaud & Jacobs 1971; Gray 1987; Huiskes *et al.* 2000). *Suaeda maritima* is also a species displaying a noticeable ecotypic variation (Gray 1974). All these examples suggested that the genetic diversity within saltmarsh species is strongly structured across a macro-geographical scale.

Studies using modern molecular markers to investigate the structure of the genetic diversity within these species showed that genetic differentiation existed between saltmarsh plant populations. (*Aster tripolium*: Krüger *et al.* 2002; Brock *et al.* 2007; *Armeria maritima*: Baumbach & Hellwig 2007; *Suaeda maritima*: Prinz *et al.* 2009; *Spergularia media*: Prinz *et al.* 2010; *Triglochin maritima*: Lambracht *et al.* 2007). However, these studies largely focused on populations collected in inland saline habitat. Because these populations are highly fragmented and sometimes small, they are particularly sensitive to founder effects or genetic drift. Consequently, they give little information about the genetic structure of these species along a coastal system and the putative mechanisms shaping it.

In their population genetic study of *Spergularia media*, Prinz *et al.* (2010) noticed that coastal populations showed an overall lower ϕ_{ST} than inland populations. In *Elytrigia atherica*, populations separated by only few hundred meters but experiencing contrasting selective pressure were less genetically related than distant populations sharing the same environmental conditions (Bockelmann *et al.* 2003). Moreover, other studies looking at the genetic structure around Europe of multiple coastal plant species closely associated with sandy or rocky habitats highlighted extensive geographical clustering (Kadereit *et al.* 2005; Weising & Freitag 2007) and that genetic distance between populations was correlated with the coastal geographic distance separating them (Clausing *et al.* 2000). Altogether, these results suggest that long distance gene flow along the coast is likely to be of critical importance in controlling the dispersal processes that shape the population genetic structure of saltmarsh species.

During the past decade, the emerging field of landscape genetics aimed to investigate the impact of landscape features on structuring the genetic diversity of a species (Manel & Holderegger 2013). In marine or coastal species, specific features will have an impact on the genetic structure (e.g. ocean circulation, tidal regime, wind direction, salinity gradient). The integration of these parameters into the analysis of genetic diversity within a species was named "seascape genetics" (Galindo *et al.* 2006; Selkoe *et al.* 2008). This approach has rarely been used to study the genetic structure of western European saltmarsh species, although research into the genetic structure of sea beet (*Beta vulgaris* ssp. *maritima*) by Fievet *et al.* (2007) provides a notable example.

We sought to identify the effect of coastal environmental processes on the genetic connectivity among UK saltmarshes. The overall aim of such work is to inform saltmarsh restoration practice through developing a greater understanding of dispersal and colonization dynamics around the UK coasts. Here, we used a seascape genetics approach in two common, but ecologically contrasting, saltmarsh species *Puccinellia maritima* and *Triglochin maritima*. Using microsatellite markers designed for these species, we asked (i) What is the level of genetic connectivity between populations? (ii) Is the genetic structure comparable between species? (iii) What factors best explain the population genetic structure of both species (e.g. isolation by distance, tidal currents)?

Material and methods

Study species

Puccinellia maritima is a perennial grass naturally occurring from the early stages of saltmarsh succession (Gray & Scott 1977). This colonist species is considered to be an engineer species that permits sediment accretion, which in turn facilitates plant community development (Langlois *et al.* 2003). Colonization by *P. maritima* is therefore of primary importance for the development of the biotic and abiotic environment of a saltmarsh. The sexual reproduction of this species is predominantly outbred with caryopses being dispersed by the tides. Asexual reproduction also occurs through dispersion of uprooted tillers (Brereton 1971; Gray & Scott 1977). Morphogenetic analysis of this species showed that this plant is morphologically variable across its range and that much of this variation is under genetic control (Gray & Scott 1980; Gray 1985, 1987).

Triglochin maritima is also a perennial species but typically occurs once sediments are stabilized. Although self-compatible (Lambracht *et al.* 2007), its flowers are strongly protogynous, preventing auto-pollination. Dispersal mainly occurs by seeds, which show good viability after a floatation time of several months in sea water (Davy & Bishop 1991). Asexual propagation occurs only via centrifugal expansion of individuals producing characteristic rings (Davy & Bishop 1991). Under uniform glasshouse conditions, differences in growth

between populations collected at different elevations on the same saltmarsh have been hypothesized to be genetically based (Jefferies 1977).

Sample collection and molecular work

Samples of *P. maritima* and *T. maritima* were collected from 15 and 14 populations respectively across the UK over two successive field seasons in summer 2011 and 2012 (Table 1). Samples were collected randomly on each saltmarsh where the species occurred allowing a minimum distance of at least 5 m between samples. The average distance between adjacent sampled individuals per site was 16–36 m for *T. maritima* and 15–43 m for *P. maritima*. In one exception to this sampling regime, the Lochgoilhead site, the minimum distance between two adjacent individuals was reduced to one 1 m, with average distance between individuals sampled for *T. maritima* and *P. maritima* of 5 and 6 m respectively due to the small size of this population. Care was taken to avoid collection of physically linked individuals within any site. Samples were dried immediately in fine-grained silica gel and stored in a dry and dark place until analysed.

Genomic DNA was extracted from approximately 10 mg of dried leaf tissue using the DNeasy 96 plant kit (Qiagen) following the manufacturer's instructions. DNA was quantified using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific) and adjusted to 5–20 ng/μl using ultra-pure PCR water (Bioline). PCR protocols are detailed in Rouger *et al.* (2014) for *P. maritima* and Rouger & Jump (2013) for *T. maritima*. Fragment analysis was conducted by DNA Sequencing and Services (University of Dundee, UK) using an ABI 3730 DNA Sequencer (Applied Biosystems). Fragment sizes were scored using the software STRand (Toonen & Hughes 2001) and alleles allocated to their respective size classes using the package MsatAllele (Alberto 2009) in R (R Core Team 2013). Loci Pm27 (*P. maritima)* and Tm07 (*T. maritima*) were not included in this analysis due to high amplification failure rates. The full analysis was therefore based on 11 loci in *P. maritima* and 19 loci in *T. maritima*.

Data analysis

Puccinellia maritima and *Triglochin maritima* are both polyploid species. Although variable in ploidy across their range, the two species are reported to be octoploid in the UK (Scott & Gray 1976; Davy & Bishop 1991). Population genetics analysis of polyploids is still challenging due to diverse technical and statistical issues among which the difficulty to characterize allelic dosage of each individual or differing inheritance pattern between loci (Dufresne *et al.* 2014). To circumvent these problems, each allele was scored as present or absent in each individual. Each individual was then characterized by a binary vector as long as the total number of alleles detected across all individuals. The presence/absence matrix obtained was then comparable to a data set obtained with classic genetic fingerprinting method such as AFLP. Although part of the genetic information is lost (e.g. it is not possible to calculate allelic frequency), this method is known to give satisfactory results in recent population genetic work in polyploids (Sampson & Byrne 2012; Vallejo-Marin & Lye 2013).

Genetic parameters. GenAlEx 6.5 (Peakall & Smouse 2012) was used to calculate the number of alleles

Table 1 Location and number of samples collected in each population of *Triglochin maritima* and *Puccinellia maritima*. The number of samples successfully amplified is given in parentheses

Population	Code	Sampling year	*T. maritima*	*P. maritima*	Longitude	Latitude
Brancaster	B	2011	30 (29)	30	0.6230°E	52.9721°N
Goosemoor	G	2011	30	30	3.4541°W	50.6836°N
Lepe	L	2011	22	30	1.3860°W	50.8621°N
Nigg Bay	N	2011	30 (29)	30 (29)	4.0166°W	57.7374°N
Ryan's field	R	2011	30	30 (28)	5.4328°W	50.1768°N
Seal Sands	SE	2011	30	30	1.2140°W	54.6233°N
Skinflats	SK	2011	30	30	3.7320°W	56.0553°N
Walborough	W	2011	30	30	2.9847°W	51.3140°N
Hemley	H	2012	30	30 (29)	1.3389°E	52.0324°N
Loch carron	LC	2012	30 (29)	30	5.4517°W	57.4179°N
Lochgoilhead	LG	2012	20 (19)	30	4.9142°W	56.1601°N
Morecambe	M	2012	0	30 (29)	2.8082°W	54.1412°N
Paull Holme Strays	P	2012	30 (29)	30	0.1749°W	53.6821°N
Tollesbury	T	2012	30	30	0.8333°E	51.7699°N
Welwick	WE	2012	30 (29)	30	0.0203°E	53.6477°N

detected per population. The number of genotypes within each population was calculated by detecting individuals sharing the same genotype using the software GenoType (Meirmans & Van Tienderen 2004). Occurrence of identical multilocus genotypes (clones) in our data set was variable between populations in *P. maritima* and absent in *T. maritima* (Table 2). Consequently, all sampled individuals were included in subsequent analysis, and measures of diversity within or between populations were chosen accordingly. Genetic diversity in each population was measured using the Kosman index of diversity within populations following the equation:

$$KW_\rho(P) = 1/n\, Ass^\rho_{\max}(P,P)$$
(Equation 5 from Kosman & Leonard 2007),

where each individual of the population P of size n is paired to another individual from the same population as to maximize the sum of distance between pairs ($Ass^\rho_{\max}(P,P)$). The distance between individuals (ρ) was calculated from the presence/absence matrix using Dice dissimilarity index commonly used to calculate genetic distance in polyploids (Vallejo-Marin & Lye 2013; Cidade *et al.* 2013). Dice dissimilarity coefficient between individuals was calculated in R using the package ade4 (Dray & Dufour 2007). KW was calculated with a custom R script (Appendix S1, Supporting information) using the assignment problem algorithm implemented in the function *solve_LSAP* of the package clue (Hornik 2005).

Table 2 Genetic diversity parameters calculated for sampled populations of *Triglochin maritima* and *Puccinellia maritima*

	T. maritima				*P. maritima*			
Population	*N*	N_A	N_G	KW	*N*	N_A	N_G	KW
Brancaster	29	107	29	0.7375	30	89	23	0.7289
Goosemoor	30	115	30	0.7321	30	85	27	0.7309
Lepe	22	107	22	0.7088	30	92	30	0.7545
Nigg Bay	29	114	29	0.7375	29	91	19	0.6986
Ryan's field	30	95	30	0.7046	28	65	26	0.6271
Seal Sands	30	117	30	0.7409	30	81	21	0.7408
Skinflats	30	104	30	0.7123	30	66	18	0.6602
Walborough	30	116	30	0.7380	30	103	30	0.7627
Hemley	30	111	30	0.7277	29	101	29	0.7142
Loch Carron	29	91	29	0.6808	30	89	30	0.7584
Lochgoilhead	19	87	19	0.6440	30	88	30	0.7213
Morecambe	—	—	—	—	29	87	19	0.6601
Paull Holme Strays	29	106	29	0.6993	30	79	18	0.6196
Tollesbury	30	112	30	0.7201	30	96	30	0.7510
Welwick	29	110	29	0.7167	30	82	22	0.6805
Total	396	182	396		445	175	369	

N: Number of individuals successfully amplified; N_A: Number of alleles detected; N_G: Number of multilocus genotypes identified.

Genetic structure. A discriminant analysis of principal components (DAPC) was used to assign individuals to a predefined number of genetic clusters with the R package adegenet (Jombart 2008; Jombart *et al.* 2010). This method offers a good alternative to Bayesian analysis of assignment such as STRUCTURE (Pritchard *et al.* 2000). This multivariate approach is particularly suitable for polyploids as it does not assume populations to be at Hardy–Weinberg equilibrium and does not require assumptions about the inheritance pattern of each locus (Dufresne *et al.* 2014). We used sequential K-means clustering (all PCs retained, 100 starts of 10^6 iterations each) on our data set to characterize the most likely number of clusters detected in each species based on the Bayesian information criterion. The analysis was run for K spanning from 1 to 25. DAPC was then run using values of K around the most likely number of clusters as *a priori* clusters. The posterior probabilities of assignment for each individual were then input into distruct (Rosenberg 2003) to help visualize results at different values of K.

The partitioning of genetic variation between groups was assessed based on a two-level hierarchical AMOVA (Excoffier *et al.* 1992). The first level was defined by the clusters discriminated with the DAPC, while the second level was defined according to the populations sampled. The AMOVA was constructed for different values of K, and significance levels were tested using 999 permutations following the procedure given by Excoffier *et al.* (1992) and implemented in the package ade4 in R (Dray & Dufour 2007). The matrix of genetic distance between individuals used for the AMOVA was the Dice dissimilarity matrix calculated previously.

Even though the AMOVA framework is very flexible to test divergence between populations under different evolutionary scenarios, it makes assumptions that are very likely to be violated for the two species studied (no inbreeding, no migration, pure drift, random sampling at each level) (Excoffier *et al.* 1992). Therefore, the Kosman distance between population (KB) was preferred over pairwise ϕ_{ST} to measure distance between populations based on the matrix of genetic distance between individuals (Kosman & Leonard 2007). This dissimilarity index is particularly well suited for the study of organisms using clonal reproduction and/or which are likely to depart from Hardy–Weinberg equilibrium. This time, each individual of a population P_1 is paired to an individual of a population P_2 as to minimize the sum of distance between pairs (noted $Ass^\rho_{\min}(P_1,P_2)$), where ρ is the between individuals dissimilarity coefficient used). This sum is then divided by the number of pairs:

$$KB_{\rho}(P1,P2) = 1/n\, Ass^{\rho}_{\min}(P_1,P_2)$$

(Equation 5 from Kosman & Leonard 2007).

This requires population size to be the same between populations. As this is not always the case, KB was calculated from the average of 1000 bootstrap replicates of 30 individuals. Again, the matching of individuals giving the minimum sum was found using the assignment problem algorithm implemented in the function *solve_LSAP* of the R package clue (Hornik 2005) using a custom script (Appendix S1, Supporting information).

This measure of dissimilarity between populations was used to conduct a nonmetric multidimensional scaling (NMDS) ordination of populations with the R package vegan (Dixon 2003). We examined the solution along three axes using a maximum number of random starts of 100. To assist the pattern of genetic structure observed both on the DAPC and on the NMDS ordination, a Mantel test of correlation between the matrixes of population dissimilarity of the two species was made using the package ade4 in R.

Factors shaping the genetic structure. Isolation by distance between populations was tested using Mantel test in the R package vegan using 999 permutations. The KB dissimilarity between populations was tested against great circle geographical distance (d_{GC}) calculated using the function *distMeeus* implemented within the R package geosphere and against coastal distance between saltmarshes (d_C) calculated manually from a 1:250 000 map of the UK. Unmodified and log-transformed distance were used.

The correlation between latitude and population dissimilarity was also tested. Latitudinal distance (d_{lat}) between populations was calculated as the great circle distance between the projections of the population coordinates onto the prime meridian. Unmodified and log-transformed latitudinal distance were tested.

Aquatic dispersal in saltmarsh plants occurs primarily through sea currents around the coast. Current dynamics around the UK must therefore play a key role in gene flow between saltmarshes. This hypothesis was tested using the isolation-by-resistance framework implemented in CIRCUITSCAPE (McRae 2006). This method uses circuit theory seeing the landscape as a conductive surface. The landscape is divided into cells of equal dimensions and characterized by a resistance (or conductance) value, the most permeable cells to movement or gene flow having the least resistance (or highest conductance). Based on this landscape grid, the programme calculates pairwise resistance between each pair of populations. This matrix of pairwise resistance can then be tested for correlation with population dissimilarity using a classic Mantel test. Three models were built and tested

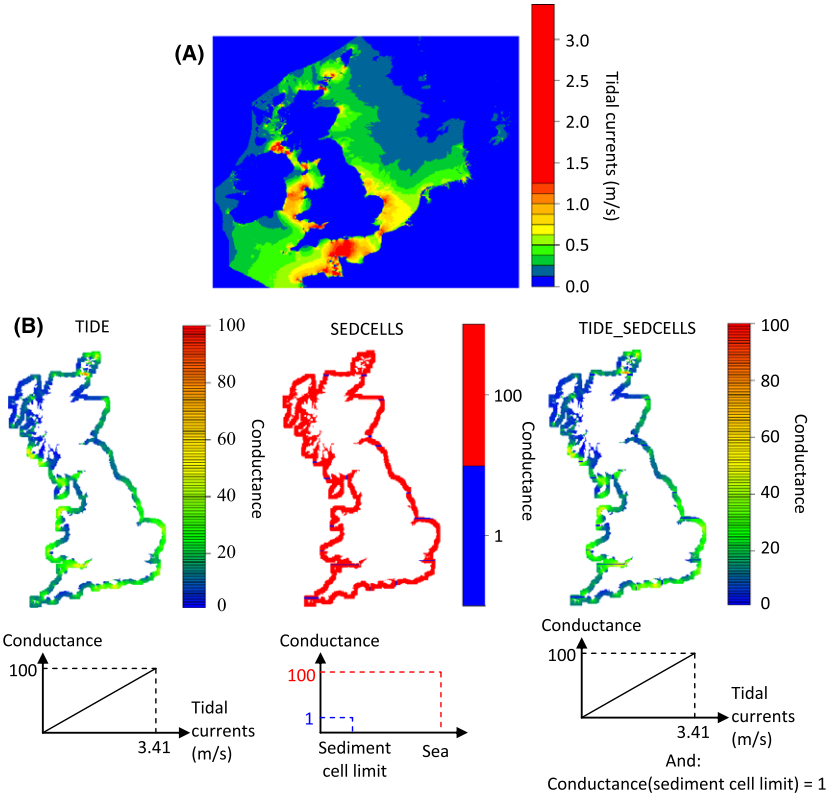

Fig. 1 Map of (A) tidal currents velocities around the UK and of (B) the three isolation-by-resistance models built in this analysis (TIDE, SEDCELLS, TIDE_SEDCELLS).

following this method (Fig. 1B). Instead of using coastal currents which are generally weak around the UK, the first model (TIDE) used tidal current velocities around the UK to determine landscape conductance. Tidal current data were provided on a 0.025° longitude by 0.0167° latitude grid (J. Polton, National Oceanography Center) (Fig. 1A). We restricted our analysis to a 10-cell wide band around the UK coastline. Values of tidal currents within this band were divided into centiles, and a value of cell conductance was allocated to each centile on a scale from 1 to 100. The second model (SEDCELLS) was based on the sediment units defined in May & Hansom (2003). The exchange of sediments between these units is understood to be very limited, dividing points between sediment cells being headlands around which almost no sediments can pass or embayments which act as sediments sinks due to converging long-shore currents (May & Hansom 2003). The model SEDCELLS hypothesizes that such limited exchange occurs also for plant propagules, thus restricting gene flow between populations belonging to different sediment units. In this second model, the same geographical grid as for the 'TIDE' model was used. Each cell was given a conductance value of 100 apart from the sediment cell boundaries where a three-cell wide band was given a lower conductance value of 1. The third model (TIDE_SEDCELLS) aggregates the two first models. Each cell had the conductance value allocated in the 'TIDE' model except at the sediment unit boundaries where a three-cell wide band was given a conductance value of 1.

Grids of landscape conductance for each model were produced out of the tidal current data using a custom script in R. They were then imported into the software CIRCUITSCAPE and pairwise landscape resistance between populations were inferred using a cell connection scheme of eight neighbours (McRae 2006). Similarly to isolation-by-distance models, unmodified and log-transformed resistance were used to test correlation with genetic dissimilarity between populations.

When comparing models, the best model should not only show the best correlation to genetic distance but also a significant partial correlation when controlling for the other competing models (McRae & Beier 2007). Therefore and in order to compare the different models investigated in this study, we used partial Mantel tests implemented in the package vegan in R.

Results

Genetic parameters

The 19 microsatellite loci used in *T. maritima* yielded 182 alleles overall with the number of alleles detected in each population varying from 87 to 117 (only 19 individuals were successfully amplified in the population of Loch Carron where 87 alleles were detected). In *P. maritima*, the 11 microsatellite loci used yielded 175 alleles. The number of alleles per population spanned from 65 to 103 (Table 2). The average genetic diversity within populations measured with KW was of 0.7143 and 0.7100 for *T. maritima* and *P. maritima*, respectively. The number of different multilocus genotypes detected within *P. maritima* was lower than the number of samples amplified in 9 of 15 populations. Furthermore, the populations of Brancaster, Welwick and Paull Holme Strays were shown to share common genotypes (Brancaster–Welwick: 1 shared genotype, Brancaster–Paull Holme Strays: 1 shared genotype, Welwick–Paull Holme Strays: 2 shared genotypes). In *T. maritima*, each multilocus genotype was represented by a unique sample (Table 2).

Genetic structure

The sequential *K*-means clustering showed that the most likely number of clusters for both species was around $K = 5$. Although, based on the BIC score, this value was clear for *T. maritima*, it was more ambiguous for *P. maritima* (Fig. 2). This pattern was further confirmed with *T. maritima* showing a clear separation between segregated clusters, while the limits between groups in *P. maritima* were not as distinct.

Two particularly important observations can be made from the DAPC analysis. First, a regional clustering of the genetic structure was observable along the coast for both species. This was confirmed by the two-level hierarchical AMOVA using regions indicated by the DAPC. The amount of genetic variation explained among regions was significant in both species for all values of *K* considered (Table 3). However, at any value of *K*, the genetic variation explained between populations within regions remained significant, indicating that differentiation between populations remains for both species.

Second, the regional organization of the genetic structure showed similarities between species, as confirmed by the Mantel test comparing the matrices of population dissimilarity between the two species ($r = 0.608$, $P < 0.001$). These similarities in genetic structure between species were further developed looking at the NMDS ordination where the groups segregated previously for both species on the DAPC were coherent with the results of this analysis. (Fig. 3). In *T. maritima*, the populations grouped by the DAPC within an eastern group (Paull Holme Strays, Welwick, Brancaster and Seal Sands) were shown to be close to a cluster incorporating most southern populations (Walborough, Lepe, Hemley, Tollesbury and Goosemoor) (Fig. 3). A similar pattern was found in *P. maritima*. Interestingly, the

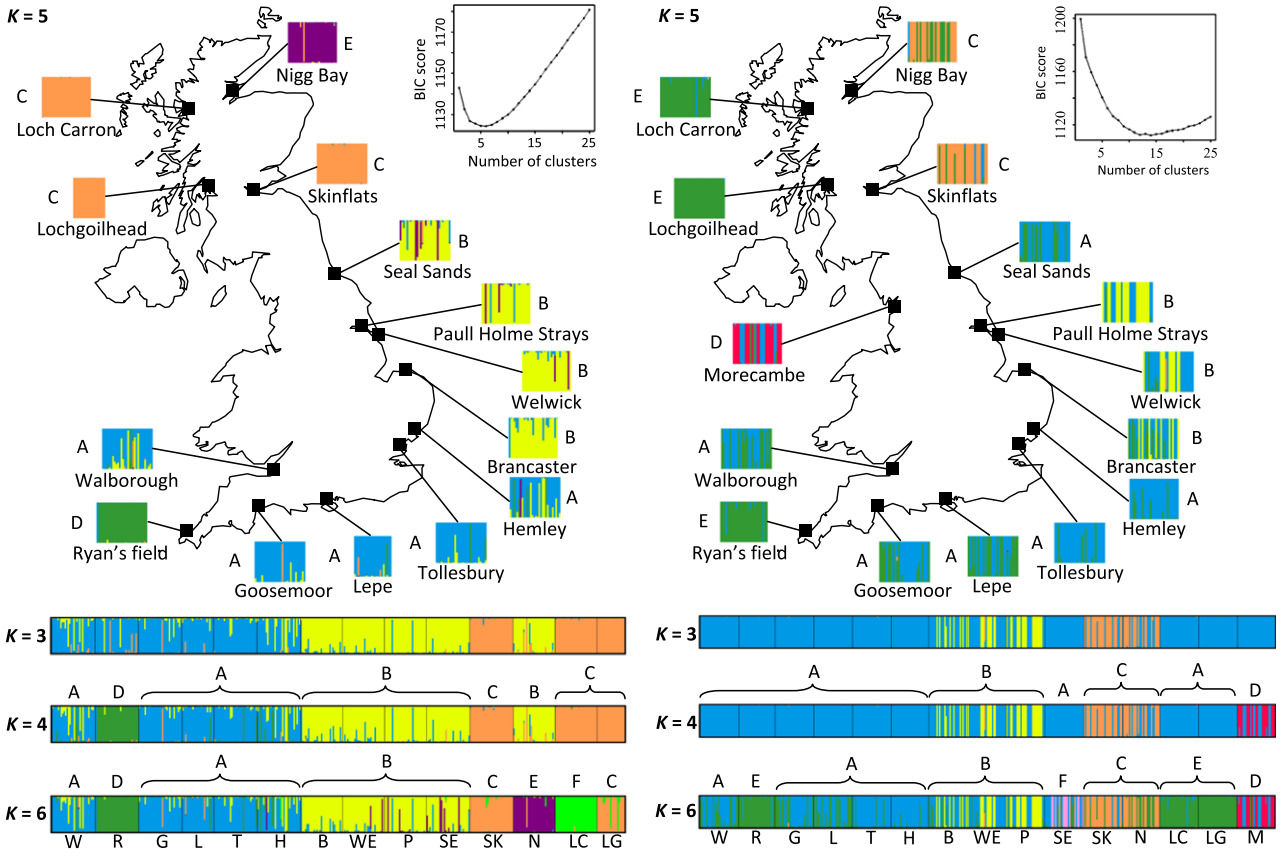

Fig. 2 Discriminant analysis of principal components showing the genetic clustering of populations of *Triglochin maritima* (left) and *Puccinellia maritima* (right) at successive values of K. The letters define the regions used as input into the subsequent AMOVA. Population codes used here are given in Table 1.

Table 3 Two-level AMOVA for *Puccinellia maritima* and *Triglochin maritima*. Regions were segregated based on discriminant analysis of principal components at successive values of K

	Triglochin maritima						*Puccinellia maritima*					
	d.f.	SS	MS	Est. Var.	%	*P*	d.f.	SS	MS	Est. Var.	%	*P*
K = 4												
Among regions	3	6.37	2.12	0.015	4.17	***	3	10.19	3.40	0.025	6.81	***
Among populations within region	10	7.57	0.75	0.015	4.28	***	11	14.07	1.28	0.033	8.98	***
Within populations	382	124.83	0.33	0.327	91.54	***	430	131.95	0.31	0.307	84.21	***
K = 5												
Among regions	4	7.65	1.91	0.017	4.65	***	4	12.19	3.05	0.023	6.27	***
Among populations within regions	9	6.29	0.70	0.013	3.72	***	10	12.07	1.21	0.030	8.42	***
Within populations	382	124.83	0.33	0.327	91.63	***	430	131.95	0.31	0.307	85.31	***
K = 6												
Among regions	5	8.91	1.78	0.019	5.39	***	5	13.94	2.79	0.024	6.59	***
Among populations within regions	8	5.03	0.63	0.011	3.02	***	9	10.32	1.15	0.028	7.88	***
Within populations	382	124.83	0.33	0.327	91.59	***	430	131.95	0.31	0.307	85.53	***

***statistically significant at $P < 0.001$.

NMDS permitted us to explain some of the differences observed between species on the DAPC. For example, the DAPC allocated the *P. maritima* population of Seal Sands within the southern group although it is part of the eastern cluster for *T. maritima*. However, the NMDS indicated that the *P. maritima* population of Seal Sands

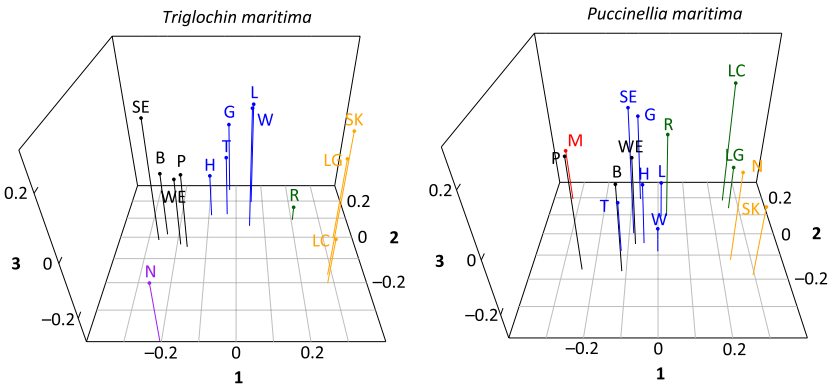

Fig. 3 nonmetric multidimensional scaling (NMDS) ordination of *Triglochin maritima* and *Puccinellia maritima* based on Kosman genetic distance between populations. The solution using three dimensions gave a stress value of 10% and 7% for *P. maritima* and *T. maritima*, respectively. Colour of each population is based on the colours obtained with the DAPC at $K = 5$, with yellow replaced by black for clarity. Population codes used here are given in Table 1.

is one of the closest populations to the eastern group, being only differentiated from Welwick along the dimension 3 of the NMDS (Fig. 3). Similarly, the DAPC indicated that populations of Ryan's field were either segregating out of the southern group for *T. maritima* or related to the populations of Loch Carron and Lochgoilhead for *P. maritima*. However, the NMDS indicates that for both species, the population of Ryan's field is more or less equidistant to these two options confirming the similarity of genetic structure between these two species.

Although the genetic structure of these species is globally similar, one incongruence could still be identified. The *P. maritima* population of Skinflats showed high similarity with the northern Scottish population of Nigg Bay. In *T. maritima* on the contrary, Skinflats and Nigg Bay were well separated both on the DAPC and on the NMDS.

Factors shaping genetic structure

Isolation-by-distance models. For any geographic distance investigated, correlation with genetic dissimilarity obtained with log-transformed and untransformed distances were compared using partial Mantel tests. Log-transformed distance always showed the better correlation of the two (Table 4). Subsequent results are therefore only given considering log-transformed distances.

Mantel tests assessing correlation between either coastal (d_C) or great circle distance (d_{GC}) to genetic dissimilarity between populations (KB) showed a strong correlation in both species (Fig. 4). Interestingly, the correlation between d_{GC} and KB showed a higher Mantel's r than the correlation between d_C and KB in *T. maritima*. A partial mantel test showed that this difference was significant (Table 4). Latitudinal distance (d_{lat}) was also significantly correlated to genetic dissimilarity between populations within both species (using log-transformed distance; *T. maritima*: $r = 0.6430$, $P < 0.001$; *P. maritima*: $r = 0.5073$, $P < 0.001$).

Table 4 Mantel test of isolation by resistance and partial Mantel test comparing competing models

	Triglochin maritima		*Puccinellia maritima*	
	r	*P*	*r*	*P*
Mantel test				
KB~log(TIDE)	0.5611	***	0.5744	***
KB~log(SEDCELLS)	0.6076	***	0.7017	***
KB~log(TIDE_SEDCELLS)	0.5877	***	0.6243	***
Partial Mantel test				
Comparison log vs unmodified				
KB~log(d_{GC}),d_{GC}	0.336	**	0.5964	***
KB~log(d_C),d_C	0.3938	***	0.6099	**
KB~log(d_{lat}),d_{lat}	0.3209	***	0.4538	***
KB~log(TIDE),TIDE	0.4232	**	0.3471	*
KB~log(SEDCELLS), SEDCELLS	0.4264	***	0.6288	***
KB~log(TIDE_SEDCELLS), TIDE_SEDCELLS	0.4205	**	0.4106	**
Comparison between competing models				
KB~log(d_{GC}),log(d_C)	0.4118	**	0.1707	0.071
KB~log(d_{GC}),log(SEDCELLS),	0.4395	**	0.137	0.121
KB~log(SEDCELLS),log(d_C)	0.1272	0.192	0.2909	**

Statistically significant at *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Isolation-by-resistance models. Similar to isolation-by-distance models, partial Mantel tests indicated that log-transformed resistance showed the best correlation with genetic dissimilarity between populations for any isolation-by-resistance model tested. Therefore, results are only given using log-transformed resistance.

The three models of isolation by resistance used in this study (TIDE, SEDCELLS, TIDE_SEDCELLS) were all shown to be significantly correlated with genetic dissimilarity between populations (Table 4). For each species, the model taking into account only sediment cells (SEDCELLS) showed the best correlation with genetic dissimilarity between populations. The best models of isolation by distance and isolation by resistance were compared for both species using a partial Mantel test (Table 4). For

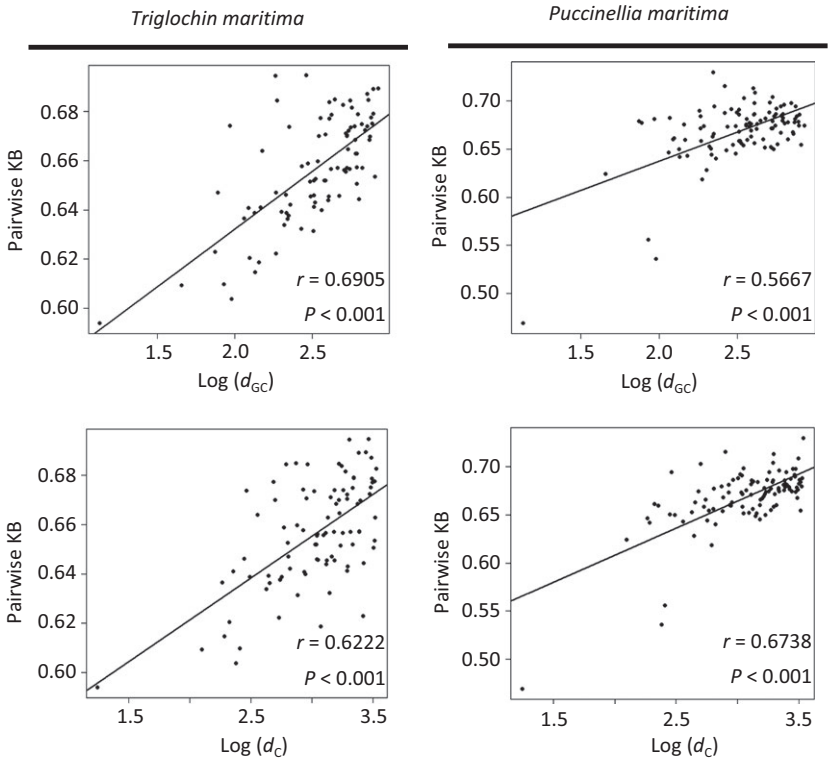

Fig. 4 Mantel test of isolation by distance between populations of *Triglochin maritima* and populations *Puccinellia maritima*. KB: Kosman distance between populations, d_{GC}: great circle distance, d_C: coastal distance. Linear regression lines were added for clarity.

T. maritima, the model of isolation by distance using the log-transformed great circle distance had a significantly better correlation to genetic dissimilarity between population than the isolation-by-resistance model SEDCELLS. Contrastingly, for *P. maritima*, the isolation-by-resistance model SEDCELLS showed a significantly better correlation than the isolation-by-distance model using the log-transformed coastal distance between populations.

Discussion

Instinctively, we might expect the action of the sea on saltmarshes to act as a powerful agent of dispersal leading to genetic homogenization of populations across a broad geographical scale. Strengthening this hypothesis, previous studies have suggested that the exchange of genetic material between isolated saltmarshes was possible due to the action of tidal currents dispersing seeds that retain good viability even after a prolonged floatation or even immersion in sea water (Koutstaal *et al.* 1987). Nevertheless, Huiskes *et al.* (1995) showed that more propagules were exported out of the saltmarsh than imported within. The strength of this source-sink asymmetry of propagule exchange between saltmarshes is an important parameter structuring plant genetic diversity. Therefore, knowing how this genetic diversity is organized should enable us to make useful inferences about connectivity between saltmarshes.

Genetic structure

In the case of our two species, the greatest part of the genetic variation was shown to be nested within populations, indicating that gene flow occurs at such a rate that genetic divergence between populations is limited around the UK. However, significant patterns of genetic structure were highlighted. The AMOVA and the DAPC both converged towards a geographical organization of the genetic diversity within these two species, indicating a stronger gene flow between populations located within the same geographical region. The strength of this genetic exchange was nonetheless still limited as suggested by the small but still significant differentiation between populations belonging to the same regions. Previous investigation showed a similar pattern in the perennial and sea-dispersed species *Spartina alterniflora*, a species dominating North American saltmarsh communities. The genetic diversity of this species was shown to be regionally structured along the Atlantic coast although genetic differences between populations were still maintained within each region (O'Brien & Freshwater 1999; Blum *et al.* 2007).

In order to estimate the strength of gene flow between populations, migration rates between populations are classically calculated from F_{ST} estimates (Wright 1949). Unfortunately, this method relies on the measurement of allelic frequencies within populations,

which is not possible here because of the ploidy level of the two species. However, the *P. maritima* genotypes found in common between the populations of Brancaster, Paull Holme Strays and Welwick are a good indication of effective exchange of propagules between populations within the same region. Detailed work aiming to estimate more precisely the strength of this propagule exchange is necessary to allow us to better understand dispersal dynamics at this most local scale.

Comparison of genetic structure between species

In a study comparing the phylogeography of five coastal plant species, Kadereit *et al.* (2005) found that genetic structure was conserved between species, suggesting that all these species were under similar processes of coastal dispersal. A strong correlation between the genetic structures of the two species was shown here by a Mantel test and graphically confirmed by the NMDS ordination of the populations. This similarity indicates that both species we investigated are likely to share similar dispersal vectors, although our data confirm that asexual propagation occurs more frequently in *P. maritima* than *T. maritima* (Table 2).

Factors shaping the genetic structure

Isolation by distance. A strong correlation was found between genetic and geographic distance for both *T. maritima* and *P. maritima* confirming the regional organization of the genetic diversity found earlier with the DAPC and the AMOVA. A similar pattern was reported for *Spartina alterniflora* along the Atlantic coast of North America (O'Brien & Freshwater 1999; Blum *et al.* 2007; Travis & Grace 2010) and, more weakly, in the invasive European species *Elytrigia atherica* (Bockelmann *et al.* 2003).

Here, although coastal distance explains genetic dissimilarity between *P. maritima* populations significantly better than great circle distance, this is not the case for *T. maritima,* where great circle distance explains genetic dissimilarity significantly better. This pattern typified in both the DAPC and the NMDS ordination by the *T. maritima* population of Skinflats located on the east coast of Scotland (Figs 2 and 3). This population is genetically closer to Lochgoilhead and Loch Carron (both located on the west coast) than to its neighbouring east-coast populations of Nigg Bay and Seal Sands.

In the UK, the re-treat of the ice sheet following the last glacial maximum followed a latitudinal gradient (Siegert 2001). The strong correlation between genetic and latitudinal distance for both *T. maritima* and *P. maritima* might, therefore, be a signature of the sequential UK colonization by these two species following a latitudinal gradient after the last glaciations maximum (LGM). However, this result must be taken cautiously due to the strong correlation between latitudinal and coastal distance in our study (Mantel test: $r = 0.518$, $P < 0.001$).

Isolation by resistance. One issue with isolation-by-distance model is that they ignore landscape heterogeneity when used to predict expected gene flow between two populations (McRae 2006). The isolation-by-resistance framework was developed to overcome this issue, and its application has permitted the testing of more precise scenarios to explore the importance of landscape features acting as barriers to gene flow (e.g. Goulson *et al.* 2011). In our study, the model SEDCELLS, only considering sediment cells around the UK, gave the best results among the three tested here, suggesting that the same processes that shape the geomorphology of the UK coastline are also important in shaping its biodiversity. Tidal currents do not seem to play the most important role at the scale investigated. However, the effect of tidal currents was only tested at a large geographical scale; its effect on mixing the genetic pool and therefore having an impact on a finer scale spatial genetic structure (i.e. within an estuary) needs further investigation.

In *T. maritima*, although the resistance matrix obtained with the SEDCELLS model was significantly correlated with genetic distance between populations, the correlation coefficient was significantly higher when using great circle distance between populations. This confirmed the impact of other than strictly coastal processes on shaping the genetic structure of this species. In their phylogeographic analysis of *T. maritima,* Lambracht *et al.* (2007) suggested that this species colonized the Baltic sea after the LGM from an inland refuge habitat. It is therefore possible that the overland pattern of dispersal highlighted here is due to a stepping-stone dispersal process through inland habitat connecting apparently distant populations. However, these inland populations are rare in the UK (Davy & Bishop 1991), and their effectiveness in connecting distant populations may be questionable. Similarly or in conjunction with this last hypothesis, zoochory may connect distant populations of *T. maritima*. Indeed, migrating geese or ducks have been reported to feed on this species (Charman & Macey 1978; Davy & Bishop 1991).

In contrast, the resistance matrix obtained with the SEDCELLS model in *P. maritima* was significantly better correlated overall to genetic distance between populations. Sediment cell boundaries may therefore act as a strong barrier to dispersal of this species. This finding confirms that dispersal in *P. maritima* is primarily through a coastal process as already suggested for other coastal species around Europe (Kadereit *et al.* 2005).

Moreover, our findings in *P. maritima* are comparable with work exploring the influence of marine currents on the genetic structure of sea beet (*Beta vulgaris ssp. maritima*) along the north-western coast of France (Fievet *et al.* 2007), where the separation between the two genetic groups discriminated follows the direction of a marine current similar to the ones delineating sediment cells around the UK.

The differential dispersal strategy between our two species may also be explained by their ecology. Recourse to sexual reproduction is known to vary between populations of *P. maritima* (Gray & Scott 1977; Erfanzadeh *et al.* 2010), whereas it is the principal means of propagation in *T. maritima*. Interspecific differences in the prevalence of sexual reproduction may therefore partially explain the association of *P. maritima* with coastal processes via the long-shore dispersal of uprooted fragments, while more abundant gene dispersal through the smaller units of seed and pollen may connect more distant populations of *T. maritima*.

Conclusion

Genetic diversity around the UK for *Puccinellia maritima* and *Triglochin maritima* is organized regionally; however, different parameters are at the origin of this structure. While the genetic organisation of *P. maritima* is shaped by a coastal process, our data indicate a stronger ability of *T. maritima* to disperse overland. Multispecies seascape genetic analysis such as that presented here is highly valuable for ecosystem management as it helps to designate coherent units of conservation and barriers to ecosystem connectivity (e.g. Kelly & Palumbi 2010; Coleman *et al.* 2011). Furthermore, it can inform saltmarsh restoration strategy by demonstrating the likely extent of and barriers to dispersal processes underpinning colonization of target restoration sites.

Saltmarsh is a species poor ecosystem, and molecular tools are now rapidly available for nonmodel species. Future research should exploit the opportunity to take a community genetics approach to understanding genetic diversity and structure in this ecosystem and thereby provide valuable information on habitat connectivity and the development of plant communities in newly restored saltmarsh sites.

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R.R. and A.S.J designed the research. R.R. conducted practical work and data analysis. A.S.J supervised this work. R.R. and A.S.J wrote the article.

Data accessibility

Microsatellite data, raw data for dispersal analysis and R codes used in the analysis: Dryad entry doi:10.5061/dryad.dc56n.

Supporting information

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

Appendix S1 'Kosman.txt', R script used to calculate KB and KW.