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ORIGINAL PAPER

Fine-scale spatial genetic structure across a strong environmental gradient in the saltmarsh plant *Puccinellia maritima*

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Abstract Saltmarsh forms the transition between maritime and terrestrial environments where biotic and abiotic conditions vary substantially along a gradient in elevation. Theoretical and empirical population genetics studies have focused on the influence of environmental gradients on intra-specific genetic variation. Contrastingly, only a few studies have focused on genetic variation in saltmarsh plants, despite the potentially strong influence of environmental gradients shaping diversity in these species. In the present paper, we assess the genetic structure of the saltmarsh plant *Puccinellia maritima* collected across an elevation gradient in restored and natural saltmarsh. Both spatial autocorrelograms of genetic variation and spatial analysis of principal components detected genetic structure in the natural saltmarsh organized along the gradient in elevation, yet no such pattern was identified considering distance between individuals without taking elevation into account. In combination with previous phenotypic analyses, our results imply that ecological divergence likely plays a key role in shaping genetic structure within saltmarsh species. Comparison of restored and natural saltmarsh indicated that interspecific competition plays an important role in shaping the genetic structure observed on the natural saltmarsh. The results of this study demonstrate that saltmarshes are valuable models in which to test effects of ecological differentiation and, by extension, provide a better understanding of the functioning of this threatened environment.

Keywords *Puccinellia maritima* · Saltmarsh · Polyploidy · Microsatellite · Genetic structure · sPCA

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Introduction

How environmental variation shapes intra-specific genetic structure in spite of gene flow has prompted much debate over the past decades (Slatkin 1987; Lenormand 2002; Räsänen and Hendry 2008; Thibert-Plante and Hendry 2010; Edelaar and Bolnick 2012; Sexton et al. 2014). On one hand, gene flow between populations is expected to homogenize the regional gene pool, thus impeding local adaptation and subsequent genetic differentiation. On the other hand, strong environmental variation can trigger pre or postzygotic barriers to gene flow facilitating divergence between populations. A classic example where the two conflicting effects of local adaptation and gene flow interact is typified along environmental gradients. This parapatric model of divergence has notably received important theoretical attention over the past decades (e.g. Endler 1977; Doebeli and Dieckmann 2003; Leimar et al. 2008). For example, mountain species provide frequent examples of such inter-population differentiation along an environmental gradient (Ohsawa and Ide 2008). For instance, Barber and Jackson (1957) highlighted a gradual differentiation in spite of gene flow between the genetically controlled green and glaucous leaf forms of *Eucalyptus urnigera* growing at low and high altitude respectively. Similarly, Gonzalo-Turpin and Hazard (2009) found adaptive morphological divergence between populations of *Festuca eskia* growing at different elevations. While Jump et al. (2006) described predictable temperature-linked changes in allele frequency along an altitudinal gradient in *Fagus sylvatica*, despite high gene flow and otherwise low differentiation across the population.

Lying at the transition between land and sea, saltmarshes also occur across a steep environmental gradient. Regularly inundated by sea tides, environmental conditions such as soil-moisture, salinity and disturbance are strongly spatially auto-correlated along an elevation gradient (Huckle et al. 2000; Boorman 2003). Besides having a strong effect on the structuring of vegetation communities on the saltmarsh (Rodwell et al. 2000), this variation in environmental conditions has also been strongly associated with plant intraspecific morphological variation (Davy and Smith 1985; Gray 1987).

Puccinellia maritima is one of the species for which such phenotypic variation was recorded to occur along the saltmarsh elevation gradient. Plants from mature populations were found to be on average larger, with longer leaves, producing more vegetative and flowering tillers, more inflorescences and panicles, and having a better seed production (Gray 1987) than plants from pioneer populations. Moreover, plants from mature populations were demonstrated better resistant against intra-specific competition (Festoc 1999). The maintenance of these differences in a common garden experiment indicated the genetic control of these characteristics.

Additionally, greater morphological variation was found to exist between individuals coming from the pioneer stage of the vegetation than between individuals from the mature population (Gray et al. 1979; Gray 1985, 1987). Selection favouring the better competitor was therefore hypothesized to reduce the morphological variability in the mature populations since interspecific competition increases with vegetation closure (Gray 1985). This hypothesis of post-zygotic selection was supported by growing seeds collected from the mature community which exhibited greater morphological variation than their parental cohorts (Gray 1987). Based on subsequent experimental work, Festoc (1999) identified that the process was most likely based on a gradual substitution of the genotypes during population maturation rather than a gradual selection for particular genotypes from the pioneer morphological pool. Festoc (1999) also outlines evidence for post-zygotic

selection acting at both pioneer and mature stages of succession based on molecular investigations using RAPD markers. While these markers are expected to be selectively neutral, recent simulations have underlined that ecological differentiation may be detected using neutral markers under specific regimes of gene flow and strength of selection (Thibert-Plante and Hendry 2010).

Following this early evidence, we sought to test for environmentally-linked genetic structuring of *P. maritima* populations using microsatellite markers. Specifically, we assessed if the elevation gradient impacts the pattern of fine-scale spatial genetic structure over and above the effect of inter-individual distance within a natural population of *P. maritima*. Secondly, we sought to determine if environmentally linked pattern of spatial genetic structure differs between this natural environment and an adjacent recently restored saltmarsh where the vegetation is still open. This comparison was expected to significantly advance earlier work seeking to determine whether the observed genetic structure in the natural saltmarsh can be related either to the raw physical–chemical characteristics of the saltmarsh environment (salinity, disturbance, inundation) that vary predictably along the elevation gradient in both restored and natural saltmarsh or to the interspecific competition increasing with vegetation closure, which differs strongly in the restored and natural saltmarsh.

Materials and methods

Study species

The halophyte *P. maritima* is a common species of north-west European saltmarshes understood to play an important role in the function of these ecosystems (Langlois et al. 2001, 2003). Often dominating the vegetation community, *P. maritima* is preferentially found at low elevation on the saltmarsh (Gray and Scott 1977). Wind-pollinated, the relative contribution of sexual reproduction is variable between *P. maritima* populations, for which asexual reproduction via stoloniferous expansion or detachment and dispersal of viable fragments also plays an important role (Gray and Scott 1977).

Study area

This study was conducted in neighbouring restored and natural saltmarsh at Skinflats on the Forth estuary, Scotland (3.7320°W, 56.0553°N) (Fig. 1). The natural saltmarsh is a good example of gradual community change along an elevation gradient. The seaward edge of the saltmarsh is occupied by the *Puccinellietum maritimae* communities (UK National Vegetation Community, NVC, SM13) where a sub community dominated by *P. maritima* (SM13a) switches progressively to a sub community *Plantago maritima-Armeria maritima* (SM13d) along the elevation gradient. The highest part of the saltmarsh is then occupied by the *Festuca rubra* community *Juncetum gerardi* (SM16) (Rodwell et al. 2000; Jump et al. 2009). Lying behind the seawall, the restored part of the saltmarsh was until 2009 a mesotrophic grassland. Engineering work took place to enable inundation by sea water entering into the site via a pipe fitted through the sea wall ("regulated tidal exchange"). Colonisation of the restoration site by saltmarsh plants among which *P. maritima* was first noted in 2010 (Jump 2010). Although plant community developed quickly inside the restored saltmarsh, the vegetation cover in 2012 was still open with much bare substrate

Fig. 1 Location map of the restored and natural sites of Skinflats in the Forth estuary, Scotland. The restored and natural saltmarshes are respectively delineated in *red* and *green* on the aerial picture. Within the restored saltmarsh, the vegetation community is distributed around two pools excavated during restoration work. (color figure online)

and so we assume interspecific competition to be much lower when compared to natural saltmarsh (R. Rouger, pers. obs.).

Sampling regime

In 2012, one hundred samples were collected from the restored saltmarsh and a further one hundred plants sampled within the natural saltmarsh. A stratified random sampling design was adopted across the area occupied by *P. maritima*. This allowed us to sample individuals in order to obtain enough pairs of samples in each spatial class for the construction of spatial autocorrelograms. When individuals were close to each other, care was taken in not sampling physically linked individuals The position of each plant was recorded using a differential global positioning system (Leica Geosystems) allowing precise measurement of Northing, Easting and elevation (±4 cm). The average distance between two *P. maritima* individuals was of 5.31 m (min 0.20 m; max 17.80 m) and 3.06 m (min 0.26 m; max 11.01 m) in the restored and natural saltmarsh respectively.

Combining elevation data obtained for our sampling together with data from additional topographical surveys by the authors (data not shown) allowed the interpolation of a three dimensional representation of both saltmarshes using the packages *akima* (Akima et al. 2009) and *rgl* (Adler et al. 2012) in R (R Core Team 2013) (Fig. 2).

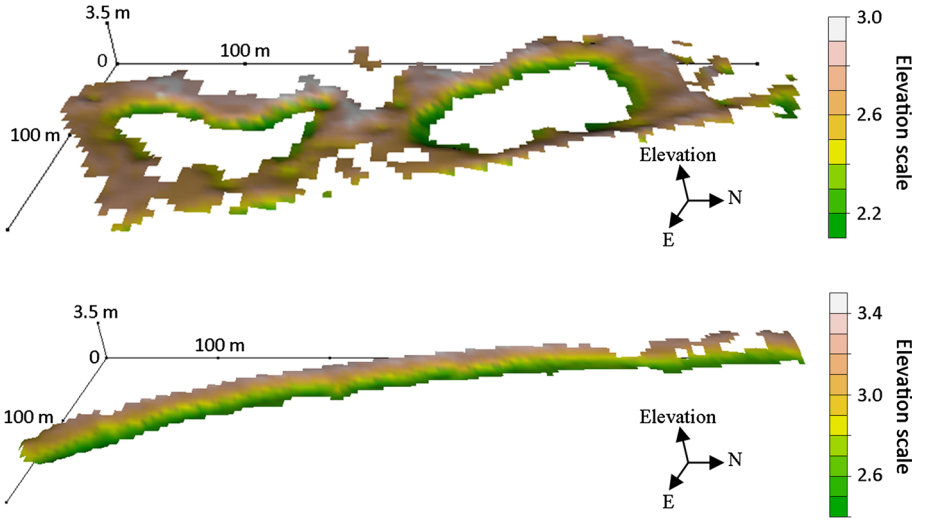

Fig. 2 Three dimensional representation of the restored (*top*) and natural saltmarsh (*bottom*). Elevation scale is given in meters

DNA extraction and analysis

DNA from each sample was extracted using the DNeasy 96 plant kit (Qiagen) following the standard protocol provided by the manufacturer. Concentration of each DNA extract was controlled on NanoDrop 2000c (Thermo Fisher Scientific) and diluted to a standard concentration of 5–20 ng/µl in ultra-pure PCR water. Each sample was then amplified using a recently developed set of microsatellite markers following the published protocol (Rouger et al. 2014). Allele scoring was made using the program STRand (Toonen and Hughes 2001). Allele binning was made using the package *MsatAllele* (Alberto 2009) in R. As observed in previous studies (Rouger and Jump 2014), locus Pm27 showed a high amplification failure rate and was therefore discarded from further analysis with a total of 11 loci used for the final analysis.

Genetic diversity

P. maritima is reported to be variable in ploidy across its range but is octoploid in the UK (Scott and Gray 1976). Population genetics of polyploid species is still challenging due to their inherent characteristics such as the difficulty to infer allelic dosage in individuals or their greater probability to deviate from Hardy–Weinberg equilibrium (Dufresne et al. 2014). Consequently, alleles within each locus were recorded as present or absent (0/1) and data analysis was then based upon methods that do not require the assumption of Hardy–Weinberg equilibrium.

Genetic diversity indices were calculated based on band frequencies, since allele frequencies are difficult to infer using co-dominant markers in polyploid species (Dufresne et al. 2014). We calculated the number of alleles (Na), the number of rare alleles having a band frequency lower than 5 % in the population (Na_5), the number of common alleles having a band frequency higher than 50 % in the population (Na_{50}). Dice dissimilarity

index was used to calculate inter-individual genetic distance using the package *ade4* (Dray and Dufour 2007) in R. This matrix of inter-individual distance was then used to calculate the Kosman index of genetic diversity within populations, which is well suited to species likely to depart from Hardy–Weinberg expectations such as polyploid or clonal species (Kosman and Leonard 2007). This index was computed using an R script designed previously (Rouger and Jump 2014). The matrix of inter-individual distances was also used as an input into the program GenoType (Meirmans and Van Tienderen 2004) in order to detect clones. Genotypic diversity was then calculated following the equation $G - 1/N - 1$ (Dorken and Eckert 2001) where G is the number of multilocus genotypes detected within the population and N, the number of individuals collected.

K-means clustering analysis

A clustering analysis was made in order to see whether a clear distinction could be made between samples collected in the natural and those collected in the restored saltmarsh. The same procedure was also replicated inside each saltmarsh in order to detect any obvious substructure. At both scales, the likeliest number of cluster in the data was determined using a k-means clustering method as implemented in the package *adegenet* (Jombart et al. 2008). This method transforms the genetic data using a principal component analysis (PCA) and subsequently runs the k-means clustering algorithm of Hartigan and Wong (1979) in order to segregate individuals in relevant clusters. This multivariate method is acknowledged to be better suited than STRUCTURE (Pritchard et al. 2000) for detecting substructure in datasets of polyploid and/or clonal species as it does not assume that clusters should meet Hardy–Weinberg expectations (Dufresne et al. 2014). We therefore used sequential k-means clustering with values of k spanning from 1 to 10 (all PCs retained, 100 starts of 10^6 iterations each) and estimated the likeliest number of clusters based on a Bayesian information criterion (BIC).

Genetic autocorrelograms

The software GenAlex was used to build spatial autocorrelograms of genetic variation (Peakall and Smouse 2012) within each saltmarsh. Two spatial distances were measured between each pair of samples. Firstly, we used the Euclidean distance between samples based on Northing and Easting coordinates. We allocated each pair of samples into evenly spaced classes (increasing by 5 m) from 0 to 100 m. Spatial autocorrelograms thus obtained allow estimation of the amount of genetic structure due to limited dispersal (e.g. centrifugal clonal propagation, limited seed dispersal). Secondly, we used the difference in elevation between samples based on precise elevation data obtained with the differential GPS. We also allocated each pair of samples into evenly spaced classes (increasing by 0.1 m). Spatial autocorrelograms computed this way permit us to estimate the amount of the genetic variability that is linked to the elevation gradient.

The autocorrelation coefficient r was computed in each distance class of the spatial autocorrelograms based on the Dice dissimilarity index between samples computed previously on R. Levels of significance were inferred for each distance class using 999 bootstraps and 999 permutations. Results obtained from GenAlex were then imported to R for plotting.

sPCA

The classic PCA previously used for k-means clustering summarizes the genetic variation present in a multivariate dataset into its principal components. Although particularly appropriate to detect obvious genetic structure, it does not take spatial information into account and may therefore miss a cryptic and spatially arranged genetic structure. In order to confirm and visualise the genetic structures detected by spatial autocorrelograms, we thus conducted a spatial Analysis of Principal Components (sPCA) (Jombart et al. 2008). This procedure is a spatially explicit multivariate analysis permitting us to focus on the part of the genetic variation that is spatially structured by optimizing not only the genetic variance between samples but also their spatial autocorrelation.

Spatial information is entered in the sPCA in the form of a row standardized weighting matrix derived from a connection network between individuals. Two types of connection network were built and tested. The first connection network, called "Distance CN", connects samples which are no more than 30 m distant. The second connection network, called "Elevation CN", connects samples between which differences in elevation are <0.1 m. sPCA was then conducted using the package *adegenet* (Jombart et al. 2008) in R for both species on each site and using the two connection networks. Tests for global and local genetic structure according to the definition given by Thioulouse et al. (1995) were made using 999 permutations. The visualisation of each significant structure detected was made by plotting the samples according to their geographic coordinates and colouring them according to their respective scores along the first sPCA component. Lastly, the contribution of each microsatellite marker to the detected genetic structure was estimated by grouping the contribution of each allele to the first component of the sPCA into per locus boxplots.

Results

Genetic diversity

101 alleles were detected overall, among which 98 were detected in the restored saltmarsh and 80 in the natural saltmarsh (Table 1). This difference was due to the higher number of rare alleles detected in the restored saltmarsh (30 were found in the restored saltmarsh against only 12 in the natural saltmarsh). The impact on levels of genetic diversity assessed using KW was therefore limited but it reduced the level of genotypic diversity within the natural saltmarsh (Table 1). Levels of genotypic diversity are relatively low overall,

Table 1 Genetic diversity parameters of *P. maritima* in both restored and natural saltmarshes

Site	N	Na	Na5	Na50	KW	R
Restored saltmarsh	100	98	30	28	0.7277	0.7373
Natural saltmarsh	100	80	12	28	0.7103	0.6465

N, number of collected samples, in brackets is the number of successfully amplified samples; Na, number of alleles; Na_5, number of rare alleles (alleles present in <5 % of individuals); Na_{50}, number of common alleles (alleles present in more than 50 %); KW, Kosman index of diversity within population; R, Genotypic diversity

meaning that multiple individuals shared the same multi-locus genotype. Interestingly, individuals sharing similar multilocus genotypes were not spatially clustered but spread inside the saltmarsh (see examples in Supplementary material 1).

K-means clustering analysis

The sequential k-means clustering procedure undertaken on all samples collected in both natural and restored sites failed to assign individuals to clusters delineating the two neighbouring saltmarshes. The BIC score steadily declined from $k = 1$ to $k = 10$ without marking an "elbow" indicating the most relevant number of cluster to describe our data (see Supplementary material 2). A similar pattern was observed inside each saltmarsh indicating the absence of clear separation between two or more discrete clusters.

Genetic autocorrelograms

Spatial autocorrelograms of genetic variation obtained for *P. maritima* using Euclidean distance between samples did not show any genetic structure in the restored saltmarsh whereas a significant structure was observed in the natural saltmarsh (Fig. 3). However, this structure was limited to the first distance class of the spatial autocorrelogram (up to 5 m). Spatial autocorrelograms using difference in elevation between samples did not detect any structure in the restored saltmarsh. In contrast, however, in the natural saltmarsh a very strong genetic structure was detected along the elevation gradient (Fig. 3).

Spatial analysis of principal components (sPCA)

The tests of significance for global and local genetic structures detected by sPCA using "Distance CN" differed slightly when compared to the results obtained by the spatial autocorrelograms using Euclidean distance between samples (Table 2). Although no genetic structure could be observed for *P. maritima* in the restored saltmarsh with the spatial autocorrelogram using Euclidean distance (Fig. 3), a significant global structure was detected using the "Distance CN" (Table 2). However, the visualisation of the original scores along the first global component of the sPCA on the saltmarsh (Fig. 4) showed that this structure occurred at a larger scale than that observable with the spatial autocorrelogram (>100 m). Along this component, original sample scores seem to be differentially distributed around the two pools dug during the restoration site development (Fig. 4). However, this pattern must be taken cautiously given the relative importance of the second global component which still gives an isolation by distance pattern of genetic structure but arranged in a different way (see Supplementary material 3). Interestingly, on the natural saltmarsh where a weak spatial genetic structure was detected by the spatial autocorrelogram for *P. maritima* (Fig. 3), no global structure was observed using the sPCA (Table 2). The connection network used here, joining only individuals which are not further apart than 30 m, is however, not appropriate to detect the very fine structure observed on the spatial autocorrelogram, thereby explaining the contrasting findings of these two analytical approaches.

Concerning the influence of elevation on the distribution of genetic diversity in *P. maritima* within the natural saltmarsh, the sPCA using 'Elevation CN' converged towards what was observed on the elevation autocorrelogram using differences in elevation between samples. The importance of the first component of the sPCA on the plot of

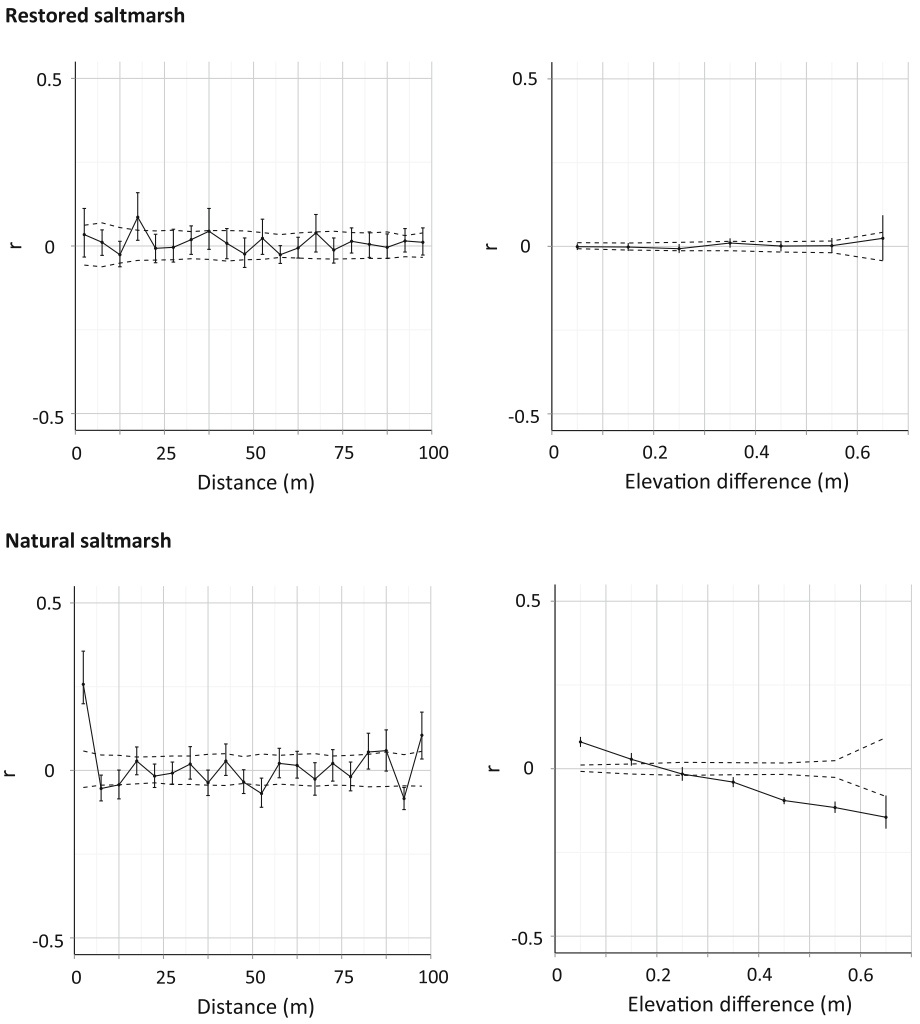

Fig. 3 Spatial autocorrelograms of genetic variation obtained in both restored and natural saltmarshes for *P. maritima*. *Left*: spatial autocorrelograms using Euclidean distance between samples. *Right*: spatial autocorrelograms using difference in elevation between samples. r: correlation coefficient of Smouse and Peakall (1999). *Dotted lines*: 95 % confidence interval determined by permutation. *Vertical bars*: 95 % confidence interval determined by bootstrapping

eigenvalues indicates that an important part of the genetic variation is summarized by this component (Fig. 4). The visualisation of this first component on the saltmarsh illustrates the extent of this genetic structure with a gradient of differentiation occurring from the low to the high part of the saltmarsh despite the very restricted spatial distance between these two zones (around 20 m, Fig. 4).

The contribution of each marker to the structure detected by sPCA was also analysed (Fig. 5). Within the natural saltmarsh using "Elevation CN", the contribution of each marker to the first global component of the sPCA is uniform with no marker identified as being preeminent in shaping the observed genetic structure (Fig. 5a). This pattern is similar

Table 2 Tests of global and local structures for the structure detected by sPCA with different connection network

Site	Distance CN		Elevation CN	
	Global structure	Local structure	Global structure	Local structure
Restored saltmarsh	$p < 0.01$ max(t) = 0.0267	$p = 0.761$ max(t) = 0.0208	$p = 0.949$ max(t) = 0.0138	$p = 0.447$ max(t) = 0.0250
Natural saltmarsh	$p = 0.086$ max(t) = 0.0250	$p = 0.509$ max(t) = 0.0254	$p < 0.001$ max(t) = 0.0789	$p = 0.267$ max(t) = 0.0277

Distance CN: connection network joining individuals no further apart than 30 m; Elevation CN: connection network joining individuals with a difference in elevation of no more than 0.1 m

when looking at the contribution of each marker to the first global component of the sPCA within the restored saltmarsh using "Distance CN" (Fig. 5b). However, the genetic structure observed in that case is driven by the strong weight of a few alleles distributed within four different loci (Pm26, Pm39, Pm61, Pm65).

Discussion

Genetic structure in the natural saltmarsh

Using microsatellite loci, our study highlighted the presence of genetic structure strongly associated with an elevation gradient in a natural population of the saltmarsh plant *P. maritima*. This structure was confirmed by the two complementary approaches of spatial autocorrelograms and sPCA (Figs. 3, 4). Contrastingly, the k-means clustering analysis failed to discriminate discrete genetic clusters congruent with the gradient in elevation. This indicates that the genetic transition between the two locations (high marsh vs low marsh) must be seen as a continuous genetic cline rather than a discrete segregation between diverging populations.

Ecological variation across a landscape can create differential selection pressure on local populations and therefore induces pre or post-zygotic barriers to gene-flow between them. Genetic divergence between populations is, in such a case, expected to be restricted to areas of the genome which are closely linked to loci under selection, recombination events allowing all other neutral loci to exchange genetic variants freely through the landscape (Barton and Bengtsson 1986). However, it was observed that this differential selection can also create higher genetic divergence at neutral loci unlinked to genomic regions under selection than that expected under the classical isolation by distance scenario alone ("isolation by adaptation", Nosil et al. 2009; Orsini et al. 2013). The spread of this divergence across the genome depends on various parameters such as time since local adaptation, number and location of loci under selection or the balance between gene flow and strength of selection (Thibert-Plante and Hendry 2010; Feder et al. 2012).

In our case, the possibility that the genetic cline highlighted for *P. maritima* within the natural saltmarsh was governed by one of the microsatellite marker being located near a region under selection was discarded when examining the contribution of each microsatellite to the first global component of the sPCA (Fig. 5a). This contribution was indeed homogeneous between loci and no locus could be highlighted as driving the genetic divergence between individuals from the low and high part of the saltmarsh. This indicates

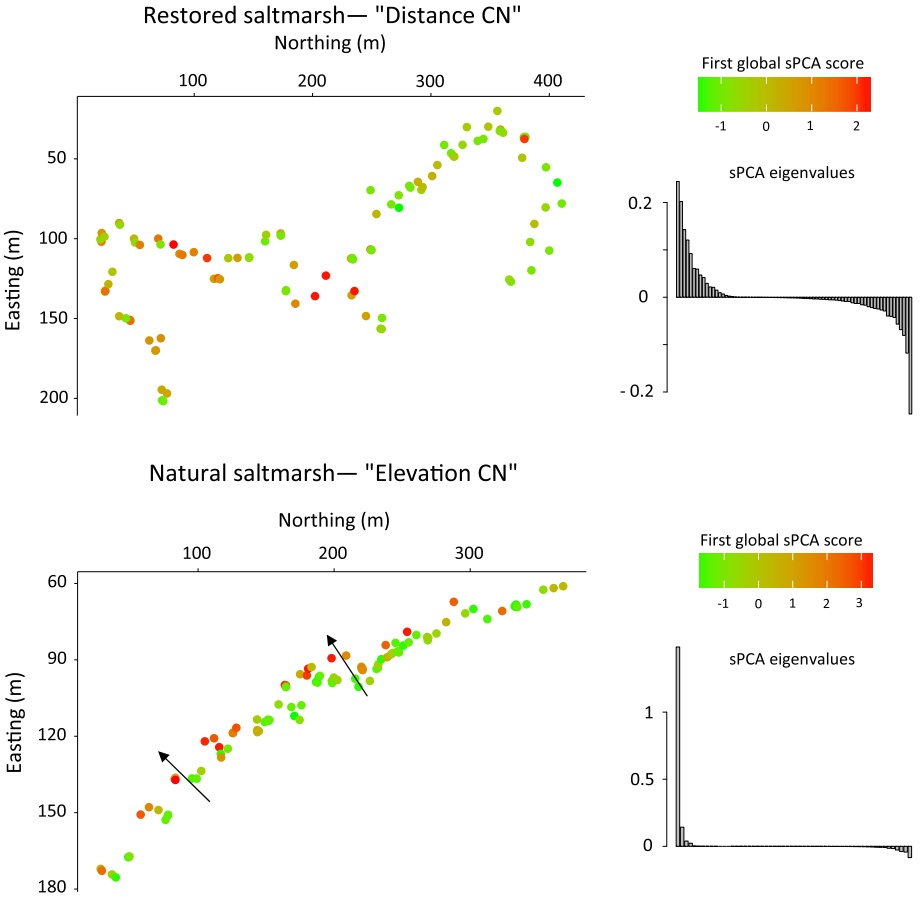

Fig. 4 Significant spatial genetic structure detected by sPCA. First global original scores of each sPCA were used. Plots of sPCA eigenvalues are shown on the *right* of each representation. *Black arrows* inside the *bottom plot* indicate the direction of the elevation gradient. Origins of Northing and Easting axis are identical to the one used in Fig. 2

that differentiation along the elevation gradient of the saltmarsh is genome-wide rather than locus-specific.

Estimating whether this homogeneous divergence across loci along the elevation gradient is due to isolation by distance or ecological divergence between habitats requires comparison of levels of gene-flow along the elevation gradient and along geographical distance. Unfortunately, the ploidy level of *P. maritima* makes the usual indirect estimates of gene-flow very difficult to obtain. Allelic dosage within each individuals, and by extension allelic frequencies, cannot be precisely measured (Dufresne et al. 2014) impeding the estimation of gene flow. However, various indications suggest that isolation by distance cannot be the only parameter explaining restricted gene-flow along the elevation gradient leading to the genetic cline observed here. First of all, the extent of this observed genetic structure was of only 10–20 m (Fig. 4). Since *P. maritima* is wind-pollinated (Gray and Scott 1977), limitation in pollen flow is unlikely to produce isolation by distance over such a small scale. Secondly, samples sharing identical multilocus genotype were not found to

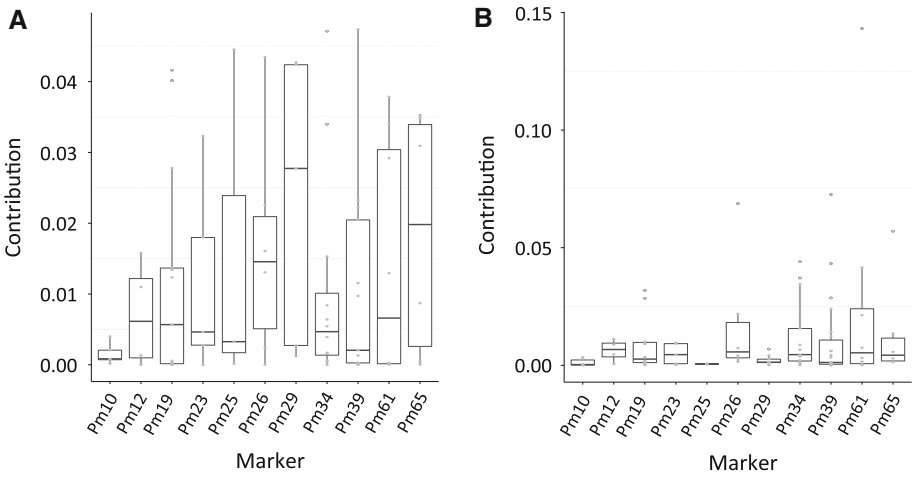

Fig. 5 Contribution of each microsatellite loci to the sPCA first global component of the detected structures. **a** sPCA of the natural saltmarsh using "Elevation CN"; **b** sPCA of the restored saltmarsh using "Distance CN". *Grey dots* represent single allele weights along the first global component of each sPCA

be spatially clustered but spread across the saltmarsh. Limitation in propagule dispersal is, therefore, also unlikely to cause the observed pattern. Lastly, spatial autocorrelograms and sPCA using Euclidean distance between samples indicated no genetic structure due to isolation by distance across a distance of several hundred meters, indicating that gene-flow in this species has the potential of mixing genetic diversity over such a scale. Consequently, processes other than isolation by distance must act to shape genetic structure of *P. maritima* along the elevation gradient.

Other studies investigating intraspecific diversity in saltmarsh species also observed that variation was arranged following a gradient in elevation. For instance, morphological and physiological variation was found to exist in *Spartina alterniflora* and *Triglochin maritima* (Jefferies 1977; Gallagher et al. 1988) along the elevation gradient. However, the most striking example concerns *Elymus athericus* for which a strong genetic differentiation was found to exist between individuals collected at high and low elevation within the saltmarsh that could not be explained by isolation by distance alone (Bockelmann et al. 2003). Authors suggested that this pattern was governed by restricted gene flow due to strong post-zygotic selection against immigrants in both habitats. This reason was also evoked by Festoc (1999) to explain the genetic cline detected using RAPD markers for *P. maritima*. Based on the genetic data presented here, ecological divergence is strongly indicated as a driving force of the genetic differentiation between samples collected at high and low elevation within the natural saltmarsh.

Comparison between restored and natural saltmarsh

The distribution of plant species in saltmarsh depends primarily upon two factors, (1) the gradient in abiotic conditions (salinity, inundation, disturbance) but also (2) interspecific interactions such as competition (Pennings et al. 2005). It has been demonstrated that species able to cope with high levels of abiotic stress make poor interspecific competitors when stress decreases (Engels and Jensen 2010). Plants are therefore limited on their lower

limit of distribution within the saltmarsh by abiotic stress and on their upper limit by competition with less stress tolerant species (Engels and Jensen 2010). These two factors influencing interspecific distribution are also likely to control intraspecific genetic variation. In the case of *P. maritima* for example, increased interspecific competition was hypothesized to be the reason why genotypes were found to be on average more vigorous at higher elevation (Gray 1987).

Both natural and restored saltmarsh of Skinflats are under tidal influence. Abiotic conditions within both saltmarsh are therefore correlated with the gradient in elevation (de Leeuw et al. 1991; Huckle et al. 2000; Wang et al. 2007). In contrast, the vegetation cover differs between saltmarshes. While vegetation density and thus interspecific density is positively correlated with the elevation gradient within the natural saltmarsh, the vegetation canopy within the restored saltmarsh remains open and interspecific competition homogeneous along the elevation gradient. The comparison of the genetic structure in both restored and natural saltmarsh therefore allowed us to assess the relative influence of interspecific competition on the genetic structure detected in the natural saltmarsh. Interestingly, no genetic structure organised along the elevation gradient was detected in the restored saltmarsh by either sPCA and spatial autocorrelograms (Fig. 3; Table 2), implying that interspecific competition might play a key role in the ecological divergence observed within the natural saltmarsh.

The only structure detected by the sPCA within the restored saltmarsh was related to Euclidean distance between individuals, with samples being spatially differentiated around the two pools dug during saltmarsh restoration (Fig. 4). However, only a few alleles contributed to the first global component of the sPCA showing this structure (Fig. 5), making its relative importance weak when compared to the other global components (Fig. 4, plot of eigenvalues). Moreover, the k-means clustering analysis which should be particularly suited to detect discrete units did not identify any grouping of individuals. This grouping of individuals into two discrete units detected by sPCA must therefore be taken cautiously and need further investigation to be confirmed.

Conclusion

This work demonstrates the organization of a genetic structure along an elevation gradient in the saltmarsh species *P. maritima*. Our data suggest that isolation by distance is insufficient to explain this pattern and that ecological divergence is likely to play a key role. In established saltmarsh, interspecific competition is implicated as an important parameter driving this ecological divergence. Consequently, we hypothesise that as the vegetation canopy closes within the restored saltmarsh a similar genetic cline should develop. Further morphological investigation between individuals in high and low area of the saltmarsh would give us a better idea of the traits directly linked with this ecological divergence and should be a priority for further research. In the light of previous work, our results provide an intriguing insight into the impacts of strong environmental gradients in shaping genetic structure within continuous populations of saltmarsh species. These results therefore advance our understanding of evolutionary ecology in general and more specifically the functioning of this threatened environment (Gedan et al. 2009).

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