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The importance of genetic make-up in seagrass restoration: a case study of the seagrass *Zostera noltei*

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ABSTRACT: Seagrass meadows are among the most important coastal ecosystems. Their ongoing decline is of concern, and transplantations are carried out in many parts of the world to restore the ecosystem services seagrass meadows provide. Several studies have highlighted the importance of genetic diversity for transplantation success in seagrasses, but this is still rarely taken into account in transplantation trials. Here we assess a transplantation experiment of the seagrass *Zostera noltei* in one of the largest saline Mediterranean lagoons 4 yr after transplantations were carried out with low success rates. We compare genetic diversity values of a transplant site, 2 relict meadows and newly appeared patches in the lagoon to genetic diversity metrics measured before the transplantation experiment inside and outside the lagoon. We show that genotypic richness of the transplant site assessed 4 yr after the transplantation is very low. Moreover, the transplants are genetically distinct from the genetic stock in the lagoon, with low migration rates, low effective population size and signs of a recent population bottleneck. Relict meadows and newly appeared patches show, in contrast, signs of high levels of sexual reproduction and are connected via gene flow. The newly appeared patches likely did not originate from the transplantation. The lack of success of transplanted shoots could be due to an adaptation mismatch of the marine donor material to lagoon conditions or to low plasticity of the transplanted shoots.

KEY WORDS: *Zostera noltei* · Microsatellites · Ecosystem recovery · Genetic diversity · Transplantation

INTRODUCTION

Seagrass meadows are recognized as one of the most important and valuable marine biomes, when considering biodiversity, economics and ecosystem services (Costanza et al. 1997, Spalding et al. 2003). Their importance stems from the fact that seagrasses are habitat-providing species as well as ecosystem engineers, i.e. species that modify their environment to an extent that the availability of resources to other species is altered (Jones et al. 1996). Ecosystem services that seagrasses provide include reducing wave impact, stabilizing the sediment, adding oxygen to the water, exporting important amounts of carbon, nitrogen and phosphorus to coastal food webs, and producing significant amounts of organic carbon (Beck et al. 2001, Costanza et al. 1997, Duffy 2006). Moreover, they provide nursery grounds and shelter for commercially important species (Beck et al. 2001, Heck et al. 2003).

Unfortunately, seagrass habitats are also one of the most threatened ecosystems on earth (Waycott et al. 2009, Short et al. 2011). Since 1990, the rate of loss of seagrasses has increased to an estimated 7% yr^{-1}, a worrying rate that is comparable to mangroves, coral reefs and tropical rainforests (Waycott et al. 2009). Seagrass meadows are very sensitive to anthropogenic impact, including, but not limited to, eutrophication, pollution, trawling and mooring (Duarte 2002, Orth et al. 2006, Boudouresque et al. 2009). This sen-

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sitivity has led to the decline of seagrasses and is also the reason why seagrasses are used as 'coastal canaries', since any change in distribution or well-being of seagrass meadows indicates changes in the associated ecosystem (Orth et al. 2006) and affects the entire coastline. In order to restore seagrasses and maintain the ecosystem services they provide, transplantation and restoration of meadows has been performed for several seagrass species at different latitudes (Paling et al. 2009, van Katwijk et al. 2009).

For a transplantation to be successful, the transplant meadow must not only be able to persist short term, but must also have sufficient evolutionary potential to adapt to changing conditions in the future (Kettenring et al. 2014). Genetic diversity is a key issue in ensuring persistence on both timescales, but local adaptation of transplanted genotypes also needs to be taken into consideration. Genetic and genotypic diversity of donor meadows and transplant sites has been linked with transplantation success in several studies over certain time frames. Procaccini & Piazzi (2001) found that increased heterozygosity of the donor population leads to better growth performance in the transplanted meadow in the Mediterranean seagrass *Posidonia oceanica*. Similarly, genetic diversity of donor material was found to be positively correlated with population growth and fitness and with the reproductive rate of the seagrasses *Zostera marina* and *P. australis* (Williams 2001 and Sinclair et al. 2013, respectively). Moreover, Reynolds et al. (2012a) found that increased genetic diversity in transplanted communities of *Z. marina* also leads to higher invertebrate density, nitrogen retention and areal productivity of the seagrass meadow ecosystem. The importance of selecting the right source material for transplantation was also stressed by Olsen et al. (2014), who suggest the presence of inbreeding at many southern Californian transplant locations, a probable result of the immense reduction of the effective population size when using only a limited number of transplants.

Another transplantation strategy involves the use of seeds instead of adult shoots, and this method seems to be able to maintain a high effective population size as well as high levels of allelic richness (Reynolds et al. 2012a,b, 2013), an important proxy for long-term evolutionary potential (Petit et al. 1998). Conversely, a risk also exists of outbreeding depression or adaptation mismatches when the source material is not collected locally (enough) (McKay et al. 2005). An extreme example for the detection of outbreeding after transplantations in seagrasses is the observed hybridization between *Z. marina* and its congener *Z. japonica* at a transplant site, a probable result of the transplant strategies used (Olsen et al. 2014). 'Intraspecific hybridization' is, in contrast, more likely to remain undetected, but the admixture of different genotypes is expected when introducing non-local transplant material (Hufford & Mazer 2003). The definition of 'local' is not necessarily associated with geographic distance, as habitat heterogeneity can also result in genetically distinct ecotypes by natural selection and adaptation to differing environments in close geographic proximity. Therefore, the transplantation of new genotypes and ecotypes, which are not well adapted to local conditions, may not only affect the success of the transplantation, but may also affect existing populations adapted to local conditions when they admix. Hence, the selection of adequate source populations based on genetic diversity metrics is crucial for transplantation success (Campanella et al. 2010, Lloyd et al. 2012, Reynolds et al. 2013, Sinclair et al. 2013, Olsen et al. 2014), but might also negatively impact the genetic diversity dynamics of meadows in the vicinity.

Z. noltei is an important intertidal seagrass species occurring along European, North African, Mediterranean, Black Sea and Azov Sea coasts (Boudouresque et al. 2009). In the Mediterranean, *Z. noltei* is mainly present in brackish conditions. It is relatively tolerant to nutrient input, but has nevertheless seen recent worldwide declines (Spalding et al. 2003, Short et al. 2011). In the Berre lagoon, one of the largest saline Mediterranean coastal lagoons, *Z. noltei* has undergone a dramatic decline during the last decades. Before the severe impacts of industrialization and urbanization in the late 19th and early 20th centuries, the Berre lagoon harbored extensive *Zostera* meadows, with an estimated cover over 6000 ha (Bernard et al. 2007). In 2007, the total surface area of *Z. noltei* in the Berre lagoon was considered to be <1.5 ha, while *Z. marina* disappeared completely (Bernard et al. 2007). Although legislation to reduce pollution and eutrophication was adopted in 1994 and re-enforced in 2006, *Zostera* spp. did not make a recovery until 2009. A genetic study was performed in order to assess genetic diversity of the relict meadows in the Berre lagoon, as well as a site in the Gulf of Fos (Carteau) (Procaccini et al. 2013) in 2009. Genetic and genotypic diversity from locations within the lagoon and the Gulf of Fos were mainly comparable, but gene flow between the lagoon and the open sea was low (Procaccini et al. 2013). In the same year, restoration of the *Zostera* meadows was identified as a key action for ecosystem recovery.

It was decided to re-enforce natural populations by transplanting *Z. noltei* from the nearby fully marine meadow located in the Gulf of Fos that was analyzed in Procaccini et al. (2013). Plants for transplants were collected at a distance of approximately 400 m from the site where plants were collected for genetic analysis (Procaccini et al. 2013). The transplantations were carried out in 2009 and were followed over a 4 yr time frame. The survival rate was initially quite good (around 50 % after 5 mo), but it was very low after a time frame of 4 yr. In 2013, when the last monitoring was performed, transplants were only present at 2 sites, and absent at 4 sites. The survival rate of *Z. noltei* at these 2 sites was 6 % for Pointe de Berre and 33 % for Arc (Bernard et al. 2013). The low success of this transplantation over a timescale of years is shared by most European transplantation projects, which, according to expert opinion, have all failed thus far (Cunha et al. 2012). In contrast, the remaining local meadows in the Berre lagoon have seen a small recovery and a progression towards the coast since 2009, and new patches of *Z. noltei* have appeared in close proximity to the transplant sites.

Here we compare genetic characteristics of the meadows collected in 2009 and studied by Procaccini et al. (2013), i.e. 1 marine meadow and 2 relict meadows in the lagoon, with the transplant site collected in 2013 and new patches that appeared in 2013 around the transplanted areas. From this comparison we aim to assess whether the low success of the transplantation trial can be explained by the genetic make-up of the transplanted shoots. In addition, we aim to assess whether the transplanted material may have represented the source for initiating the establishment of the new patches appearing near the transplant sites at Pointe de Berre and Arc, or if patches originate from relict indigenous meadows (at 1 to 2 km distance) by drifting propagules.

MATERIALS AND METHODS

Study site and sampling

Transplantation of *Zostera noltei* in the Berre lagoon was performed in 2009 at 6 distinct locations (Fig. 1), with donor material sourced at Carteau in the Gulf of Fos (Mediterranean) at 0.6 m depth on sandy and muddy bottom. Transplants were collected manually by scuba-diving and collected at a reciprocal distance of approximately 50 cm to 2 m. Each transplant represents an area of 0.04 m^2 of meadow with sediments which were carefully placed in a plastic tub to maintain sediment cohesion, and

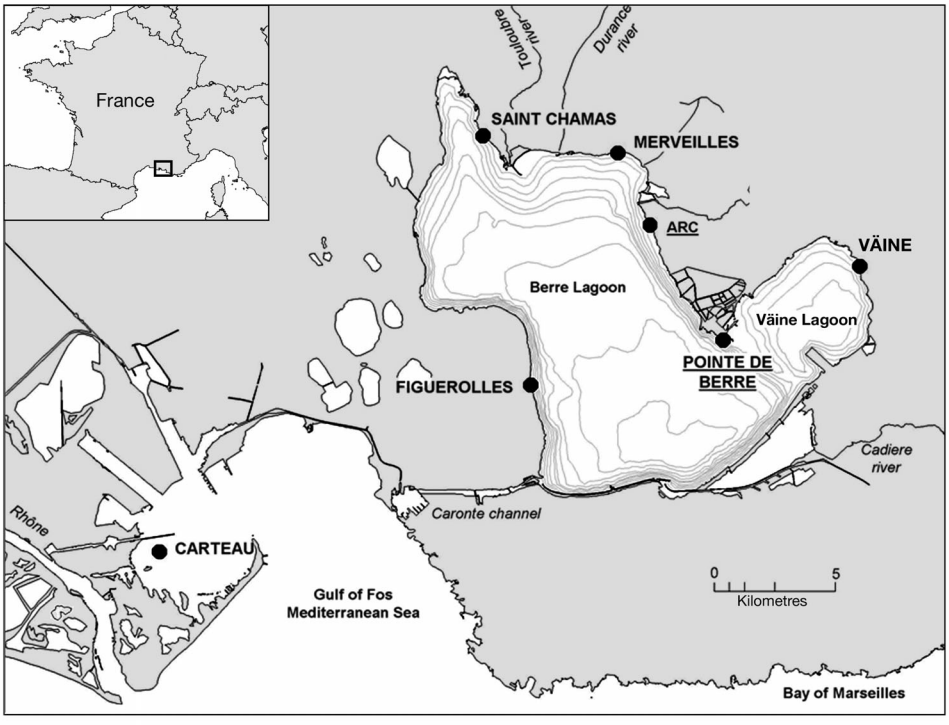

Fig. 1. Berre lagoon (43.47° N, 5.09° E) with the 6 transplantation sites within the lagoon and the donor site at Carteau. The 2 relict seagrass *Zostera noltei* meadows are underlined. Grey lines indicate depth contours, from 9 m in the central area of the lagoon to 1 m close to the shore

wet tissues were used to prevent transplant desiccation. The transplants were transported by boat to the Berre lagoon receiving sites on the day of collection. Six receiving sites were transplanted with *Z. noltei*, using 45 transplants at each site and at a depth from 0.5 to 1.0 m on sandy and muddy bottom. The transplants were placed in a pre-made hole without specific fixation systems regularly spaced and covering a total surface area of 450 m^2. Survival rates, shoot density and transplant maximal elongation were surveyed regularly over a 4 yr time frame. *Z. noltei* survived at only 2 transplant sites (Arc and Pointe de Berre), albeit with extremely high mortality rates (Bernard et al. 2013). At these 2 localities, relict indigenous meadows of *Z. noltei* still exist at a distance of 1 to 2 km from the transplant site. The relict Pointe de Berre meadow currently covers several thousand square metres at a maximum depth of 1.5 m. The *Z. noltei* meadow at Arc covers several hundred square metres and reaches a maximum depth of 2 m. In these relict meadows, as well as in the marine meadow in Carteau near the donor site (400 m and at a comparable depth), sampling was performed in 2009 (Procaccini et al. 2013). Four years later — in 2013 — 2 transplanted sites and the nearby newly appearing patches in Pointe de Berre and in Arc, as well as the newly appeared meadow of Vaïne (4000 m^2), were sampled. A total of 103 shoots were collected in the 5 localities, approximately 1 to 2 m apart within the same location (Table 1). Only 1 small transplanted patch survived in the Pointe de Berre transplanted site, and only 1 individual was sampled. Samples were dried and stored in silica crystal before genetic analysis.

DNA extraction and genetic analysis

DNA was extracted from 20 mg of dried tissue with the NucleoSpin® 96 Plant II kit (Macherey-Nagel) following a modified protocol optimized for a Biomek FX robotic station (Tomasello et al. 2009). Nine polymorphic microsatellites were used for the analysis of *Z. noltei* (Coyer et al. 2004b). Microsatellites were combined in 2 different multiplexes in 96-well plates, and all PCRs were run under the following conditions: 95°C for 15 min, 35 cycles of 94°C for 30 s, 60°C for 1 min 30 s and 72°C for 1 min, with a final extension step of 60°C for 30 min. Only samples that were successfully genotyped at all loci were used for further analyses.

Clonal discrimination and identification of multilocus genotypes (MLGs) for each sampling location was carried out using the software GenClone 2.0 (Arnaud-Haond & Belkhir 2007) by calculating P_{sex}, the probability that identical MLGs arose by chance

Table 1. Genetic measurements of the seagrass *Zostera noltei* samples collected in Berre lagoon in 2009 and 2013. *N*: number of extracted samples; N_r: number of samples successfully genotyped at all loci; MLG: number of multilocus genotypes; *R*: genotypic richness; N_a: mean number of alleles per locus; A_4: allelic richness standardized to 4 genotypes; A_{19}: allelic richness standardized to 19 genotypes; H_o: observed heterozygosity; H_e: expected heterozygosity; H_{nb4}: unbiased expected heterozygosity standardized to 4 genotypes; F_{IS}: fixation index; na: not applicable; SD: standard deviation; asterisk: significant F_{IS} value

Population	*N*	N_r	MLG	*R*	N_a (SD)	A_4 (SD)	A_{19} (SD)	H_o (SD)	H_e (SD)	H_{nb4} (SD)	F_{IS} (SD)
Arc transplant	15	15	4	0.21	2.778 (0.401)	2.778 na	na	0.583 (0.102)	0.517 (0.079)	0.427 (0.003)	−0.143* (0.095)
Arc 2013	21	21	19	0.9	3.889 (0.611)	2.711 (0.297)	3.889 (na)	0.532 (0.103)	0.535 (0.093)	0.531 (0.002)	−0.02 (0.097)
Berre transplant	1	1	1	na	1.667 (na)	na	na	0.667 (0.167)	0.333 (0.083)	na	na
Berre 2013	20	19	19	1	3.889 (0.588)	2.844 (0.324)	3.889 (na)	0.532 (0.093)	0.516 (0.087)	0.532 (0.002)	−0.035 (0.04)
Vaïne	36	36	36	1	4.444 (0.709)	2.7 0.189	4.122 (0.199)	0.429 (0.094)	0.488 (0.1)	0.496 (0.002)	0.160* (0.075)
Berre 2009	40	36	36	1	5.556 (0.456)	3.167 (0.328)	4.800 (0.261)	0.531 (0.102)	0.569 (0.1)	0.57 (0.001)	0.135* (0.091)
Arc 2009	40	35	32	0.91	4.889 (0.655)	2.844 (0.352)	4.467 (0.281)	0.59 (0.091)	0.547 (0.08)	0.555 (0.002)	−0.093* (0.079)
Carteau 2009	40	31	31	1	6.000 (0.866)	3.189 (0.292)	5.300 (0.174)	0.573 (0.099)	0.588 (0.084)	0.595 (0.002)	0.038 (0.068)

rather than from sexual reproduction (and so are actual clones). All P_{sex} values were lower than the cut-off value of 0.01, and duplicate genotypes were considered as belonging to the same MLG and were removed from the dataset for the calculation of genomic diversity. We also pooled samples from all populations to identify MLGs that might be shared among populations. Genotypic diversity (R) was estimated as: $G - 1/N - 1$, where G is the number of MLGs and N is the number of samples. Genomic diversity measurements and F statistics were calculated using GenAlex Ver. 6.5 (Peakall & Smouse 2012). Allelic richness (A) was also estimated and standardized to 4 (the minimum number in the Arc transplant population) and 19 genotypes (the second lowest number of genotypes found in a population) to account for differences in the number of MLGs per population, using the STANDARICH package (www.ccmar.ualg.pt/maree/software.php?soft=sarich) and R software (R Development Core Team 2012). Unbiased expected heterozygosity was also calculated for a standardized number of 4 MLGs using GenClone Ver. 2.0 (Arnaud-Haond & Belkhir 2007). Assignment tests were calculated using GeneClass2 Ver. 2.0 (Piry et al. 2004), using 50% probability as a cut off for the most likely assigned population. Principal component analysis (PCoA) and AMOVA, as implemented in GenAlex, were used to explore the genetic distance among populations (including duplicate genotypes). Effective population size (N_e) was estimated using LDNe V.1.31 (Waples & Do 2008), with P_{crit} = 0.05 (where P_{crit} is the minimum frequency for alleles to be included in the analysis) and using the Burrows method to calculate linkage disequilibrium (LD), which is subsequently used to calculate the N_e of each population. The number of migrants per generation (N_m) was calculated as a pairwise comparison based on F_{ST} using GenAlex 6.5 (Peakall & Smouse 2012), based on the frequency of rare alleles using GenePop (Raymond & Rousset 1995) and by a Bayesian approach using the Markov chain Monte Carlo (MCMC) technique as implemented in BayesAss 3.0.3.Windows (Wilson & Rannala 2003).

For the last approach, 3 independent runs with different starting seeds were performed using 1 million burn-in, 10 million MCMC repetitions and a sampling interval of 100. The mixing parameters were adjusted according to Rannala (2007). The reliability of migration rate estimates was assessed by checking for consistency in the estimates between runs. The MCMC Trace Analysis Package Tracer Ver. 1.6 (Rambaut & Drummond 2003) was used to confirm convergence in each run. In contrast to F_{ST}, BayesAss also gives sensible results when populations are not in Hardy-Weinberg equilibrium by calculating population-specific inbreeding coefficients (Faubet et al. 2007), but requires a high number of individuals per population (Meirmans 2014). The different methods utilized to calculate migration rates also allow assessment of the different time frames over which migration rates occur. Migration rates based on heterozygosity as in F_{ST} can — at least in partially clonal species — reflect gene flow up to hundreds of years (Lloyd et al. 2013). Migration rates based on allele frequencies have a shorter history of a few generations, because allele frequencies have been shown to change much more quickly in isolated populations than heterozygosity (Allendorf et al. 2013). The Bayesian approach implemented in BayesAss identifies first or second generation migrants (Wilson & Rannala 2003).

We tested for recent genetic bottlenecks at each location using a test for heterozygote excess in the program Bottleneck 1.2.02 (Cornuet & Luikart 1996), which computes heterozygote excess as the difference between expected heterozygosity (H_e) and heterozygosity expected at equilibrium (H_{eq}), calculated from the observed number of alleles. We tested the significance of the difference between H_e and H_{eq} using a 2-tailed Wilcoxon test under a 2-phase mutation (TPM) model. This model provides results intermediate between an infinite allele model (IAM) and a stepwise mutation model (SMM) as expected for most microsatellite loci (Di Rienzo et al. 1994).

RESULTS

Intra-population genetic diversity

Overall, 92 individuals from the 2013 samples were successfully genotyped at all loci and were combined for further analysis with the 102 individuals of the 2009 sampling. Since only 1 sample was collected at the Pointe de Berre transplantation site, data were not included in comparisons among sites. Genotypic richness was considerably lower (R = 0.21) at the Arc transplant site compared to the other sampling sites, where genotypic richness values were >90% (Table 1). No clones were shared among the different locations. Heterozygosity and allelic richness were high in all meadows, regardless of their origin and year of sampling. Values ranged from 0.427 to 0.595 for unbiased heterozygosity (standardized to 4 genotypes) and from 2.700 to 3.189 for allelic richness A_4 (standardized for 4 genotypes) (Table 1). Allelic rich-

ness estimates standardized to 19 genotypes confirmed the pattern of A_4, with highest allelic richness values at the marine site Carteau near the donor site, followed by the 2 relict meadows. Two sites (Arc transplant and the relict meadow at Arc, i.e. Arc 2009) showed a significant excess (±SD) of heterozygosity (F_{IS} = −0.143 ± 0.095 and −0.093 ± 0.079, respectively), while 2 sites (the newly appeared meadow at Vaïne and the relict meadow at Pointe de Berre, i.e. Berre 2009) showed a significant excess of homozygosity (F_{is} = 0.160 ± 0.075 and 0.135 ± 0.091, respectively).

N_e ranged from an extremely low value of 0.5 at the Arc transplant site up to infinite at the marine Carteau site (Table 2). All naturally occurring sites within the lagoon had very low N_e values, ranging from 2.2 to 11 for the relict meadows sampled in 2009 and from 1.7 to 5.9 for the newly appeared patches and meadows sampled in 2013 (Table 2).

The analysis showed no evidence of recent bottlenecks in most locations. However, the Arc transplant site, as well as the newly appeared patches at Arc (Arc 2013), showed a significant excess of heterozygotes (2-tailed Wilcoxon test, p = 0.0078 and p = 0.0195, respectively), indicating recent bottlenecks.

Table 2. Mean effective population size (N_e) estimates for the seagrass *Zostera noltei* 95% confidence intervals are also shown

Name	N_e	Lower 95%	Upper 95%
Carteau 2009	Negative/ Infinite	127.4	Infinite
Berre 2009	11	7.1	16.6
Arc 2009	2.2	1.7	2.7
Arc transplant	0.5	0.3	1.2
Arc 2013	1.7	1.3	2.2
Berre 2013	5.1	2.6	10.3
Vaïne	5.9	3.4	9.5

Inter-population assessments

The PCoA clearly showed that all natural meadows within the Berre lagoon grouped together, on the negative side of Axis 1 (variance = 45.84%). Both the transplanted site of Arc and the population in Carteau near the donor site were located on the positive side of Axis 1, coherent with the fact that material for transplants was collected in the Gulf of Fos, close to the Carteau population (Fig. 2). Nevertheless, the relict meadows and newly appeared patches in the Berre lagoon grouped closely together, while Carteau and the samples from the Arc transplant site were more distinct from each other (Fig. 2). Unfortunately, the Pointe de Berre transplant site could not be considered in the analysis, since only a single shoot was sampled from the single patch still existing.

An AMOVA was performed with 2 different scenarios of likely grouping: (1) 7 groups, with each population separately, and (2) 3 groups, with all natural lagoon sites versus the transplant site and the marine Carteau sites (Table 3). Among-population differentiation was higher in the latter scenario (variance = 19%), re-enforcing the distinctness of the transplant

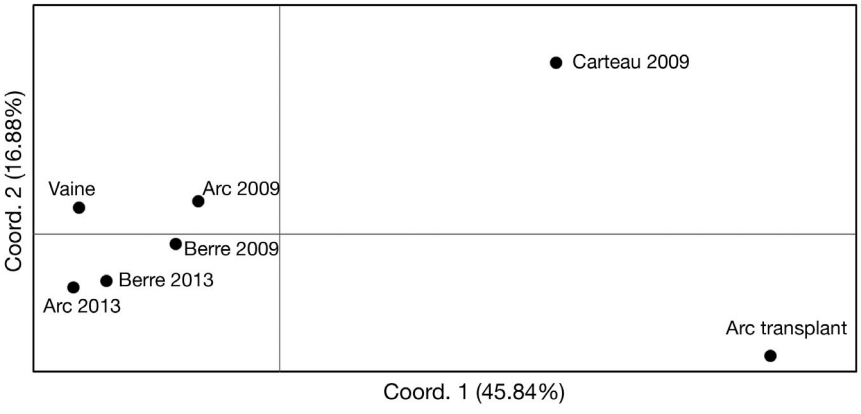

Fig. 2. Principal component analysis of samples of the seagrass *Zostera noltei* collected in the Berre lagoon in 2013 at the transplant site in Arc, at newly appeared patches in Arc and Berre and at a new meadow in Vaïne (Arc transplant, Arc 2013, Berre 2013 and Vaïne) and in relict meadows in the Berre lagoon and the marine donor site in 2009 (Berre 2009, Arc 2009 and Carteau 2009) based on mean pairwise population codom genotypic genetic distance

Table 3. Analysis of molecular variance for populations (populs) of the seagrass *Zostera noltei* in the Berre lagoon and the Golf of Fos (Car.)

Potential structure	Source	df	SS	MS	Estimated variance	Variance (%)
All populations	Among populs	6	188.497	31.416	0.534	18
	Within populs	379	918.379	2.423	2.423	82
Car. vs. lagoon vs. transplant	Among populs	2	97.907	48.954	0.618	19
	Within populs	383	1008.969	2.634	2.634	81

shoots from both the lagoon and the marine populations (Table 3).

The assignment test, as implemented in GeneClass 2 (Piry et al. 2004), assigned 84% of samples to their own population. Carteau was very distinct from all other populations, with 100% of individuals being assigned correctly, while the locations within the lagoon exhibited a higher level of connectivity (Fig. 3d, Table S1 in the Supplement at www.int-res.com/articles/suppl/m532p111_supp.pdf).

All pairwise comparisons of number of migrants per generation based on F_{ST} (N_m_F_{ST}) resulted in values >1 (Table S2 in the Supplement). Values >1 are expected to maintain a level of genetic connectivity between locations that is sufficient to avoid harmful effects of local inbreeding, although only values >10 are deemed to maintain similar allele frequencies (Lowe & Allendorf 2010). Our N_m_F_{ST} values ranged from 1.531, between Carteau and the Arc 2013 samples, to 5.276, between the relict meadow at Pointe de Berre (Berre 2009) and new patches at Pointe de Berre (Berre 2013) (Table S2, Fig. 3a). Pairwise comparisons of N_m based on the frequencies of rare alleles (N_m_freq) resulted generally in lower estimates, ranging from 0.1 to 1.8. N_m_freq, with the lowest values found between the transplants and the newly appeared patches at Arc and Pointe de Berre and the new meadow at Vaïne (Table S3 in the Supplement, Fig. 3b). N_m was also calculated using a multilocus genotype-based Bayesian assignment approach as implemented in BayesAss (N_m_Bay). Three independent runs were performed with very similar results and with migration rates differing by a maximum of 0.0024. Two runs delivered identical results, and those estimates were used for further analyses instead of using averages as recommended by Meirmans (2014). Estimates of N_m_Bay for first and second generation migrants ranged from 0.12 to 4.58. Lowest migration rates were calculated from Carteau and Berre 2009 to the Arc transplant site, and highest, between Berre 2009 and the new patches at Pointe de Berre (Berre 2013) (Table S4 in the Supplement, Fig. 3c).

Although N_m_F_{ST} estimates were considerably higher than N_m estimates from the other methods, all methods confirmed the presence of genetically distinct populations, with variable levels of gene flow within the Berre lagoon and with the transplant site having consistently lower connectivity than those among native populations in the Berre lagoon (Fig. 3). The marine site Carteau was most isolated from the other populations.

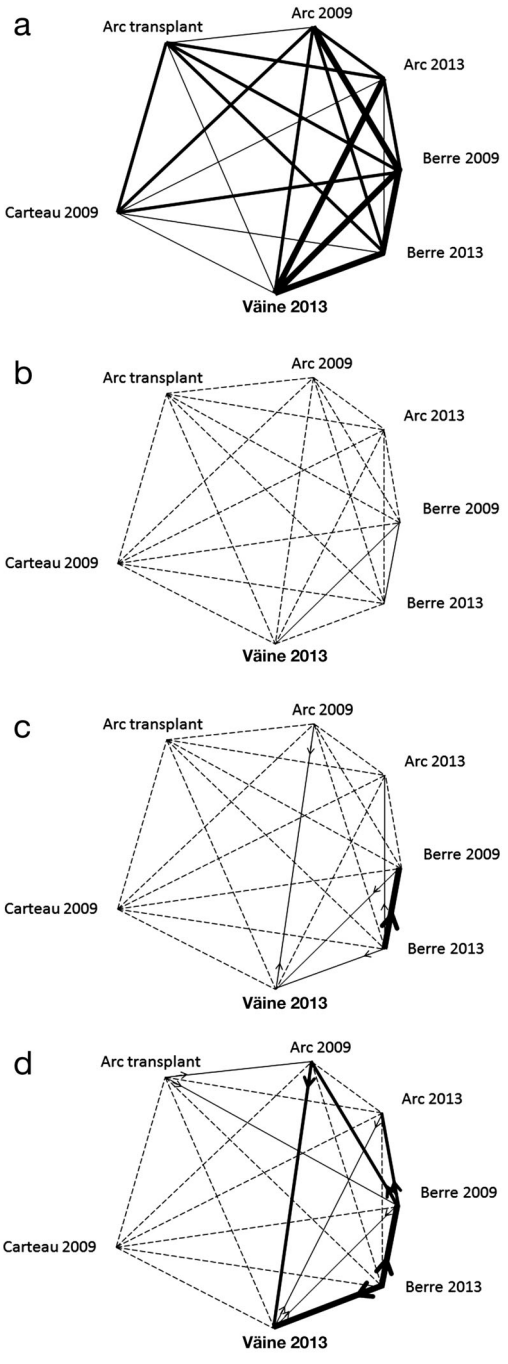

Fig. 3. Estimate of pairwise connectivity between populations of the seagrass *Zostera noltei*. (a) Number of migrants per generation (N_m) in pairwise population comparisons based on F_{ST} ranges from 1.5 to 5.3 N_m_F_{ST}. (b) N_m_freq values based on the presence of rare alleles range from 0.1 to 1.8. (c) First and second generation N_m_Bay values, including direction estimates, calculated with a BayesAss range from 0.12 to 4.58. (d) The number of individuals not assigned to their own population in an assignment test ranges from 0 to 8 in pairwise population comparisons. All population pairs are connected by a line. Dashed lines: values of N_m (migrants in Panel d) <1; thin lines: N_m (migrants in Panel d) = 1–2; medium lines: N_m (migrants in Panel d) = 2–3; thick lines: N_m (migrants in Panel d) >3

DISCUSSION

This study is one of few examples in which genetic data of both the near-to source population and receiving meadows have been available for a transplant trial in seagrasses before restoration. We show that, in 2013, the transplanted meadow within the Berre lagoon has lower genotypic diversity in comparison to relict meadows in 2009 and to newly appearing patches within the lagoon in 2013. We also show that newly appeared patches are likely derived from the lagoon gene pool (i.e. the relict meadows) and not from the genotypes used for transplantation or from outside the lagoon. We suggest that transplantation strategies should take into consideration both genetic and genotypic diversity of the donor material and the possibility of an adaptation mismatch due to environmental differences between donor and transplantation sites.

Genetic diversity can have high relevance for the success of restoration projects (Procaccini & Piazzi 2001, Williams 2001, Reynolds et al. 2012a,b, Ort et al. 2014). Theory predicts that small populations face the negative genetic consequences of increased inbreeding and reduced genetic variation caused by genetic drift, founder effects and accumulation of deleterious mutations (Procaccini et al. 2007), and signs of inbreeding have been found in many restored seagrass populations 10 yr after transplantation (Olsen et al. 2014). Reduced genetic diversity, the accumulation of mutations and increased inbreeding may decrease the evolutionary potential of a species or population to adapt to changing environments, and may reduce fitness (Leimu et al. 2006). While it has been shown that increasing the genetic diversity of a transplanted meadow by sourcing the most polymorphic donor material can be advantageous for transplantation success (Procaccini & Piazzi 2001, Williams 2001), the role of local adaptation should also be considered (Laikre et al. 2010). In this study, the donor material was sourced from a meadow in fully marine conditions, with little gene flow with the persistent meadows in the transplanting area, the Berre lagoon, as shown by F_{ST} values before transplantation. F_{ST} values were as high as 0.112 and 0.140 between the marine meadow and the 2 relict meadows at Berre and Arc, respectively (Procaccini et al. 2013). Mortality rates of the assessed restoration project were initially good, but very high after 4 yr (Dandine 2013), suggesting that these shoots were not well adapted to lagoon conditions, with higher turbidity, higher temperature and higher salinity variation than found in the source meadow.

The relict meadows, as well as the transplanted meadows, have been monitored regularly since 2009. No evidence of sexual reproduction of the transplanted shoots was detected until 2014, when flowers were reported in the remaining patch of the Berre transplanted site. In contrast, flowering was detected every year in the Arc and Berre relict meadows (P. Liger pers. comm.). The observed sexual reproduction of transplanted shoots in 2014 could result in the admixture of very distinct populations and could potentially contribute to the loss of locally adapted alleles or genotypes in the lagoon.

Genotypic diversity

We detected very high genotypic richness values at all sites assessed in the Berre lagoon, as well as in the marine Carteau meadow, but low levels at the transplant site in Arc. The low genotypic richness values at the transplant site could either be a result of the way the transplantation was performed (using bundles of shoots and therefore potentially only very few differing genotypes) or as a result of selective mortality over the 4 yr time frame after transplantation. High genotypic diversity has been associated with increased resistance (Hughes & Stachowicz 2004), resilience (Reusch et al. 2005, Ehlers et al. 2008), productivity (Hughes et al. 2008) and ecosystem services (Reynolds et al. 2012a) in the closely related species *Zostera marina*, and any transplantation project should aim to maintain levels of genotypic richness that are comparable with natural close-by meadows. Sourcing transplant shoots at a reciprocal distance of 1 to 2 m, as is done for genetic diversity assessments of *Z. noltei*, and a practice that is used for transplantations of *Z. marina* in the Wadden Sea (van Katwijk et al. 2009), might increase transplanting effort, but could help to increase levels of genotypic richness at transplant sites and ensure the presence of a higher number of alleles. Moreover, in *Z. marina* the use of seeds for large-scale transplantations seems a successful strategy for obtaining healthy meadows with high genetic diversity metrics over the time frame of years (Reynolds et al. 2013, Ort et al. 2014). This method has, to our knowledge, not been tried on *Z. noltei*, but could also provide a potentially more successful strategy in the future.

The generally high genotypic richness in the Berre lagoon compared to other locations along the distribution of *Z. noltei* (Coyer et al. 2004a, Diekmann et al. 2005, 2010, Ruggiero et al. 2005, Zipperle et al. 2009, 2011, Chust et al. 2013) indicates high levels of

sexual reproduction and local seed dispersal in the Berre lagoon. In *Z. noltei*, sexual reproduction is common at many locations, resulting in high genet turnover, aided by the fact that *Z. noltei* can form seed banks (Diekmann et al. 2005, Zipperle et al. 2011). However, some meadows of *Z. noltei* are also highly clonal, with little sexual reproduction (Ruggiero et al. 2005). It is unclear what factors influence the reproduction mode of this mainly perennial species, but low salinity (Boudouresque et al. 2009), as well as natural and anthropogenic disturbance in form of grazing by geese (Zipperle et al. 2010) and clam harvesting (Alexandre et al. 2005), have been shown to alter reproductive output.

Effective population size and bottlenecks

The effective population size at the marine site was infinite, while it was very low for all populations in the Berre lagoon, at orders of magnitude predicted to lead to a decline in heterozygosity and allelic richness (Allendorf et al. 2013). Despite the low N_e, which could be a natural consequence of the geographic isolation of lagoon populations, only the Arc transplant site and the recently naturally appearing patches at Arc showed signs of recent genetic bottlenecks. The low N_e, combined with evidence of recent population bottlenecks at the transplant site, suggests again that insufficient numbers of different genotypes were used during transplantation or that survival over 4 yr was too low to maintain allelic richness and heterozygosity.

Connectivity

The analysis of the number of migrants (based on 3 different methods), the assignment test, the AMOVA and the PCoA, all confirmed that there was a degree of connectivity among the assessed populations, albeit low. Connectivity was highest among the populations in the Berre lagoon and especially high between newly appeared patches in Pointe de Berre (measured in 2013) and the relict meadow at Pointe de Berre (measured in 2009), indicating the likely source for the newly appeared patches. The transplant site in Arc showed little gene flow with either the marine site at Carteau or all populations in the Berre lagoon. As the transplant material was sourced at the Carteau meadow this result was surprising; however, the donor material was collected approximately 400 m away from the location of the genetic assessment.

The naturally re-occurring patches in the Berre lagoon are genetically distinct from the transplanted meadows within the lagoon and from the transplant source locality, but are genetically similar to the relict meadows in the lagoon. This suggests that the newly appeared patches are derived from the lagoon gene pool and not from the genotypes used for transplantation or from outside the lagoon. Transplant sites were also considerably smaller than relict meadows, and this could reflect the decreased probability that they supplied propagules to the Berre lagoon. Nevertheless, the close proximity of transplanted sites and new patches may have favored colonization from the former, since most seeds disperse on a small scale, from centimeters to meters (McMahon et al. 2014). The distance between newly appeared patches and relict meadows, in fact, is on the order of kilometers, while it is on the order of meters between the transplant site and the newly appeared patches. Moreover, the most interesting aspect is that indigenous seeds can settle and grow naturally at exactly the same site where transplants have failed.

Conclusions

Transplantation efforts have shown extremely low success rates in Europe so far, suggesting that improving natural conditions is crucial before transplantations can be considered an option for ecosystem recovery, and restoration should not be considered the first alternative (Cunha et al. 2012). When transplantations are nevertheless deemed necessary to re-enforce local populations, genetic factors need to be taken into account. In this study, the genetic assessment showed that the transplant site at Arc had low genotypic and allelic richness values, an extremely low effective population size and showed evidence of a recent genetic bottleneck. Moreover, the transplants were genetically different from the lagoon populations, and the lack of success suggests an adaptation mismatch of the transplants that were sourced in a fully marine meadow. Research on *Z. marina* has revealed considerable intraspecific variation of morphology, physiology and phenotype among genotypes (Hughes et al. 2009), and evidence from plant restoration projects highlights the importance of using local genetic stocks for successful transplantations (McKay et al. 2005). Lagoons are confined environments, potentially highly dynamic along different time frames and highly disturbed (Basset et al. 2006), and local adaptation of genotypes might play an especially important role. The trans-

plants sourced in a marine meadow may not have enough phenotypic plasticity and may not be physiologically adapted to tolerate such harsh conditions. In conclusion, we want to highlight the need for genetic assessments in putative donor areas and in remnant meadows near the transplantation area before transplantations are carried out, in order to assess the genetic distinctiveness between donor and acceptor sites and to select genetically polymorphic material. Moreover, genetic monitoring after transplantation is also advised in order to detect unintentional genetic, ecological, or biological impacts on the ecosystem where the transplantation was performed — especially if the circumstances make it necessary to use genetically distinct material.

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