

The possible origin of *Zostera noltii* in the Canary Islands and guidelines for restoration

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Abstract Seagrasses and their habitat are declining worldwide. *Zostera noltii* in the Canary Islands has been drastically reduced, mainly by anthropogenic disturbance, to three small surviving patches in a single harbor in Lanzarote. A previous genetic study, using neutral microsatellite markers, revealed that these patches consist of a single clonal individual. Here, an assignment test, using microsatellite data, was used to locate the most likely population of origin from a set of possible donor populations. Our results show that the Moulay Bouselham population in northern Morocco is assigned as the most likely population of origin (88%), although the probability of being at one generation time distance is low (2.7%). This result, however, allows locating the most closely related stands that may be the most successful donor populations for future restoration based on shoot or seed transplantation.

Introduction

Seagrass beds are among the most valuable ecosystems in the world (Costanza et al. 1997) for their key roles in ecosystem functioning. Seagrasses are structural species; they are important for nutrient fixation, sediment trapping, erosion prevention, and function as nurseries for commercial fish and shellfish species. Yet, these seagrass-dominated systems are relatively unknown and therefore often underappreciated by the general public (Orth et al. 2006). Seagrass habitat is being lost and fragmented worldwide (Duarte 2002; Hughes et al. 2009). In many cases, this can be clearly attributed to anthropogenic disturbances such as pollution, fisheries, or coastal development (Short and Wyllie-Echeverria 1996; Holmer et al. 2008; Díaz-Almela et al. 2008). In some places, the decline has been so drastic that the recovery of the local meadows now entirely depends on transplantation programs aimed to reintroduce shoots or seeds from distant populations to give a new start to the declining or locally extinct meadow, such as in project BIOMARES (LIFE06/P/192) aimed at Restoration and Management of Biodiversity in the Marine Park Site Arrábida-Espichel.

Three species of seagrasses inhabit the shores of the Canary Islands (Pavón-Salas et al. 2000). *Cymodocea nodosa* (Ucria) Ascherson is the most abundant and can be found throughout the Canary Island archipelago, whereas *Halophila decipiens* Ostenfeld inhabits deeper water and sometimes lives in mixed meadows with *C. nodosa*; both are still relatively abundant compared to *Zostera noltii* Hornemann. Despite historical records in Fuerteventura and Gran Canaria (Pavón-Salas et al. 2000 and references herein), *Z. noltii* has now almost vanished and is restricted to three small patches in Arrecife, Lanzarote Island (Fig. 1, no 8), making it vulnerable to extinction. The population

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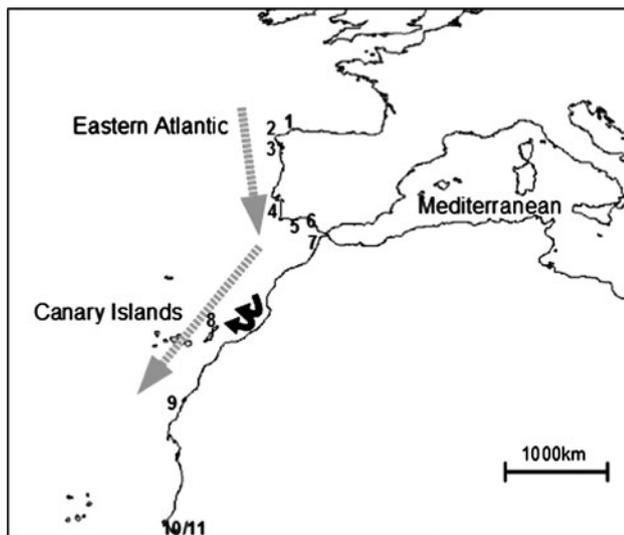


Fig. 1 Map showing collection sites of reference (possible donor) populations (1. Ortigueira, 2. Arousa, 3. Ria de Vigo, 4. Rio Mira, 5. Ria Formosa, 6. Puerto Real, Cadiz, 7. Moulay Bouselham, 8. Lanzarote, 9. Dahkla, 10. and 11. Mauritania, Banc d'Arguin). Also shown are the major currents along the Iberian Atlantic and North African coast (grey arrows), as well as the important Moroccan upwelling area (black arrows; Pelegrí et al. 2005a, b)

has decreased to a very small size (i.e., in terms of covered area) and is restricted to an intertidal area inside a harbor (Hernández and Gil-Rodríguez 2009). Besides that, a genetic analysis using microsatellite markers showed that it consists of only one single genetic individual (Rumeu et al. 2007). This further reduces the adaptive potential, and therefore the survival likelihood of these scarce patches in case of environmental change. Indeed, it has been demonstrated in the sister species *Zostera marina* L. that genotypic diversity plays an important role in the capacity to resist and recover from perturbations (Hughes and Stachowicz 2004; Reusch et al. 2005; Ehlers et al. 2008), and genetic diversity is an asset for the capacity of adaptation to changing environmental conditions (Spielman et al. 2004).

The restoration of the nearly extinct populations of *Zostera noltii* in the Canary Islands may therefore consider on increasing not only the cover and number of shoots to give a new start to the population, but also the diversity of the Lanzarote *Z. noltii* population by introducing new genotypes from healthy sources. In order to maximize the potential for adaptation to local conditions of the translocated seeds or shoots, as well as to prevent potential outbreeding depression by mixing of differently locally adapted populations, a transplantation effort should seek to introduce plants that are genetically similar to the native ones, although this is not the single criterion in selecting donor populations. The selection of donor population has been shown to be extremely important for reestablishment

success (Balestri et al. 1998; Van Katwijk et al. 2009). The present study is aimed to determine, using a Bayesian assignment method, which continental populations of *Z. noltii* are more genetically connected with the *Z. noltii* clone on Lanzarote Island. The time elapsed since the colonization of Canary Island by *Z. noltii* and the kind of demographic events that have occurred may prevent the identification of “the population of origin,” i.e., they may hamper the assignment of the unique remaining genotype in Lanzarote to an existing population. Nevertheless, our results allow us to propose the most likely geographical area where meadows would be the most closely related to the Lanzarote population, and this is one of the key biological information that can contribute to improve the success of future transplantation efforts in order to restore the *Z. noltii* population in Canary Islands.

Materials and methods

Determining the multilocus genotypes

The DNA samples of *Z. noltii* shoots from Lanzarote analyzed in this study were those characterized by Rumeu et al. (2007). Since it is very problematic to calibrate allele sizes estimated with different chemistry and electrophoresis devices, the samples from Lanzarote were re-genotyped, so we were able to compare the multilocus genotype (MLG) of the Lanzarote genet with a representative set of reference *Z. noltii* samples from Iberia previously analyzed by Diekmann et al. (2005). Several new samples from West Africa and Northern Iberia were also genotyped and included in the data set of reference populations (Table 1). Samples were genotyped using nine microsatellite loci described by Coyer et al. (2004). Fluorescent-labeled PCR Fragments were analyzed on an ABI PRISM 3130 Genetic Analyzer (AppliedBiosystems) using the GeneScan-500 LIZ standard. Raw allele sizes were scored with STRAND (<http://www.vgl.ucdavis.edu/informatics/STRand/>), binned using the R package MsatAllele (Alberto 2009) in R software (R Development Core Team 2009), and manually reviewed for ambiguities.

Genetic diversity analyses

Clonal copies (ramets) of the same MLG were considered as genets when the probability of being derived from distinct events of clonal reproduction, P_{SEX} (Arnaud-Haond et al. 2007), was lower than 0.01, as estimated using GenClone 2.0 (Arnaud-Haond and Belkhir 2007), and these were consequently removed from the samples before subsequent analyses. Genotypic Richness (R), the proportion of different genets in each population, was estimated

Table 1 Data set of the *Z. noltii* populations analyzed in this study, ordered from North to South, and relative scores of the assignment test for the different populations used as references

Population	GPS coordinates	<i>N</i>	<i>G</i>	<i>R</i>	H_E	F_{IS}	\hat{A} ($G = 5$; \pm SE)	\hat{A} ($G = 23$; \pm SE)	Relative score (%)	<i>P</i>
Spain (Ortigueira)	43°40.883'N 7°51.950'W	72	67	0.93	0.56	0.00857	3.596 (0.237)	6.2 (0.295)	0	0
Spain (Ría de Arousa)	42°32.867'N 8°51.500'W	43	38	0.88	0.47	0.08640*	3.078 (0.212)	4.887 (0.239)	0.02	0
Spain (Ria de Vigo)	42°21.043'N 8°37.714'W	48	44	0.91	0.58	0.08849*	3.509 (0.351)	6.418 (0.401)	0.01	0
Portugal (Rio Mira)	37°43.277'N 8°46.650'W	48	46	0.96	0.63	0.31469*	3.611 (0.412)	6.538 (0.486)	0.001	0.0001
Portugal (Ria Formosa)	37°0.230'N 7°59.213'W	48	48	1	0.66	0.16404*	4.038 (0.397)	7.316 (0.408)	1.9	0.001
Spain (Puerto Real, Cadiz)	36°31.305'N 6°11.112'W	48	47	0.98	0.65	0.05228*	3.971 (0.47)	6.891 (0.512)	2.0	0.002
Morocco (Moulay Bouselham)	34°52.334'N 6°17.348'W	40	40	1	0.65	0.22354*	3.718 (0.482)	6.789 (0.532)	87.9	0.027
Morocco (Dakhla)	23°52.395'N 15°47.209'W	30	5	0.14	0.42	— 0.46835*	2.222	NA	8.2	0
Mauritania (Banc d'Arguin 1)		39	23	0.58	0.55	−0.06431	3.538 (0.246)	5.778	0.04	0
Mauritania (Banc d'Arguin 2)	19°53.116'N 16°19.104'W	40	27	0.68	0.61	0.08690*	3.802 (0.29)	6.227 (0.155)	0.02	0
Canary Islands (Lanzarote)	28°57.436'N, −13°32.868'W	3	1	0	0.28	NA	NA	NA	NA	NA

N number of ramets, *G* number of genets, *R* genotypic richness, H_E expected heterozygosity, F_{IS} inbreeding coefficient, significant F_{IS} values ($P < 0.05$) are highlighted with asterisks, \hat{A} allelic richness standardized to a minimum common sample size of 5 (the lowest *G* recorded) and 23 (second lowest *G* observed) genotypes, standard error (SE) associated to the estimation of \hat{A} is shown in brackets. Relative score is indicated for assignment of the origin of the Canaries genotype, *P* probability test of correct assignment

following Dorken and Eckert (2001): $R = (G-1)/(N-1)$, where *G* is the number of genets and *N* is the number of ramets. Expected heterozygosities (H_E), inbreeding coefficient (F_{IS}), and an estimator of differentiation (F_{ST}) were calculated with Genetix v4.05.2 (Belkhir et al. 2001). We also tested for linkage disequilibrium (LD) and for deviations from Hardy–Weinberg equilibrium (HWE). Allelic richness (\hat{A}), average number of alleles per population, was calculated standardized for two different sample sizes using the StandArich package (<http://www.ccmr.ualg.pt/maree/software.php?soft=sarich>) and R software (version 2.9.0). The problem of unequal sample size is typical in clonal species where *G*, the number of genotypes or genetic individuals, is the relevant sample size for allelic richness estimates. Independently of the sampling design used for clonal organisms, *G* is unpredictable before genotyping, even when *N*, the number of sample units, is kept constant across samples. To test for isolation by distance (IBD) across reference populations (Wright 1943; Slatkin 1993), estimates of $F_{ST}/(1-F_{ST})$ (Rousset 1997) using the θ estimator (Weir and Cockerham 1984) in GENETIX 4.05.2 were correlated with the geographical

distance using matrix correlation methods based on the Mantel test (Manly 1994) in the isolation by distance web service (IBDWS) Version 3.16 (Jensen et al. 2005) with 10,000 randomizations. The strength of the isolation by distance relationship was determined with reduced major axis (RMA) regression, which is more appropriate than standard ordinary least squares regression when the independent axis (geographic distance) is measured with error (Sokal and Rohlf 1981; Hellberg 1994) and calculated with IBDWS 3.16 (Jensen et al. 2005).

Assignment testing

An assignment test was performed using GenClass 2.0 (Piry et al. 2004) to find the most likely population of origin for the Lanzarote clone, where a Bayesian method is used that assigns individuals to their possible population of origin on the basis of the composition of their genotypes (Baudouin et al. 2004). It is noteworthy that by testing in this way, an individual will always be assigned to a possible population of origin, even though the 'real' one may not be present in the putative sources available.

A probability test is therefore needed to estimate the probability that the assigned population is the actual population of origin. Probability testing was performed using a Monte Carlo resampling procedure (Paetkau et al. 2004) to compute the probability that an individual belongs to each reference population (Testing for Type I error). The number of resampled individuals was set to 10000, and the Type I error threshold was set to 0.01.

Results and discussion

Genotypic richness (R) varied greatly in our reference meadows, ranging from 0.14 in the Dahkla meadow to 1 across the Iberian and North African meadows, and was 0 in Lanzarote since the remaining patches consisted of only 1 genet (Table 1). Expected heterozygosity ranged from 0.28 to 0.66 and tended to be particularly lower in the samples with low genotypic diversity. We found no evidence for LD. Several samples showed significant deviations from Hardy–Weinberg equilibrium. Both significant positive and negative values were observed. The high F_{IS} values found for Moulay Bousselham, Ria Formosa and Rio Mira suggest significant inbreeding, or a Wahlund effect, the mating between differentiated subgroups within the population. Low effective population size is likely in clonal populations and may contribute to biparental inbreeding and to selfing, the most extreme type of inbreeding (Delmotte et al. 2002; Billingham et al. 2007). Allelic richness did not vary much among all these reference populations, with the highest values observed in the central range (Ria Formosa population; Table 1). Pairwise F_{ST} values ranged from 0.06 to 0.43 (Table 2), and all possible donor locations were significantly differentiated ($P < 0.001$), therefore corresponding to distinct, dynamically independent populations. Significant (Mantel test) IBD was observed ($P < 0.0001$) among the Atlantic

reference populations. Populations at distances as small as 4 km (the two Mauritania populations) are differentiated, as shown by significant F_{ST} , and differentiation increases with increasing distance (Fig. 2).

Our results show that *Z. noltii* does not disperse widely at the scale of 10–100 km and confirm the expectation that the depleted meadows from the Canary Island are not likely to be recolonized through natural dispersion from continental meadows. Regardless of whether or not some of the continental populations have a more recent common history with the vanished meadows from Canary Islands, and/or are more likely sources for further reintroduction or natural recolonization, the high level of differentiation between populations also suggests that divergence may hamper a very significant assignment of the last remaining Lanzarote genet to any of the reference populations, particularly because assignment tests are typically set for one generation distance to the putative parental pool.

Among all reference meadows, the one from northern Morocco (Moulay Bousselham) was assigned as the most likely population of origin (Table 1). The probability test confirmed this relative closeness of the Moroccan and Lanzarote genetic compositions, with, however, a low probability (0.027) for the first one to be at one generation distance from the Lanzarote genet. This is not surprising because, as discussed above, both the original source(s) and the Lanzarote population have had long and likely independent histories since colonization. Considering the high values of F_{ST} reported here for populations separated by similar geographic distances, an independent evolution of gene pools at relatively small distances may be expected and it is therefore not possible to confidently identify the population of origin. However, the use of an assignment test indicates that the surviving Lanzarote genet is genetically closer to the Moroccan population than to any other reference populations and therefore that (1) the area of origin of the population that gave rise to that genet might

Table 2 Matrix showing pairwise F_{ST} values (above diagonal) and pairwise distances in kilometers (below diagonal)

	Ortigueira	Arousa	Ria de Vigo	Rio Mira	Ria Formosa	Puerto Real	Moulay B.	Dahkla	Mauritania 1	Mauritania 2
Ortigueira		0.18387	0.18766	0.25805	0.25910	0.25767	0.19822	0.39676	0.36252	0.34254
Arousa	250		0.15805	0.17611	0.17293	0.20918	0.14282	0.42765	0.38798	0.36773
Ria de Vigo	300	50		0.18822	0.14035	0.16985	0.18495	0.37617	0.34491	0.31977
Rio Mira	850	600	550		0.08987	0.12905	0.16171	0.32207	0.28134	0.26616
Ria Formosa	1,050	800	750	200		0.09315	0.15381	0.32135	0.28183	0.25739
Puerto Real	1,250	1,000	950	400	200		0.15477	0.34503	0.29326	0.25897
Moulay B.	1,450	1,200	1,150	600	400	200		0.29561	0.27169	0.25054
Dahkla	3,050	2,800	2,750	2,200	2,000	1,800	1,600		0.22459	0.21375
Mauritania 1	3,600	3,350	3,300	2,750	2,550	2,350	2,150	550		0.05964
Mauritania 2	3,604	3,354	3,304	2,754	2,554	2,354	2,154	554	4	

All F_{ST} were significant ($P < 0.05$, 10000 permutations and after Bonferroni correction)

have been located along the Moroccan coast, and more importantly (2) that the genetically closest source for future translocation programs should be searched for in this area. We excluded from this study a possible donor population from another part of the *Z. noltii* distribution i.e., Northern Europe or the Mediterranean, because a study by Coyer et al. (2004) showed that both northern populations and the Mediterranean are very different compared to the Iberian and West African coast. The same pattern is found in *Cymodocea nodosa* (Alberto et al. 2008) where Mediterranean populations are different from Atlantic populations, and particularly from the Canary island populations.

Establishment of migrating seagrasses in subtidal regions is likely to occur by seeds transported in the migrating ramets (as shown for *Zostera marina* by Reusch 2002), yet since *Z. noltii* meadows are mainly intertidal, it may be easier for drifting shoots to become buried in appropriate habitat, as they reach shorelines at low tide. The minimal distance between Morocco and Lanzarote is 110 km (Fig. 1). The Canary Current is moving southwards along the Moroccan coast (Pelegrí et al. 2005a). Part of the current passes west of the Canary Islands, while the other part moves between the Moroccan coast and the Archipelago. The upwelling from the Moroccan coast extends away from the coast and reaches the Eastern Islands of the Archipelago, Fuerteventura, and Lanzarote (Pelegrí et al. 2005b). Therefore, it could have served as a possible route of colonization or have maintained a minimum level of gene flow between the Moroccan and Canary Island

populations when those had a sufficient effective population size. In any case, the present day pattern of sea surface currents makes colonization from areas further southwards on the African coast less likely. Indeed, the relative scores of the assignment tests decrease drastically from Moulay Bousselham to Dakhla *Z. noltii* populations, which are located northwards and southwards of the Canary Islands, respectively. The distance between the northern Moroccan population and Lanzarote is still quite large (1,000 km), and there might be areas between Moulay Bousselham and Lanzarote that could function as more appropriate donor populations.

Restoration of seagrass habitat will be successful only when the stress factors have been identified and eliminated. The first rule in the guidelines for restoration (van Katwijk et al. 2009) and the most important rule for any seagrass restoration attempt is to reverse the habitat degradation. A recent paper by Ferrer and Gil-Rodríguez (2009) shows that *Z. noltii* is now listed in a category “Risk of Extinction” in the *Catálogo de Especies Amenazadas de Canarias* (BOC 2001/097). Several actions must be taken urgently: to prevent discharges to improve the quality of seawater and to reduce the transit of the people through the area of seagrasses. However, some measures have been taken such as industrial and water treatment resulting in increased water quality, but not specifically for the protection of the seagrass in the area. These measures probably helped the remaining meadows or patches to survive.

A second important guideline involves site selection. Preferably, the site should have a history of seagrass growth although there are examples where seagrass restoration was successful in areas which did not have a seagrass history (Campbell and Paling 2003). In Lanzarote, *Z. noltii* is still present so this second guideline is also met. The Lanzarote *Z. noltii* meadow is a special case because it consists of one single genetic entity or clone. This genotype might have survived by chance or it might instead have survived due to being the most resistant to the stresses it is exposed to. The patches did, however, not increase in size for the past years although large clones have been found elsewhere in natural populations for this (Coyer et al. 2004) and other species of seagrass (Reusch et al. 1999; Arnaud-Haond et al. 2007; Alberto et al. 2008). In order to increase the chance of meadow extension, it is advisable to increase genetic variation by introducing novel genotypes by transplanting either plants or seeds. This brings us to guideline number three: the selection of appropriate donor population(s). In general, donor populations for restoration should be (1) adapted to similar environmental conditions so that survival and meadow expansion are enhanced at the transplantation site and (2) have sufficient adaptive potential for future environmental changes meaning that sufficient genetic variation should be present in the donor

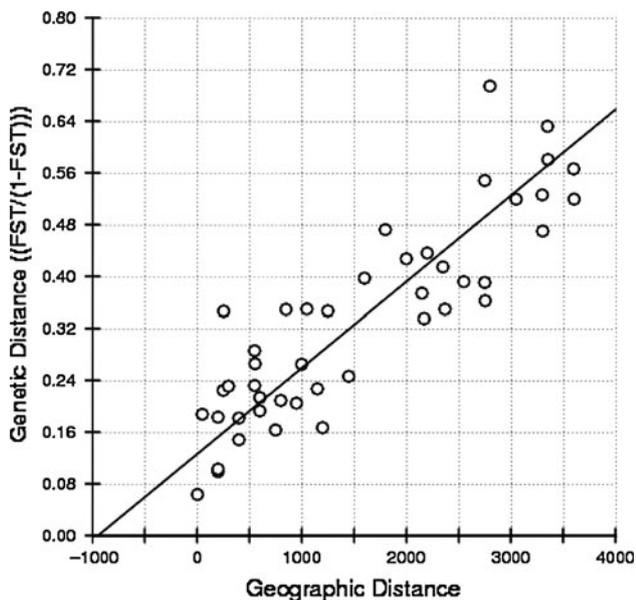


Fig. 2 Isolation by distance. Pairwise comparisons of genetic and geographic distance (km) among the 10 Atlantic reference populations. The genetic and geographic distance matrices were significantly correlated (Mantel test; $P < 0.0001$ and $r = 0.878$). The regression estimates are: $y = 1.330e-04 x + 0.1259$, $r^2 = 0.771$

population. In the present study, we focused on selecting the donor population that best resembles the original genotype. Transplanting plants or seeds from this population increases the chance of a highly genetically diverse restored population. There are risks associated to the transplantation. First, there is of course the risk that the original genotype is lost because it is outcompeted by the introduced genotypes. Second, there is also the risk of outbreeding depression. Crosses between the Lanzarote genotype and the transplants may result in hybrids that have a lower fitness. Eventually, this could result in replacement of the original genotype by the introduced population. But the opposite is also possible where the crosses between the local genotype with introduced genotypes have an advantage (heterosis). This would be of course the best scenario because then the local alleles would be preserved. The important question is whether the objective is to preserve the local genotype or the species on Lanzarote. If the aim is to preserve the remaining Lanzarote genotype, additional studies are needed to test for outbreeding depression/heterosis effects through breeding experiments. Ideally, such hypotheses of inbreeding/outbreeding depression should be experimentally tested in controlled conditions before any transplantation efforts.

Another important guideline proposed by Van Katwijk et al. (2009) is that of spreading the risk of transplant loss over space and time, especially in a highly dynamic coastal environment influenced by storms, temperature fluctuations, and sediment movement. For the Lanzarote site, risk could be spread by not limiting transplantation to the surviving *Z. noltii* meadow but by replicating at various distances from that site. Transplantation could even be extended to the other islands where *Z. noltii* previously existed, i.e., Gran Canaria and Fuerteventura. For long-term transplantation success, it might be wise to include transplanting into suboptimal sites, which may be particularly selective, in order to keep genetic variability in space, maintaining more diverse genotypes that might be advantageous for survival when the environment changes (van Groenendael et al. 1998).

Conclusion

Our study shows that *Z. noltii* populations from the Moroccan coast northwards from the Canary Islands are so far the closest ones to Lanzarote population in genetic terms. These Moroccan populations should ensure the best survival as donors in future restoration plans since genetically similar individuals may maintain preexisting adaptive traits to the local environment (Van Katwijk et al. 2009). Furthermore, the Moulay Bousselham population is bearing one of the highest genotypic and allelic diversities

characterized in the present study. These two genetic parameters have been proven to be important for resistance and resilience to perturbations (Hughes and Stachowicz 2004; Spielman et al. 2004; Reusch et al. 2005; Ehlers et al. 2008) and may, therefore, provide the transplanted population with a better adaptive potential on the mid- and long-term.

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