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High genetic diversity and strong differentiation in dramatically fluctuating populations of *Zostera japonica* (Zosteraceae): implication for conservation

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Abstract

Aims

Seagrasses provide a variety of ecosystem goods and services, but they are subjected to frequently anthropogenic disturbances. In this study, we genotyped samples collected from *Zostera japonica* meadows with dramatic fluctuations in the area in order to understand the distribution of genetic variation within and among populations.

Methods

We collected samples from eight extant populations along coastal areas of southern China. Ten polymorphic microsatellites were adopted to genotype the samples. Parameters of genetic diversity and differentiation were calculated with general software.

Important Findings

High levels of genetic diversity were found in the studied populations, suggesting that the effective population size has not decreased significantly, which was supported by no signs of recent bottlenecks. High genetic diversity reflects an important role of sexual seedling recruitment in *Z. japonica* populations. We found a significant relationship between genetic differentiation and the shortest sea surface distance of populations, suggesting that ocean currents play a critical role in shaping the genetic structure of *Z. japonica* populations. STRUCTURE software analysis clustered the eight populations into two groups: western and eastern populations separated by the Qiongzhou Strait/Leizhou Peninsula, hinting that there was very limited gene flow through the narrow strait in this marine plant. Four populations had high contribution diversity and, thus, high priority for *in situ* conservation.

Keywords: Seagrass, *Zostera japonica*, Genetic diversity, Genetic differentiation, Microsatellites

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INTRODUCTION

Seagrasses are monocotyledons that complete their full life cycle submerged in marine environments (den Hartog 1970). The seagrass ecosystems provide a variety of ecosystem services, such as maintenance of fisheries and other marine animals, carbon sequestration, and coastal protection by reducing wave energy (Barbier *et al.* 2010). Seagrasses are found in all coastal regions of the world except along the Antarctic shores (Short *et al.* 2007), but their distribution is narrow, usually restricted to near-shore shallow water zones with a maximum distribution of ~80 m in depth (den Hartog 1970). However, these coastal areas are subjected to tremendous direct and indirect anthropogenic effects, resulting in an accelerating

loss of seagrass meadows and the ecosystem services they provide (Short *et al.* 2011; Waycott *et al.* 2009). In China, seagrass meadows have also experienced dramatic declines due to anthropogenic disturbances (such as aquaculture, water pollution, reclamation and channel dredging) accompanying China's rapid economic development in recent decades (Zheng *et al.* 2013). Therefore, several national and provincial reserves were established in order to protect the extant seagrasses, and some projects were initiated to restore seagrass meadows (Zheng *et al.* 2013). Unfortunately, most restoration efforts ended in failure because of low seagrass survival rates and improper protocols for restoration (Qiu *et al.* 2014).

Accompanying habitat loss, remaining populations may have experienced population bottlenecks and then loss of genetic variation due to genetic drift (Lu *et al.* 2006; Xiao *et al.* 2015). The extent of reduction in genetic diversity depends on both the duration of and the population size at the bottleneck (Nei *et al.* 1975). However, recruitment via sexual reproduction may increase novel genotypic diversity (Eriksson 1993; Reusch 2006), offsetting the reduction of genetic variation in perennial clonal plant populations (Araki and Kunii 2006; Benson and Hartnett 2006). Impact of disturbance on genetic diversity clonal plants, therefore, is complex, and is relatively less understood (Banks *et al.* 2013).

Zostera japonica Aschers. et Graebn. (Zosteraceae) is a small monoecious seagrass with a typical leaf size of 20 cm in length and 0.8–1.2 mm in width (Moore and Short 2006). This species inhabits sandy or muddy intertidal and shallow subtidal zones. It is one of the very few seagrasses that is distributed from tropical to colder temperate zones. *Zostera japonica*-dominated meadows had experienced fluctuations in area (Huang *et al.* 2006; Zheng *et al.* 2013), due to rapid economic development in Southern China. *Zostera japonica* is a seagrass with mass flowering and seed production. In the introduced regions, high seed densities (>600 per m^2) were observed in sediments in the winter (Bigley 1981; Nielsen 1990), and the proportion of new shoots arising from seeds in spring was high (Harrison and Bigley 1982; Ruesink *et al.* 2010). In general, *Zostera* seagrasses have a transient rather than a persistent seed bank (Jarvis *et al.* 2014; Orth *et al.* 2006). However, seeds of *Z. japonica* buried in deep sediments may be maintained viable over one year, though the density was very low (Bigley 1981); and this species has the ability to develop a persistent seed bank (Kaldy *et al.* 2015). Thus, we expected that extant *Z. japonica* populations, though dramatically fluctuating sizes, might have no sign of recent bottlenecks and a high level of genetic variation comparable to that in populations with relatively stable sizes due to its repeated seedling recruitment.

To test the above hypothesis, we collected samples from the extant *Z. japonica* populations in the southern distribution range that have experienced dramatic loss and fluctuation in areas of seagrass meadows (Fan *et al.* 2007, 2011; Wang *et al.* 2012). We analysed genetic composition using polymorphic microsatellite markers developed for *Z. japonica* (Jiang *et al.* 2011). Specifically, our aims were to: (i) estimate the level of population genetic diversity; and (ii) describe and analyse the genetic differentiation among the studied populations.

MATERIALS AND METHODS

Study sites and sample collection

Zostera japonica is a slender seagrass that distributes in intertidal and/or shallow subtidal zones. The northern distribution ranges are located in Far Eastern Russia, Korea, Japan, and North and East China, and the southern distribution edges are located in South China and North Vietnam. Although it was considered to be an invasive seagrass along the Pacific Coast of North America (Harrison and Bigley 1982; Shafer *et al.* 2014), the native ranges of *Z. japonica*-dominated meadows have been declining due to anthropogenic disturbances (e.g., Lee *et al.* 2004).

Zostera japonica has been recorded in 10 sites along the coastal regions of southern China (Fan *et al.* 2011; Huang *et al.* 2006). The average surface water temperature is about 20°C in February and 28–29°C in August (Fang *et al.* 2006), and typhoons occur frequently in the summer and fall. Areas of the extant *Z. japonica* have experienced large fluctuations. Seagrass meadow in Zhenzhu Bay was 12.7 ha in the autumn of 2007, and 41.6 ha in the summer of 2008 (Fan *et al.* 2011), but 10 ha in the winter of 2011 when we collected the samples (Table 1). Seagrass meadow in Hepu was 14.3 ha in 2001 but 225 ha in 2003 (Fan *et al.* 2007). The seagrass meadow area in site DJ was 5 ha in the summer of 2010 (Table 1), but no *Z. japonica* meadow could be found via extensive investigation from 2004–2009 (Wang *et al.* 2012). The area of seagrass meadow located in Shangchuan archipelago was very small when we conducted the sample collection (Table 1), but we were told by local villagers that the whole bay (SZ) were covered with seagrasses several years before our sampling. Density of *Z. japonica* varied among seagrass meadows, ranging from 33 (GS) to 4690 (Island Shangchuan) in the studied populations (Fan *et al.* 2011).

After carefully surveying these sites from 2008 to 2011, we only found eight remnant populations along the tropical coasts of China. We collected samples from these eight populations (SX, DC, GS, JD, BG, DJ, YT and SZ) (Table 1; Fig. 1). Populations SX and DC were located in the same bay, i.e., Zhenzhu Bay in Guangxi Autonomous Region. Populations GS and JD were separated by a river and located in Hepu county in Guangxi Autonomous Region. Populations BG and DJ were located in Hainan Province, south of the Qiongzhou Strait (Fig. 1). Populations YT and SZ were collected from seagrass meadows located west and north, respectively, of Shangchuang Island in Guangdong Province (Fig. 1). The position of each population was recorded using eTrex H GPS (UniStrong, Inc., Beijing, China). Between 13 and 50 samples were collected haphazardly with a minimal distance interval of 2 m between any two samples (Table 1). We collected 251 samples, and all individual samples were cleaned and then dried with silica gels.

Table 1: sampling information of *Zostera japonica* populations located in coastal regions of South China

Population ID	Geographic coordinates	Sample interval (m)	Sample size	Area of seagrass meadow (ha)	Composition of seagrass meadow
SX	N21°34′29.6″, E108°11′15.6″	8	50	25	Monospecific
DC	N21°35′51.6″, E108°12′45.5″	10	33	50	Monospecific
GS	N21°29′45.8″, E109°41′14.1″	2	14	<1	Mixed with *Hb*
JD	N21°28′34.1″, E109°42′44.8″	2	28	10	Monospecific
BG	N20°00′44.0″, E110°33′38.8″	20	37	25	Mixed with *Ho*
DJ	N19°31′33.6″, E110°51′14.5″	5	44	5	Mixed with *Cr*, *Th*, *Ho*, *Cs*, *Si*
YT	N21°39′21.8″, E112°45′47.5″	2	32	<2	Monospecific
SZ	N21°43′05.0″, E112°45′34.8″	2	13	<1	Monospecific

Abbreviations: *Hb* = *Halophila beccarii*, *Ho* = *Halophila ovalis*, *Cr* = *Cymodocea rotundata*, *Th* = *Thalassia hemprichii*, *Cs* = *Cymodocea serrulata*, *Si* = *Syringodium isoetifolium*.

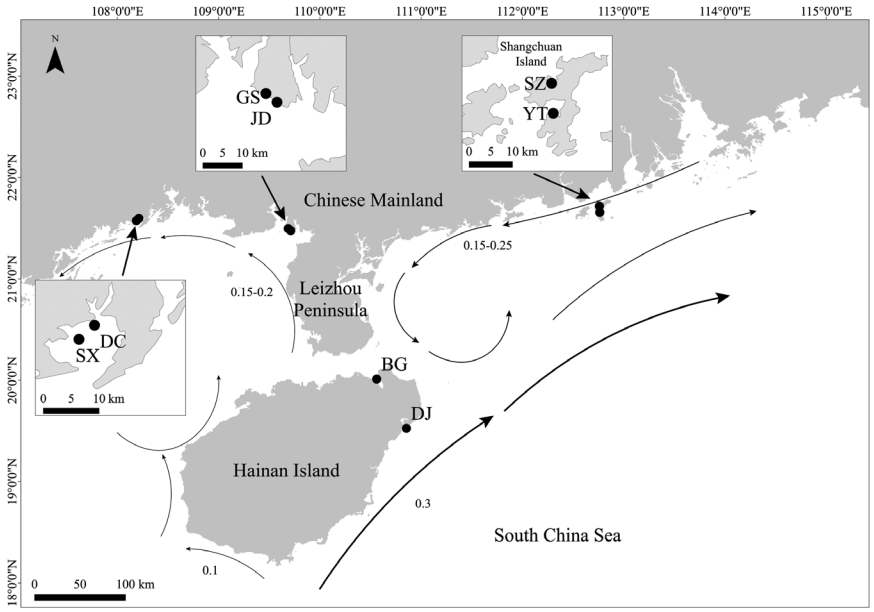

Figure 1: map of the eight sampling sites of *Zostera japonica* located in coastal regions of South China. Major surface currents occurring during reproduction season are drawn following the map by Bao *et al.* (2005). The number beside the arrow indicates current speed in m/s.

Microsatellite amplification and genotyping

Total genomic DNA was extracted from approximately 35 mg dried leaf tissue using a Plant Genomic DNA Kit (Tiangen, Inc., Beijing, China). We genotyped all 251 samples using 12 microsatellite primers (*ZJ5*, *ZJ48*, *ZJ178*, *ZJ198*, *ZJ207*, *ZJ397*, *ZJ542*, *ZJ706*, *ZJ896*, *ZJ1069*, *ZJ1345* and *ZJ1368*) (Jiang *et al.* 2011). We performed PCR following the conditions proposed by Jiang *et al.* (2011). Fluorescent-labelled PCR fragments were analysed on an ABI3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using an internal lane standard (GS500 (-250 LIZ)). We then conducted allele binning and calling using GeneMapper 4.0 (Applied Biosystems).

Analyses of genetic diversity

Zostera japonica can reproduce both sexually via seed production and asexually via rhizomes and, thus, we first assessed the number of genets based on multi-locus genotypes (MLGs) of all samples. However, observation of identical MLGs can result from either sampling of the same genet or genetic recombination arising from sexual reproduction (Arnaud-Haond *et al.* 2007). To solve this problem, we calculated the probability of a given MLG occurring more than twice as a consequence of recombination events (P_{sex}) and tested the significance of P_{sex} via a Monte Carlo simulation (10^3 iterations) approach using MLGsim v2.0 (Stenberg *et al.* 2003).

We performed an outlier test with the 12 loci using Fdist2 software (Beaumont and Nichols 1996). Loci *ZJ48* and *ZJ1368* were potentially under directional selection as suggested by the outlier test and, thus, were excluded from subsequent analyses. To quantify the power of the remaining 10 loci, we estimated the unbiased probability of identity ($P_{ID(unbiased)}$) and the P_{ID} among sibs ($P_{ID(sib)}$) (Waits *et al.* 2001) over all

loci using GIMLET v1.3.3 (Valière 2002). In addition, a test of the 10 loci combinations was performed with GenClone v2.0 software (Arnaud-Haond and Belkhir 2007).

In clonal plants, two or more sampling units may be the same MLGs and bias the conventional genetic diversity indices, such as Nei's gene diversity. Thus, we calculated genetic diversity based on the MLGs. The number of alleles (N_A), number of private alleles (P_A), inbreeding coefficients (F_{IS}), allelic richness (A_R) (standardised to the minimum sample size) and observed and expected heterozygosity (H_O and H_E, respectively) using FSTAT v2.9.3.2 (Goudet 1995). To understand genotypic diversity, Simpson diversity complementary index (probability of two units randomly taken in the sample belonging to different MLGs, D*) and the corresponding evenness index (ED*) and clonal diversity (fraction of distinct clonal lineages relative to the number of sampling units, P_d) were calculated using GenClone 2.0 (Arnaud-Haond and Belkhir 2007).

On the basis of the MLGs, we tested the linkage disequilibrium and Hardy-Weinberg equilibrium using GENPOP'007 (Rousset 2008).

Genetic differentiation

To assess population differentiation, pairwise F_{ST} values and their significance between populations were calculated using FSTAT. Because F_{ST} may underestimate genetic differentiation when using markers of high levels of allelic variability, we estimated the standardised parameter of genetic differentiation F'_{ST} (=F_{ST}/F_{STmax}). F_{STmax} was obtained after recording the data with RECODEDATA (Meirmans 2006). In addition, using SMOGD v1.2.5 (Crawford 2010), we calculated D_{EST}, which may be more accurate than traditional measures, especially for highly polymorphic markers such as microsatellite loci (Jost 2009).

To test isolation by distance (IBD), the Mantel test was used to analyse the relationship between genetic differentiation and geographical distance matrices using IBD v1.53 software (Bohonak 2002) with 4000 replicates. We used $F_{ST}/(1\text{-}F_{ST})$ (Rousset 1997) as the parameter of genetic differentiation. The geographical distance between populations was based on the shortest sea surface distance other than the shortest spatial distance assuming that diaspores (seeds and fragments) of *Z. japonica* can only be dispersed via sea currents or marine animals.

To detect population genetic structure, individuals were assigned to infer clusters through the 10 microsatellite loci using STRUCTURE v2.3 software (Hubisz *et al.* 2009). The admixture ancestry model was used, and allele frequencies were set to be correlated. In each run, the burn-in length was set as 1×10^5, and the number of Markov chain Monte Carlo replications was 1×10^6. To obtain the best estimate of K, we set K = 1–10, performed 15 runs of each K and calculated ΔK, a statistic proposed by Evanno *et al.* (2005). We then performed 15 additional runs with the optimum K value using the same parameters to classify all genets.

We assigned the amounts of variation between groups and among populations by hierarchical AMOVA using Arlequin v3.5 (Excoffier and Lischer 2010). Nonparametric permutation procedures with 10 000 permutations were used to calculate significant differences.

Test of recent bottlenecks

Evidence for population fluctuations in the eight sites was evaluated with BOTTLENECK v 1.2.02 software (Piry *et al.* 1999). We used the two-phase model (TPM) and Wilcoxon sign-rank test to determine whether the average standardized difference between expected and observed heterozygosities significantly differed from zero (Cornuet and Luikart 1996). We set stepwise mutations as 79% with a variance of 9%, with 1000 simulations for each population (Wyllie-Echeverria *et al.* 2010).

Identifying populations for conservation priority

To identify the populations with a high priority for conservation, we assessed the contribution of each population to the whole genetic diversity in terms of allele number using the contribution diversity approach (Lu *et al.* 2007) with PGCA v1.0 software.

RESULTS

Population genetic diversity

A total of 234 individuals were genotyped successfully with these 10 loci. The 10 loci were moderately polymorphic, and their combination had sufficient resolution to discriminate different genets. A minimum of 8 loci would be powerful enough to identify all MLGs in whole populations (see online supplementary Fig. S1). P_{ID} values derived from each population ranged from 2.001 e^{-8} (DC) to 7.546 e^{-5} (YT), also suggesting that the 10 microsatellites were sufficiently polymorphic to identify genets. $P_{ID(sib)}$ values derived from each population ranged from 8.129 e^{-4} (DJ) to 1.044 e^{-2} (SZ). Using GenClone, we found that seven MLGs included repeated individuals (one genet with three repeats and six genets with two repeats) and, thus, there were 226 MLGs in total. With MLGsim, we found the P values of those P_{sex} that were shared by individuals with the same MLGs to be significantly smaller than the expectation of random mating. Therefore, we rejected the null hypothesis that those ramets with the same MLGs belonged to a different genet. Finally, 226 MLGs were identified. Based on these 226 MLGs, no significant linkage disequilibrium was observed between any pair of loci in the population, and the global test across populations and loci showed that two loci (*ZJ198* and *ZJ397*) were significantly biased from the Hardy-Weinberg equilibrium.

All of the sampled populations showed a high level of clonal diversity, ranging from 0.742 (YT) to 0.980 (SX) (Table 2). Moreover, Simpson diversity complementary indices were high in these populations, and Simpson diversity uniformity complementary indices were low except in population YT

Table 2: Parameters of clonal and genetic composition of *Zostera japonica* populations located in coastal regions of South China

			Clonal composition				Genetic composition					
Population	*N*	G	L	P_d	*D**	*ED**	H_E	H_O	A_R	F_{IS}	N_A	A_P
SX	50	50	49	0.980	1.000	-	0.487	0.533	4.694	-0.095	68	6
DC	29	28	28	0.964	0.998	0	0.491	0.550	4.699	-0.122	59	2
GS	13	13	12	0.917	1.000	-	0.401	0.454	3.435	-0.139	35	0
JD	27	26	24	0.885	0.997	0	0.463	0.504	3.518	-0.092	42	1
BG	36	36	35	0.971	1.000	-	0.561	0.620	3.758	-0.108	48	7
DJ	34	34	33	0.970	1.000	-	0.621	0.694	4.199	-0.120	51	6
YT	32	28	24	0.742	0.992	0.778	0.373	0.363	2.826	0.028	33	4
SZ	13	11	11	0.833	0.962	0	0.413	0.473	2.800	-0.153	28	2

Abbreviations: *N* = number of sample units, *G* = number of multilocus genotypes, *L* = number of multilocus lineages, P_d = (G-1)/(N-1), *D** = Simpson diversity complementary index, *ED** = Simpson diversity uniformity complementary index, H_E = expected heterozygosity, H_O = observed heterozygosity, A_R = allelic richness, F_{IS} = inbreeding coefficient, N_A = number of alleles, A_P = number of private allele.

(Table 2). The mean number of alleles per locus ranged from 2.8 (SZ) to 6.8 (SX). Private alleles were found in seven populations, but not in population GS, with population BG having a maximum number of seven alleles. Expected heterozygosities (H_E) ranged from 0.373 (YT) to 0.621 (DJ), and observed heterozygosities (H_O) ranged from 0.363 (YT) to 0.694 (DJ). Allelic richness was moderate, ranging from 2.800 (SZ) to 4.699 (DC) (Table 2).

Genetic differentiation

The overall F_{ST} value was 0.181, similar to that of D_{EST} (0.205). The value of the standardised genetic differentiation F'_{ST} was 0.355. Values of pair-wise F_{ST} ranged from 0.006 between populations GS and JD to 0.356 between YT and SZ (online supplementary Table S1). There was a significant relationship between genetic distance ($F_{ST}/(1-F_{ST})$) and geographical distance (the shortest sea surface distance) in *Z. japonica* populations along the coastal region of southern China (Fig. 2), showing a clear IBD pattern.

The STRUCTURE software indicated that the maximum ΔK value occurred when $K = 2$. All MLGs were grouped into two clusters separated by the Leizhou Peninsula: the western cluster, composed of populations SX, DC, GS and JD, and the eastern cluster, which included populations BG, DJ, YT and SZ (Fig. 3). AMOVA analysis detected that most of the molecular variance (67%) could be explained by intra-population genetic variation. In addition, differentiation among populations was small but significant ($P < 0.01$). Significant genetic differentiation was detected between the western and eastern groups ($P < 0.01$) (Table 3).

No significant genetic signature of recent demographic bottlenecks was detected in any of the populations under the TPM (online supplementary Table S2).

Populations of high priority of conservation

Based on allele richness, population SX provided the largest contribution to the number of alleles and distinctiveness due to its high uniqueness, resulting in the highest contribution to the total genetic variation of the studied populations (Fig. 4). Populations DJ, BG and DC also had positive values of Crs, Crd and Crt. However, the other four populations (SZ, GS, YT and JD) showed negative values of Crs, Crd and Crt, coinciding with their fewer numbers of alleles and private alleles (Fig. 3).

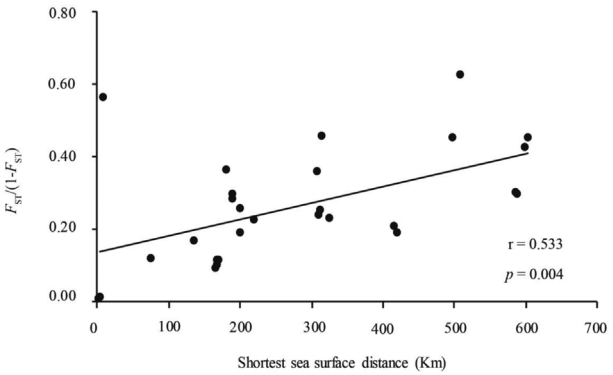

Figure 2: relationship between the shortest ocean surface distance and genetic distance for *Zostera japonica* as tested by the Mantel test.

DISCUSSION

Genetic diversity of *Z. japonica* in tropical populations

In the present study, we revealed high levels of genetic diversity in *Z. japonica* populations comparable to those in its northern distribution range (Hodoki *et al.* 2013), though the former had experienced dramatic fluctuations in area. An explanation is that *Z. japonica* usually form dense meadows, and population size was very large even in an area of several hectares. However, because *Z. japonica* is not a fast expanding seagrass (Huong *et al.* 2003) and the genotypes usually form an aggregated pattern, loss of most areas unavoidably results in loss of genotypes and reduction of genetic diversity, if no novel genotypes were added in. Thus, this reason only could

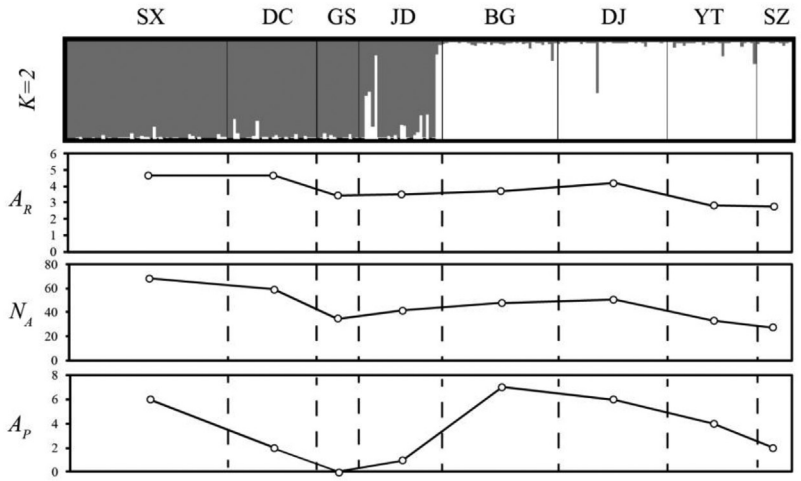

Figure 3: cluster analysis of *Zostera japonica* populations ($K = 2$) using Structure (top panel, in which different colors represent different groups), and the values of A_R (allelic richness; second panel), N_A (number of alleles per locus; third panel) and A_P (number of private alleles; fourth panel) in different populations.

Table 3: AMOVA analysis for eight *Zostera japonica* populations

Source of variation	*df*	Variance component	%Total variance	*P* value
Total				
Among populations	7	2.170	33	<0.01
Within population	218	4.418	67	
Eastern and western groups				
Between groups	1	0.906	13	<0.01
Among populations	6	1.640	24	<0.01
Within population	218	4.418	63	<0.01

not explain the high level of genetic diversity in the studied populations that had experienced frequently disturbances.

High genetic diversity in all of the studied *Z. japonica* populations may also reflect an important role of sexual seedling recruitment (Eriksson 1993), which can balance the loss of genetic diversity via removal of ramets by disturbances. This is consistent with the observation of a high seed reproduction rate in *Z. japonica* populations (e.g., Harrison and Bigley 1982). Contrary to clonal plants of initial seedling recruitment, which have the highest genetic diversity at the establishment stage, clonal plants of repeated seedling recruitment (RSR) can accumulate genetic diversity via continuous seedling recruitment (Eriksson 1993). *Zostera japonica* is a clonal seagrass with RSR, and can form dense patches. Disturbances can remove some ramets, providing space for seeds to approach sediments and germinate and for seedlings to grow. As a result, disturbances may increase number of genets per m^2, which had been observed in congener *Z. marina* (Reusch *et al.* 1999). Thus, disturbances, if not too severe, can balance the loss of or even increase genetic diversity (Reusch 2006).

Seed banks are common in seagrasses (e.g. Hammerstrom *et al.* 2006; Jarvis *et al.* 2014; Orth *et al.* 2006), and at least four genera of seagrasses have persistent seed banks (Orth *et al.* 2006). *Zostera japonica* is a clonal plant with mass flowering and seed production. Due to the characteristics of physiological dormancy (Kaldy *et al.* 2015), only a few seeds germinate without cold pre-treatment, and most seeds enter a seed bank (e.g. >1000 viable seeds/m^2 at Roberts Bank, British Columbia) (Bigley 1981). As a result, seedlings consist of a rather high proportion of shoots in the spring. Furthermore, disturbances can increase *Zostera japonica* flowering (Park *et al.* 2011) and, thereby, seed production and seed banks. The seed banks may maintain the effective population sizes and also contribute to the high level of genetic diversity in studied *Z. japonica* populations.

Among-population gene flow may also help in the maintenance of within-population genetic diversity (Carlson *et al.* 2014; Liu *et al.* 2015). When ripened, the seeds of *Z. japonica* drop from the flowering shoots and fall onto sediments, usually close to the maternal shoot. However, reproductive stems and viable seeds of *Z. japonica* may be transported to a long distance by the tides and sea currents (Nielsen 1990; Shafer *et al.* 2014). Thus, even though local populations experience sexual reproductive failure, sea current-mediated seed dispersal may maintain within-population genetic diversity. Hodoki *et al.* (2013) found high genetic diversity in *Z. japonica* populations along a tidal river, whereas, several marginal populations in a lake had low genetic diversity due to low gene flow from other populations in combination with a failure of sexual reproduction. However, among-population dispersal of seeds via vegetative fragments is usually not far in *Z. japonica* (Britton-Simmons *et al.* 2010). Thus, the role of seed dispersal in increasing genetic variation may occur among populations isolated by short distances, such as populations located within the same bay (e.g. SX and DC) or along the same coast (e.g. GS and JD) in the present study. However, for populations isolated by a long distance,

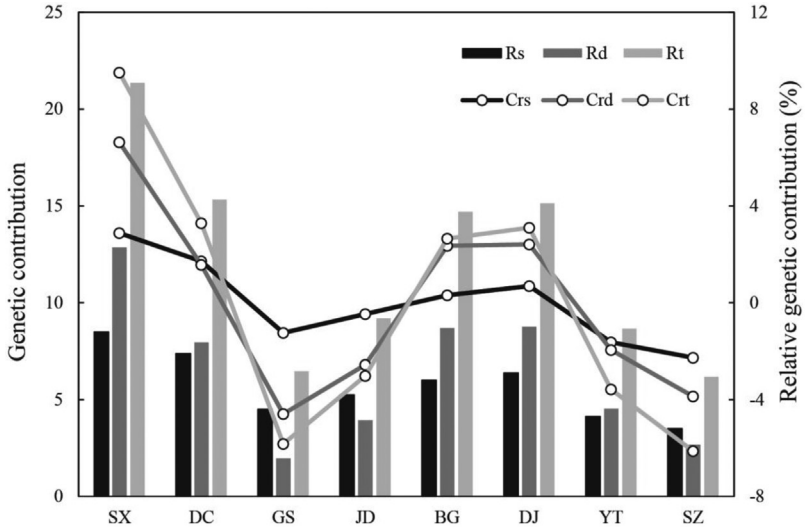

Figure 4: genetic contribution of each *Zostera japonica* population based on allelic richness. Rs and Rd are genetic contributions based on genetic variation and genetic distinctiveness, respectively. Rt represent the total genetic contribution considering both genetic variation and distinctiveness. Crs and Crd are relative genetic contribution rate based on genetic variation and genetic distinctiveness, respectively. Crt represents the total relative genetic contribution rate considering both genetic variation and distinctiveness.

among-population gene flow plays a restricted role in affecting the levels of genetic variation.

Genetic differentiation and gene flow

Low to moderate genetic differentiation was found between *Z. japonica* populations, and a significant relationship was found between genetic differentiation and the shortest distance across the sea, suggesting that gene flow played a critical role in genetic composition. Low genetic differentiation was only observed between populations in the same bay, i.e. SX vs. DC, or along the same coast, i.e. GS vs. JD, due to the short distances (3.2–4.8 km) and no dispersal barrier between these paired populations. Two populations (YT and SZ) were also close (9.56 km), but they were highly differentiated (F_{ST} = 0.356), which might be due to their geographic positions. The two populations located north and southwest of the island of Shangchuan were affected by splits of the sea current from the southwest and, thus, there was limited direct seed dispersal between these two populations, resulting in moderate to large differentiation (Hamrick *et al.* 1993).

In clonal plants, gene flow can be mediated by dispersal of clonal fragments, seed and/or pollen grains. No shared genotypes were found between any two populations, indicating that among-population dispersal via vegetative fragments was very rare, if not absent, although long-distance dispersal via vegetative fragments is frequently detected in seagrasses (e.g. Waycott and Barnes 2001). In seagrass species, pollen grains are transported on or beneath the water surface and show a sharp leptokurtic dispersal function (Cox 1988); thus, successful mating usually occurs within a seagrass meadow, resulting in a short distance of pollen dispersal. Therefore, seed was the main vector of dispersal between *Z. japonica* populations.

In the southern distribution range of *Z. japonica*, a clear segregation existed between the east and west groups with a split at the Qiongzhou Strait/Leizhou Peninsula (Fig. 1) that was most likely to have been caused by local sea currents. In the western and eastern groups, respectively, the number of alleles and number of private alleles declined from west to east (Fig. 3). Compared to the eastern group, populations of the western group had more alleles but fewer private alleles per population on average. High differentiation between populations located to the west and east of the Leizhou Peninsula/Qiongzhou Strait was also observed in the threatened seagrass *Halophila beccarii* (Jiang *et al.* 2014). These findings suggest that there was very limited gene flow through the narrow strait in some marine plants, which also was observed in other studies (Arnaud-Haond *et al.* 2007; Olsen *et al.* 2004; Serra *et al.* 2010).

Implications for *in situ* conservation of seagrass meadows

Although *Z. japonica* is an invasive species in North America, its native distribution ranges have been declined. For example, the area of *Z. japonic*a meadows in the Hii River in southwest Japan began to decrease in the 1950s, probably because of herbicide input and major reclamation works (Hodoki *et al.* 2013). Although sexual reproduction and seed banks may ameliorate the consequences of disturbances, frequent disturbances before seed maturation may decrease seed production and seed bank, and ultimately decrease the effective population size and genetic variation, thus, increasing the risk of local extinction. Therefore, the protection of extant populations and the reintroduction of *Z. japonica* in locally extinct sites are urgently needed.

Contribution diversity suggested that population SX had the highest priority of *in situ* conservation, followed by populations DJ, BG and DC (Fig. 4). This is consistent with the gradients of number of alleles and private alleles of each population (Fig. 3). Fortunately, three of the four populations are located in nature reserves. A reserve should be set up to protect *Z. japonica* in DJ, in which several other seagrasses, such as *Halophila ovalis*, *Cymodocea rotundata*, *C. serrulata*, *Thalassia hemprichii* and *Syringodium isoetifolium*, are also found. Although slight disturbances may benefit flowering and, thus, seed production (Park *et al.* 2011), frequent disturbances, especially those during flowering and seed maturation, may decrease seed production or even lead to the failure of sexual reproduction. Because genetic variation has not yet been seriously decreased, the seagrass meadows might be rapidly recovered once the disturbances are have stopped.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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