



Spatial Scales of Geographical Isolation by Distance and Barriers, and Heterogeneity in the Genetic Structure of a Seagrass

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Abstract

Gene flow is a crucial concept in the delineation of conservation units for natural populations of a species. Seagrasses are marine species targeted for conservation because their abundance has declined worldwide during the last century. However, knowledge of how to delineate seagrass conservation units based on their genetic structure is inadequate. In this study, the genomes of *Zostera marina* L. (eelgrass) populations in three semi-enclosed areas with different spatial scales in Japan were analyzed using single nucleotide polymorphisms within genome-wide loci, and their genetic structures were explored in the context of their isolation caused by geographical distance and barriers. Isolation by distance was apparent at spatial scales of 200–2000 km. A barrier apparently due to a narrow strait in an area with linear dimensions less than 200 km led to greater genetic isolation than did distance. On a more local scale, we found that heterogeneity among sites and intra-site variability rather than isolation by distance or barriers governed the genetic structure of eelgrass on spatial scales less than ~20 km. Our results imply that future seagrass conservation efforts should make more use than before of genomic tools so that greater attention can be focused on the complex structures formed on local scales. Identifying the ecological importance of genetic heterogeneity and the functional meaning of genetic differences on local scales will be among the next challenges to the delineation of seagrass conservation units.

Keywords Conservation unit · Eelgrass · Gene flow · Genetic distance · High-throughput sequencing

Introduction

Seagrasses are foundation species that form a habitat for many marine organisms and provide important ecosystem services (Costanza et al., 1997). Because the abundance of seagrasses has been declining worldwide during the

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last century (Dunic et al., 2021; Orth et al., 2006; Waycott et al., 2009), there has been a global effort to conserve seagrass beds and recover their ecosystem services (van Katwijk et al., 2016). Whether it is a natural or anthropogenic process, gene flow promotes the mixing of populations (Ellstrand, 2014; Lowe & Allendorf, 2010). Restoration efforts are expected to reduce further loss of genetic diversity (Shaw et al., 2025) and to increase the resilience of seagrasses to future global climate change (DuBois et al., 2021, 2022). However, restoration efforts involve the risk of causing outbreeding depression by admixing natural populations within a species (Allendorf et al., 2010; Funk et al., 2012; Hoelzel, 2023; Hohenlohe et al., 2021). Biological conservation units are delineated to suppress outbreeding depression and to prioritize populations for investment of limited resources (Allendorf et al., 2010; Funk et al., 2012; Hoelzel, 2023; Hohenlohe et al., 2021).

The genes of marine species are widely distributed in water bodies through diaspores such as larvae, pollen, and seeds. However, the limited distance of dispersal influences the genetic structure of marine species. Examples include isolation by distance (IBD) of seagrass species (Coyer et al., 2004; Olsen et al., 2004). Physical barriers associated with seascape topography in water currents also create isolation by resistance (IBR) in the genetic structure of marine species (e.g., D'Aloia et al., 2014; Thomas et al., 2015). Conversely, environmental heterogeneity contributes to genetic structure because marine organisms adapt to their environment (Nanninga et al., 2014; Sandoval-Castillo et al., 2018). The consequence of such environmental heterogeneity is that the genetic structure is not always related to the patterns expected on the basis of physical processes.

In seagrasses, the dispersal of pollen and seeds is considered to make a small contribution to gene flow because pollen and seeds travel only tens of meters (Hosokawa et al., 2015; Kendrick et al., 2012, 2017). However, seeds may be buoyant if they contain buoyant tissues, such as spathe and shoot (Hosokawa et al., 2015). Seeds can be transported more than 100 km by currents if the currents are sufficiently swift and the conditions that cause the seeds to remain positively buoyant persist (Kendrick et al., 2012, 2017; Orth et al., 1994). Vertebrates are potential biotic vectors of long-distance dispersal if they consume and excrete whole, viable seeds (Kendrick et al., 2017). Conversely, genetic structure within seagrass populations can also form within a distance of a few kilometers because of adaptations to water temperature and salinity (Nguyen et al., 2023; Oetjen et al., 2010; Schiebelhut et al., 2023). The present genetic structure of seagrasses has resulted from the history of gene flow among populations through dispersals and the demographic dynamics of populations, including mutation, genetic drift, and adaptations to local environments (Kendrick et al., 2017).

Exploration of the spatial scales of the genetic structures of seagrasses has included a wide range of scales from global scales to fine scales. IBD is a well-known pattern found in seagrasses on a global scale (Coyer et al., 2004; Olsen et al., 2004; Talbot et al., 2016; Yu et al., 2023a), on scales of 100–1000 km (Arnaud-Haond et al., 2007; Kim et al., 2017; Nakajima et al., 2014; Stafford-Bell et al., 2019; Tanaka et al., 2002), and on scales less than 100 km (Hori & Sato, 2021; Martínez-García et al., 2021; Sinclair et al., 2014; Tanaka et al., 2011). Conversely, the genetic structure that results from environmental heterogeneity is apparent on a spatial scale less than 10 km in seagrasses, between intertidal and subtidal populations (Oetjen et al., 2010), between the seagrasses within and outside a lagoon with extremely high temperatures (Nguyen et al., 2023), and within a bay (Harenčár et al., 2018; Schiebelhut et al., 2023). In the case of Tomales Bay, the populations that were distributed within mosaic environments on scales of 2–12 km had adapted to their home sites to survive and grow but not to other sites (DuBois et al., 2022). The genetic structure of the populations had formed in a way that allowed them to adapt to their heterogeneous environments (Schiebelhut et al., 2023). Meanwhile, their genetic structures can be formed along environmental gradients on scales of more than 100 km (Jeffery et al., 2024; Phair et al., 2019; Ries et al., 2023).

Efforts to conserve seagrasses have been conducted by sowing seeds and transplanting vegetative shoots (van Katwijk et al., 2016). However, the examples of natural genetic structures on a fine scale imply that such anthropogenic efforts can admix natural genetic structures. The perspective on seagrass conservation has become local in Japan. Seeds of a seagrass species, *Zostera marina* L. (eelgrass), have been sown in a fishing town in the Seto Inland Sea (SIS) of Japan, and no disturbance of the natural genetic structure was detected (Hori & Sato, 2021). In that case, although the admixture of genetic structures was not initially a concern, the assessment was performed after the risk had been recognized. Conversely, a migration was allowed before an assessment had been performed based on the absence of a statistically significant difference in genetic structures in Tokyo Bay (Tanaka et al., 2011). Measurements of genetic structure will be necessary to judge the feasibility of anthropogenic migrations among seagrass populations for which there is concern about the admixture of natural genetic structures.

Although we know the patterns of IBD and IBR and the heterogeneity of genetic structure in seagrass species, there is still a question of the spatial scales associated with the control of the genetic structure of seagrasses by these patterns or heterogeneity. If we know the spatial scales associated with the formation of predictable patterns and unpredictable structures, the costs of measuring genetic structure in seagrass conservation can be decreased. This

study sought to provide that information by targeting eelgrass, which is a seagrass species distributed widely in the northern hemisphere (Short et al., 2007). We used single nucleotide polymorphisms (SNPs) to investigate the genetic structures of eelgrass populations within three local areas of different spatial scales, located along a 2000-km coastline in Japan. We explored how the genetic structures had evolved within these areas by focusing on the heterogeneity and patterns of IBD and IBR within the whole area and within local areas.

Materials and Methods

Study Areas and Populations

Eelgrass, *Zostera marina* L., is distributed in the coastal waters of Japan between temperate and subarctic seas (Nakaoka & Aioi, 2001). The study areas were the SIS, Tokyo Bay, and the coastal waters of the town of Akkeshi, all of which are located along the coastline of the North Pacific Ocean (Fig. 1a). The SIS and Tokyo Bay on the one hand and the coastal waters of Akkeshi on the other hand are

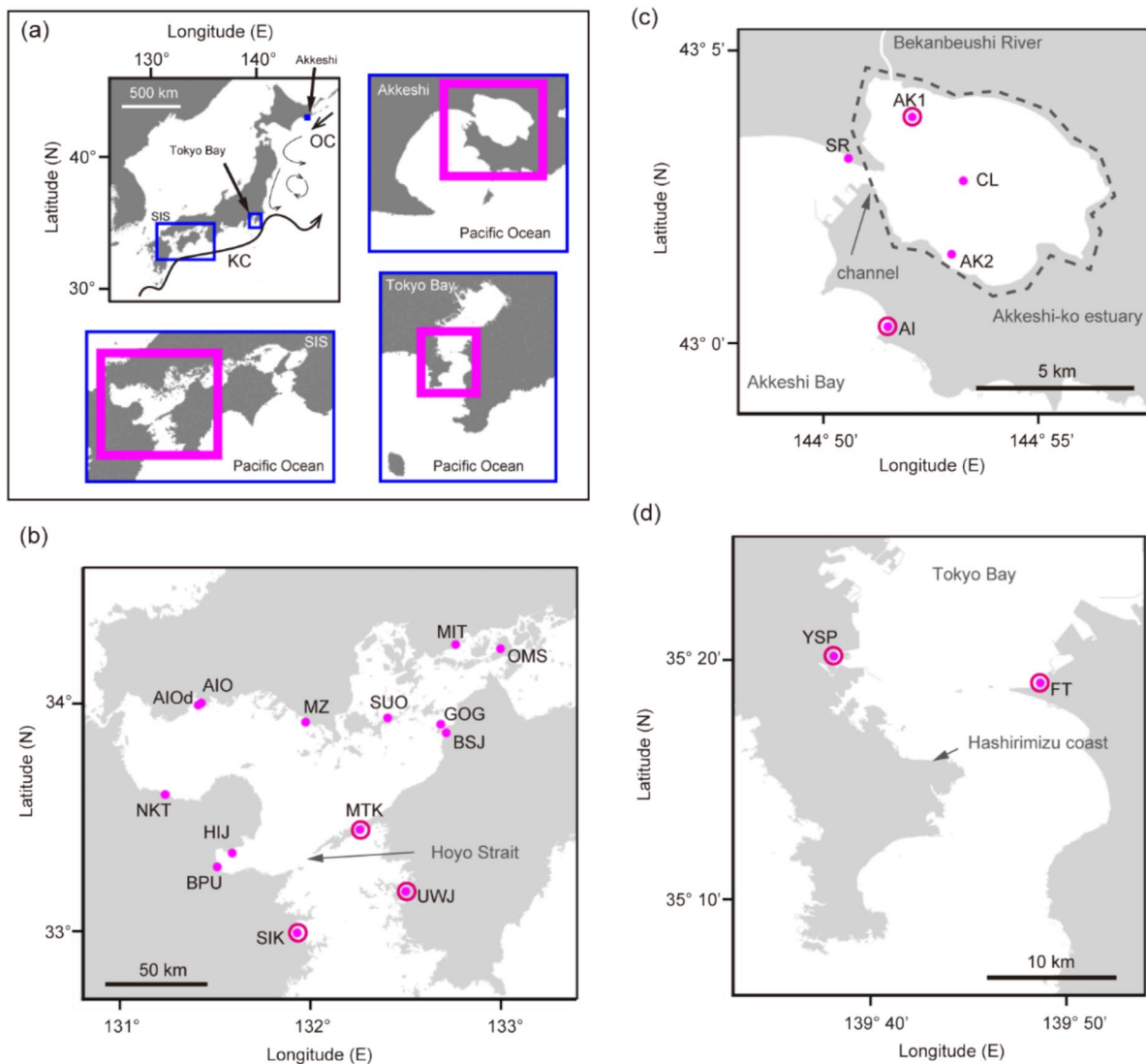


Fig. 1 Locations of study areas and sampling sites. **a** The locations of three study areas in Japan and the geographic relationships between them and the Kuroshio Current (KC) and Oyashio Current (OC) in the North Pacific Ocean. Study areas were **(b)** the Seto Inland Sea, **(c)** Akkeshi, and **(d)** Tokyo Bay. Magenta points indicate sampling sites. The points with circles around them are the sites chosen for

analyzing the whole area. One sampling site, MTK, is located inside the Seto Inland Sea near a long, narrow peninsula. The maps are based on coordinates obtained from the National Spatial Planning and Regional Policy Bureau, Ministry of Land Infrastructure, Transport and Tourism (https://nlftp.mlit.go.jp/ksj/gml/datalist/KsjTmplt-N03-v2_3.html#prefecture00)

located along the Kuroshio Current and Oyashio Current, respectively.

The area of eelgrass in the SIS began to decline in the 1970 s because of the degradation of water quality and the reclamation of sites for coastal development and port construction (Komatsu, 1997). Restoration efforts in the 2000 s and natural reproduction have allowed the eelgrass beds to largely recover (Hosokawa et al., 2019; Morita et al., 2002). We focused on the populations on the western side of the SIS (spatial scale < 200 km), which include those in the outer SIS that are geographically isolated by the narrow Hoyo Strait from the inner SIS (Fig. 1b). The Akkeshi site spanned less than ~ 10 km (Fig. 1c) and consisted of the Akkeshi-ko estuary and Akkeshi Bay. The semi-enclosed Akkeshi-ko estuary is downstream of the Bekanbeushi River and connects to Akkeshi Bay by a narrow channel. Akkeshi Bay faces the Pacific Ocean. Tokyo Bay is an area where eelgrass beds were widely distributed in the 1900 s (Morita, 2013) but are presently fragmented because much of the eelgrass disappeared after the urbanization of the coastline (Fig. 1d). In Tokyo Bay, we focused on a natural eelgrass bed at Futtsu (FT), which has been there since before urbanization (Yamakita & Nakaoka, 2009; Yamakita et al., 2011), and an artificial bed at the Yokohama Seaside Park (YSP), 17 km from the FT. The YSP site has been among the sites chosen for the restoration program (https://www.spf.org/opri/newsletter/120_3.html), and the eelgrass seed that was sown probably came from the Hashirimizu Coast (see Fig. 1d). However, there is no definitive record of the source.

The range of salinities is lower in the Akkeshi-ko estuary than in Akkeshi Bay, the SIS, and Tokyo Bay (Table 1). The water temperatures are lower in the Akkeshi study area than in the other two study areas. Water outflows from the inner to outer parts via surface currents in Tokyo Bay and Akkeshi.

Sampling Methods

Eelgrass samples were obtained from 21 sites: 14 sites in the SIS, five sites in Akkeshi, and two sites in Tokyo Bay (Table 2). The sampling sites were chosen in sheltered areas near the offshore ocean in each study area. The eelgrass samples were collected from beds at six sites in the SIS by SCUBA divers and at all sites in Akkeshi and Tokyo Bay by foot at low tide (Table 2). The individual samples were collected from locations more than 5 m away from each other within a site to avoid collection of clonal individuals. The samples at the YSP site were categorized into three sub-sites—YSP1, YSP2, and YSP3—which were 20–40 m from each other. The goal was to analyze the genetic structure across individuals within the YSP site. In the SIS, eight sites were chosen in addition to the six original sites to complement the collections from the sparse sites in that wide study area. Rafted samples, which were collected to reduce the cost of sampling, were collected on a transect along the coast at sites where the presence of eelgrass beds had been reported or visually identified and where the beds were semi-enclosed geographically. We collected at least 20 samples at

Table 1 Environmental information in three study areas

Study area	Water temperature	Salinity	Current
SIS	7–29 °C (*)	28–35 PSU (*)	Water currents in the SIS are relatively complex because of its topography as a basin–strait system. The sub-basins are dominated by geometrically constrained basin-scale circulation and intensive tidal mixing in the straits (Kosako et al., 2025)
Akkeshi	6–24 °C (Momota & Nakaoka, 2018) (**)	17–29 PSU in the Akkeshi-ko estuary (Momota & Nakaoka, 2018) (**) 30–33 PSU at the AI site (Momota & Nakaoka, 2018) (**) See Abe et al. (2024) for the spatial distribution of the salinity	Currents driven by tides but restricted by the vegetation in the Akkeshi-ko estuary (Hasegawa et al., 2008) Outflow from the estuary to the bay at the surface via estuarine circulation (Hasegawa et al., 2018)
Tokyo Bay	10–30 °C (Hosokawa & Okura, 2022; Watanabe et al., 2024)	< 20–34 PSU (Hosokawa & Okura, 2022; Watanabe et al., 2024)	Currents driven by tide, but the residual current from the inner to outer bay at the surface (Hosokawa & Okura, 2022)

*The data source is the Chugoku Regional Development Bureau, Ministry of Land Infrastructure, Transport and Tourism (<https://www.pa.cgr.mlit.go.jp/suishitu/>)

**Water temperature and salinity are measured between May and November in Akkeshi because it is frozen during the winter

Table 2 Samples used in this study. Type of sample: A = sample collected from bed and B = rafted sample

Study area	Site	Type of site	Abbreviation	Sampling		No. samples taken	Type of sample collection
				Year	Month		
SIS	Nakatsu	Inner	NKT	2022	Aug	10	B
	Aio	Inner	AIO	2022	Dec	20	A
	Aio	Inner	AIOd	2022	Aug	20	B
	Murozumi	Inner	MZ	2022	Aug	13	B
	Beppu	Inner	BPU	2022	Aug	10	B
	Hiji	Inner	HIJ	2022	Dec	20	A
	Mitsukue	Inner	MTK	2022	Dec	20	A
	Suo-oshima	Inner	SUO	2022	Dec	20	A
	Gogo-shima	Inner	GOG	2022	Dec	20	A
	Baishinji	Inner	BSJ	2022	Aug	16	B
	Mitsukuchi	Inner	MIT	2022	Dec	20	A
	Omishima	Inner	OMS	2022	Aug	15	B
	Saiki	Outer	SIK	2023	Jun	20	B
	Uwajima	Outer	UWJ	2023	Jun	20	B
Akkeshi	Akkeshi-ko1	Inner	AK1	2022	Jul	39	A
	Akkeshi-ko2	Inner	AK2	2022	Jul	41	A
	Central lake	Inner	CL	2022	Jul	20	A
	Shinryu	Outer	SR	2022	Jul	20	A
	Aininkappu	Outer	AI	2022	Jul	20	A
Tokyo Bay	Futtsu	Inner	FT	2022	May	40	A
	Yokohama Seaside Park	Inner	YSP	2022	May	29	A

each site from within the bed or 10–20 samples from rafted individuals (Table 2). The sampling was performed during 2022 and 2023.

The youngest and/or second-youngest eelgrass leaves were cut from each individual plant to mitigate potential DNA contamination from epiphytes on the eelgrass leaves (Hosokawa et al., 2009). The leaf samples were rinsed with distilled water, wiped with paper towels, and packed in silica gel for transport to the laboratory.

Genetic Analysis

The samples were ground in liquid nitrogen, and the genomic DNA was extracted using an Isospin Plant DNA extraction kit (Nippon Gene Co., Ltd., Tokyo, Japan). Genotyping by Random Amplicon Sequencing-Direct (GRAS-Di) was used for genome-wide sequencing. The GRAS-Di method involves a two-step polymerase chain reaction (PCR) with random primers to generate an amplicon library (Enoki, 2019; Enoki & Takeuchi, 2018).

The concentration of the extracted DNA was measured, and the first round of PCR was performed with 63 multiplexed random primers (Table S1 in Supplemental Methods). The PCR products were mixed with the adaptor primers for the second round of PCR. The final PCR products from the second round were mixed in equal volumes across

all samples. The mixed product was purified, and library concentrations were measured. The quality of these libraries was examined. The details of the method are described in Patent ID P2018 42548 A. The sequencing was performed using a DNBSEQ-G400 (MGI Tech Co., Ltd., Shenzhen, China) with a 150-bp paired-end protocol. The libraries were constructed and sequenced by GeneBay, Inc. (Yokohama, Japan). The reads generated by next-generation sequencing were mapped to the reference genome (Joint Genome Institute: *Zostera marina* v3.1; Ma et al., 2021).

Analytical Strategy and Filtering

The genetic structure of the eelgrass was assessed in the whole area and three local areas. The assessment in the whole area was based on sites MTK, SIK, and UWJ in the SIS, sites FT and YSP in Tokyo Bay, and sites AK1 and AI in Akkeshi. The difference in genetic structure between the two sites in Tokyo Bay was assessed using the whole-area dataset, which included them. However, the local assessments in the SIS and Akkeshi were performed on the corresponding datasets separated from the whole-area dataset to retain more SNPs.

Individual samples were chosen for each dataset, and SNPs were called from the samples (Fig. S1 in Supplemental Methods). If clonality was detected using the

shared heterozygosity index (Yu et al., 2023b), the clones were removed except for the individual with the most SNPs with read depths ≥ 5 .

The genetic structure of the eelgrass was assessed using statistical tools, principal component analysis (PCA), and admixture analysis (ADMIXTURE) (Alexander & Lange, 2011). We used different filters to calculate statistics and to carry out the PCA and ADMIXTURE analyses. Because SNP reduction is known to decrease statistical power and bias calculated statistics (Weir & Goudet, 2017), we retained the SNPs for calculating statistics without filtering and pruning them (Fig. S1 in Supplemental Methods). Conversely, for the PCA and ADMIXTURE analyses, the SNPs with a genotyping rate < 0.7 and minor allele frequency (see Table S2 for the values of minor allele frequency) were filtered to suppress noise, and SNPs with a pairwise distance of 3000 bp to any other SNP were pruned to reduce bias by possible linkage disequilibrium (Table S2). The SNPs without the above filtering and pruning were used to reduce bias when we calculated statistics.

Calculating Statistics

Calculations of nucleotide diversity and the population-specific fixation index (F_{ST}) and inbreeding coefficient (F_{IS}) were based on the SNPs without filtering and pruning. We used nucleotide diversity to assess the ancestral populations, which we considered to have high genetic diversity because there would have been more opportunities for mutations (Liu et al., 2006; Prugnolle et al., 2005). The population-specific F_{ST} was also used to assess evolutionary history by taking low values in the population with high heterozygosity to achieve high genetic diversity (Kitada et al., 2021). The population-specific F_{IS} is a relative inbreeding coefficient (Weir & Goudet, 2017) that has a low value when the degree of heterozygosity is high. In addition to the population-specific F_{ST} , we calculated the pairwise F_{ST} to determine the genetic distance between sites. These four statistics were estimated with a bootstrapping method, which randomly resampled 2% of the SNPs. This resampling rate was chosen to ensure that the pairwise physical distance between the resampled SNPs became large (see the “Results” section for the length of the pairwise distance that was achieved). We then obtained 200 bootstrap samples and calculated their means and 95% confidence intervals (CIs).

We calculated nucleotide diversity with the `pi.dosage` function in `hierfstat` version 0.5–11 of the R package. The population-specific F_{IS} , population-specific F_{ST} , and pairwise F_{ST} were calculated using the `fs.dosage` function in the `hierfstat` version 0.5–11 of the R package (Goudet et al., 2022).

Analysis of Genetic Structure

Tests of the occurrences of IBD and IBR were based on the genetic distances in each study area. The geographic distance was defined as the length of the coastline according to other studies of seagrass population genetics (Hori & Sato, 2021; Tanaka et al., 2011). The resistance to gene flow was defined in this study as a binary based on the geographical restriction of the ocean current against the coastline. Although this definition of resistance is simpler than considering realistic connectivity by ocean currents (e.g., Thomas et al., 2015), it is sufficient to test for the presence of a difference in genetic distance. In the SIS, because the two-way connectivity of rafting eelgrass seed between the inner and outer portions of the SIS is restricted by the Hoyo Strait (Kosako et al., 2025), resistance was hypothesized to be present on the coastline crossing the strait. In Akkeshi and Tokyo Bay, the estuarine circulation occurs between the Akkeshi-ko estuary and Akkeshi Bay (Hasegawa et al., 2018) and between the inner parts of Tokyo Bay (two sites) and the ocean (Hosokawa & Okura, 2022). Because estuarine circulation includes a residual current toward the ocean in the surface layer of the inner bay (Geyer & MacCready, 2014; MacCready & Geyer, 2010), we predicted a one-way connectivity of rafting eelgrass seed between the estuary and ocean in Akkeshi and Tokyo Bay. Although the estuarine circulation may not be a barrier, we hypothesized that it regulates gene flow rather than provides two-way connectivity.

The genetic distance between sites was quantified by the value of the pairwise $F_{ST}/(1 - \text{pairwise } F_{ST})$. The geographic distance was calculated using a method of least cost (van Etten, 2017) based on the bathymetric data from the National Oceanic and Atmospheric Administration server. The calculation was performed using the functions in the `marmap` version 1.0–10 of the R package (Pante et al., 2023) (see Fig. S2 in Supplemental Methods for the paths between site pairs drawn by the `marmap` package). Resistance (value of 1) was considered to be present on the coastlines crossing the Hoyo Strait and a narrow channel in Akkeshi, as well as on the coastlines crossing between two sites in Tokyo Bay and the ocean.

A multiple matrix regression with randomization (MMRR) was used to test for differences in the genetic distances between sites with and without the possibility of resistance (Wang, 2013). We tested for the possibility of a single pattern of IBD or IBR as well as multiple patterns. The number of actual permutations was set to 5039 at the seven sites in the whole area and to 119 permutations for the five sites in Akkeshi. The number of random permutations was set to 9999 in the SIS because the total number of actual permutations was large at the 14 sites. The test was not performed in Tokyo Bay because there were only two sites. The Mantel test was performed using the `mantel`

function in *vegan* version 2.6–4 (Oksanen et al., 2022). In addition to the IBR based on geography, we tested the difference in the genetic distance between site AI and other sites because site AI was distinctive with respect to salinity and was geographically distant (Momota & Nakaoka, 2018; Abe et al., 2024; see Table 1). The difference between site AI and other sites was tested by the nonparametric Wilcoxon rank sum test using the *wilcox.test* function in R.

The PCA and ADMIXTURE results were analyzed to determine the detailed genetic structure after SNP filtering and pruning. The PCA used a count datum of 0 when the alleles matched the reference genome in homozygous individuals at marker j and individual i , 1 when the alleles matched the reference in heterozygous individuals, and 2 when the alleles did not match the reference but the individuals were homozygous (Patterson et al., 2006). The count data were normalized, and the analysis was performed on a matrix consisting of the count data. A scree plot was used to determine the number of significant principal components in the PCA.

ADMIXTURE analysis is a statistical technique for estimating individual ancestries in K genetic clusters based on the likelihood of an allele occurring at locus j in individual i (Alexander et al., 2009). ADMIXTURE analysis was conducted with genetic clusters ranging from $K = 1$ to $K = 10$. This range of K was expected to be enough to find the number of genetic clusters in the study areas. A fivefold cross-validation was carried out for each genetic cluster. We considered the number of genetic clusters with the minimum

cross-validation to be the optimized model (Alexander & Lange, 2011). We used the model with minimum cross-validation and models with cross-validation close to the minimum cross-validation to find the genetic structure in the three study areas. The PCA and ADMIXTURE analyses were performed using the R package *pcadapt* version 4.3.3 (Luu et al., 2017) and the ADMIXTURE software version 1.3.0 (Alexander & Lange, 2011), respectively. Missing data were inputted using the pairwise covariance approach as the default process in the R package and ignored in ADMIXTURE.

Results

Sequence Mapping and SNPs Analyzed

The median of GRAS-Di sequences mapped on the reference genome varied between 4,538,751 and 8,681,332 reads at the sites (Fig. S3). The number of covered bases tailed when depth was relatively high (Fig. S4). The SNPs were obtained from the six chromosomes and scaffolds and were distributed widely on each chromosome (Fig. S5).

Clonemates were detected on the basis of the shared heterozygosity index calculated using the SNPs (Fig. S6). At most, 13 individuals were removed at a site (FT) (Table 3). The number of individuals rejected due to a low rate of genotyping was at most 2 (at site MZ). The number of SNPs

Table 3 Summary of the results of filtering. The numbers of individuals remaining/before filtering and the number of single nucleotide polymorphisms (SNPs) for principal component analysis (PCA) and ADMIXTURE analysis are included in parentheses for Tokyo Bay

Target	# of individuals removed due to clonemates	# of individuals rejected by filtering	# of individuals remaining/before filtering	SNPs for calculating statistics	SNPs for PCA and ADMIXTURE analysis
All three areas (Tokyo Bay)	5 at MTK, 2 at SIK, 8 at UWJ, 13 at FT, 11 at YSP, 5 at AK, 7 at AI	1 at FT, 1 at YSP	135/188 (43/69)	115,343	909 (668)
SIS	1 at AIOd, 3 at HIJ, 5 at MTK, 1 at SUO, 3 at GOG, 2 at MIT, 1 at OMS, 2 at SIK, 8 at UWJ	2 at MZ, 1 at BSJ, 1 at OMS	214/244	117,344	962
Akkeshi	4 at AK1, 5 at AK2, 1 at CL, 2 at SR, 7 at AI	1 at AK1	120/140	76,271	911

varied between 76,271 and 117,344 for statistical purposes; it was between 668 and 962 for the PCA and ADMIXTURE analyses. The SNPs for the PCA and ADMIXTURE analyses were retained at all six chromosomes in all study areas and scaffolds in the SIS (Fig. S5). The bootstrapping method based on resampling 2% of the SNPs led to a median distance between SNPs of more than 3000 bp (Fig. S7).

Genetic Structure in the Whole Area

The genetic distances between sites (the bootstrap mean of the pairwise $F_{ST}/(1 - \text{pairwise } F_{ST})$ ratio) in the whole area ranged between 0.053 and 1.112. The model including IBD and IBR was statistically significant (MMRR, $r^2 = 0.85$, $p = 0.001$, with 5039 permutations), with statistically significant

IBD at geographic distances up to 2000 km ($p = 0.001$) and not statistically significant IBR ($p = 0.147$) (Fig. 2a; n.b., this figure was drawn by single matrix regression with IBD, $r^2 = 0.83$, $p = 0.002$). The PCA revealed a two-dimensional genetic structure, and the clusters were related to the three local areas in the first two dimensions of principal component space (PC1 and PC2) (Fig. 2b; see Fig. S8a for the scree plot). The outlier SNPs that determined the genetic structure were distributed genome-wide (Fig. S8b), and the rate of genotyping was high at each site (Fig. S8c). Despite variability among sites within local areas on the PC3 and PC4 axes (Fig. 2c), the proportion of the variance was more than one-third lower in the second two dimensions than in the first two dimensions of principal component space. The ADMIXTURE analysis also showed clear genetic clusters

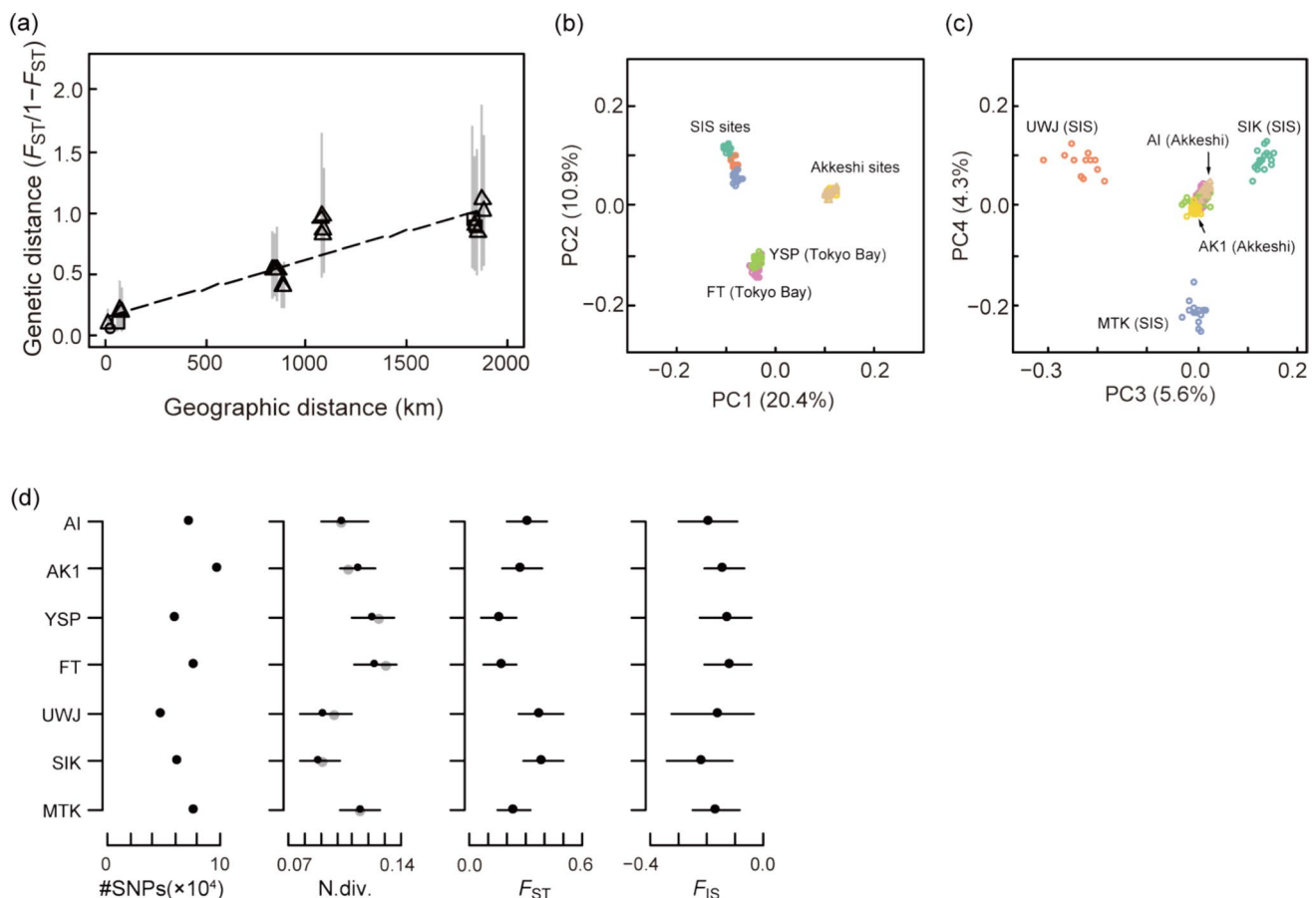


Fig. 2 Genetic structure of the eelgrass populations in the whole area. **a** Genetic distance (the bootstrap mean of the pairwise $F_{ST}/(1 - \text{pairwise } F_{ST})$ ratio) versus against geographic distance. Circles and squares are pairs of inner and outer sites, respectively, without possible resistance on their coastline. Triangles are pairs with possible resistance of any sites on their coastline. The dashed line is a statistically significant IBD based on a single matrix regression. Gray vertical bars show the bootstrap 95% confidence intervals of genetic distance. The two-dimensional plots shown are **(b)** the first and sec-

ond principal components (PC1 and PC2) and **(c)** the third and fourth principal components (PC3 and PC4). **d** Left to right: the numbers of single nucleotide polymorphisms (#SNPs) used to calculate nucleotide diversity at each site; the nucleotide diversity (N. div.; black circles and bars); the population-specific F_{ST} ; and the population-specific F_{IS} . Symbols and bars show the means and bootstrap 95% confidence intervals, respectively. Gray circles in N. div. are calculated at 907 SNPs shared among the individuals

that distinguished three local areas ($K = 3$), but the clustering was inexact within local areas for $K > 3$ (see Fig. S8d). There were site-specific variations of the nucleotide diversity and population-specific F_{ST} (Fig. 2d). The nucleotide diversity was lower and population-specific F_{ST} was greater at the SIK and UWJ sites than at the MTK, FT, and YSP sites. The population-specific F_{IS} was negative at all sites; the maximum (i.e., least negative) value was -0.114 (95% CI between -0.205 and -0.041) at FT.

Genetic Difference Between the Two Sites in Tokyo Bay

Although the difference between the two sites in Tokyo Bay was not apparent from the variation within the whole area (Fig. 2b and c, and Fig. S8d), the PCA and ADMIXTURE analyses that focused on the two sites revealed a difference between them and an intra-site variability within the YSP site in the space defined by PC1 and PC2 (Fig. 3; see Fig. S9a for the scree plot and Fig. S9b for the fivefold cross-validation). The genetic structure was determined by the outlier SNPs that were distributed genome-wide (Fig. S9c); the rate of genotyping was high at each site (Fig. S9d).

Genetic Structure Within the SIS

The genetic distances within the SIS ranged between 0.051 and 1.074 and were statistically significant for the IBR ($p = 0.010$) but not for the IBD ($p = 0.348$) based on multiple matrix regression (MMRR, $r^2 = 0.895$, $p = 0.001$, with 9999 permutations). Although single matrix regressions revealed the statistical significance of both the IBD and IBR, the coefficient of determination was greater for the IBR model ($r^2 = 0.893$, $p = 0.012$, and see Fig. 4a) than for the IBD model ($r^2 = 0.091$, $p = 0.011$).

PCA separated three clusters—SIK, UWJ, and the inner SIS sites—in the space defined by PC1 and PC2 (Fig. 4b; see Fig. S10a for the scree plot), and their differences were apparent in the space defined by PC3 and PC4 of the analysis for the whole area (Fig. 2c). The sites with different colors accounted for the genetic structure on the PC3 and PC4 axes (Fig. 4c), despite the fact that the percentage of the variance accounted for by the eigenvalues in this space was only 4.9%. The homogenizations among the sites where samples were collected from the bed and raft were not found visually (see Table 2 for the type of sample collection). The outlier SNPs that determined the genetic structure based on PCA were distributed genome-wide (Fig. S10b), and the rate of genotyping was high at each site (Fig. S10c). Although the ADMIXTURE analysis separated three clusters that differed from the clusters separated by PCA at $K = 3$ (Fig. 4d; see Fig. S10d for the fivefold cross-validation), the analysis revealed that similar structures were formed by different-colored sites in the inner SIS based on PCA if there were five or more genetic clusters. The nucleotide diversity was lower and population-specific F_{ST} greater at the SIK and UWJ sites than at the inner sites in the SIS (Fig. 4e). The population-specific F_{IS} was negative at all sites.

Genetic Structure Within Akkeshi

Isolation by distance and IBR were not statistically significant by multiple matrix regression in Akkeshi ($r^2 = 0.83$, $p = 0.075$, with 119 permutations) (Fig. 5a). Single matrix regressions also showed no statistical significance for the IBD ($r^2 = 0.76$, $p = 0.075$, with 119 permutations) and the IBR ($r^2 = 0.025$, $p = 0.47$, with 119 permutations). However, PCA revealed a genetic structure (see Fig. S11a for the scree plot) that was determined by the outlier SNPs that were distributed genome-wide (Fig. S11b), and there was a high rate

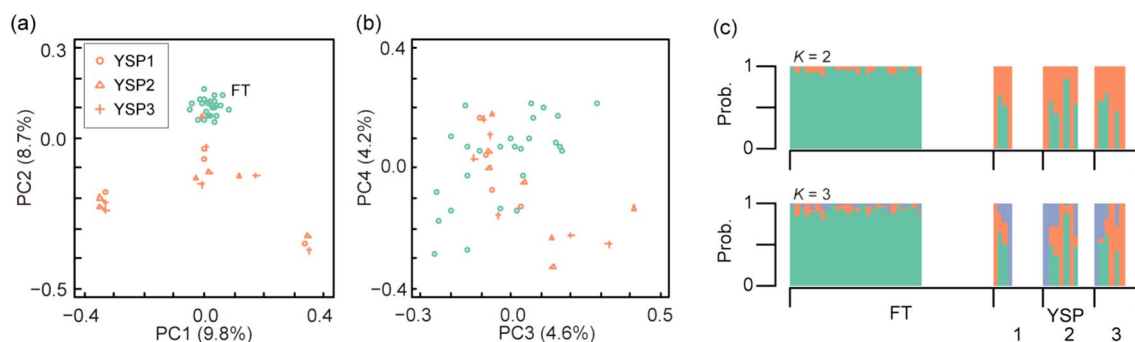


Fig. 3 Genetic structure of the eelgrass populations in Tokyo Bay. **a**, **b** Results of principal component analysis; **c** ADMIXTURE analysis results for two (upper) and three (lower) genetic clusters. Each color and vertical bar in panel (c) represent genetic cluster and an individ-

ual, respectively. Each individual is partitioned into genetic clusters. White individuals were removed by filtering. See Fig. 2 for detailed explanations

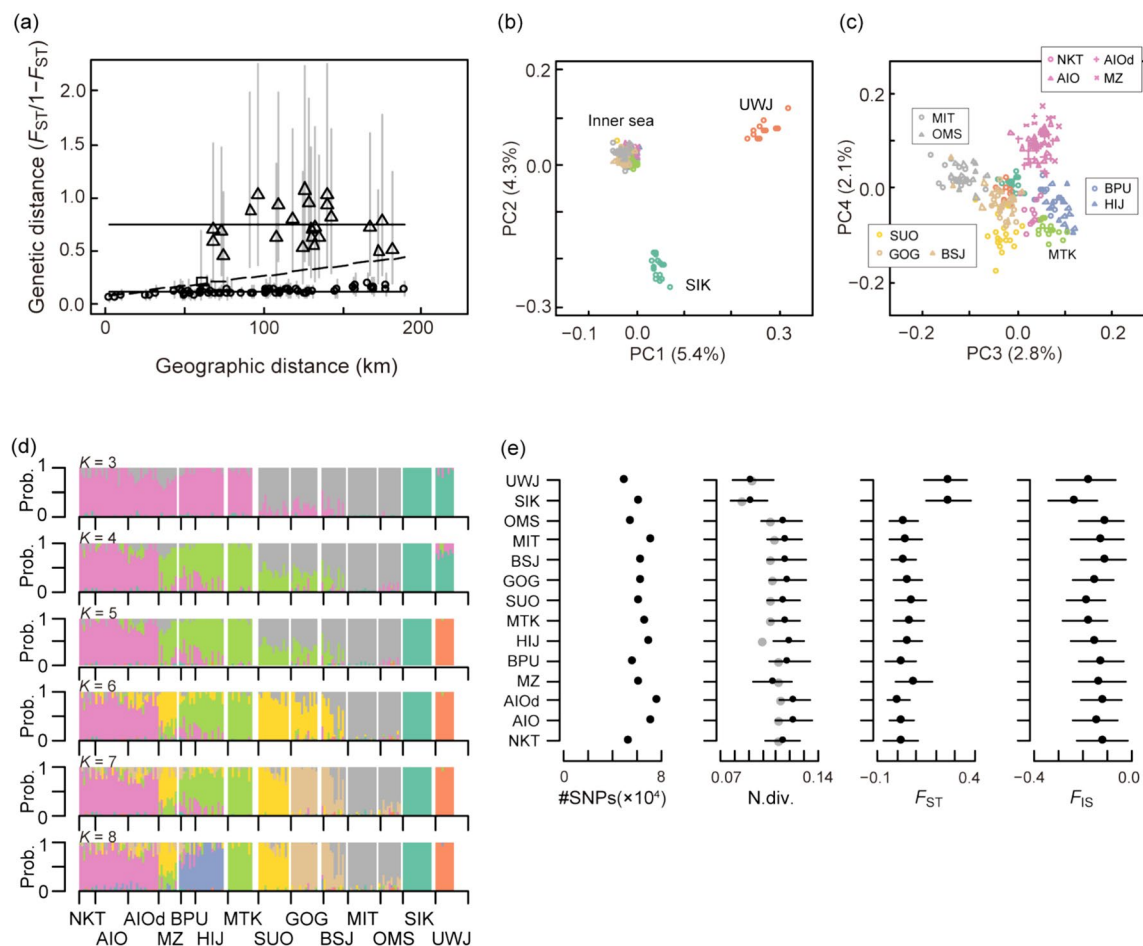


Fig. 4 Genetic structure of the eelgrass populations in the Seto Inland Sea. **a** Genetic distance versus geographic distance; two-dimensional plots showing **(b)** the first and second principal components (PC1 and PC2) and **(c)** the third and fourth principal components (PC3 and PC4). **d** ADMIXTURE analysis results for three (upper), four (mid-

dle), and five (lower) genetic clusters, and **(e)** statistics similar to Fig. 2d (gray circles were calculated at 919 SNPs shared among the individuals). The dashed line and solid lines in panel **(a)** are statistically significant IBD and IBR by single matrix regression, respectively. See Fig. 2 for detailed explanations

of genotyping at each site (Fig. S11c). The geographically outer site in Akkeshi (AI) was distinguished from the other four sites on PC1 (Fig. 5b). The genetic distance between site AI and the other sites was significantly greater than the genetic distance between other pairs of sites (Wilcoxon rank sum test: $W = 24$, $p = 0.0095$, and see Fig. 5a). The ADMIXTURE analysis at $K = 3$ also revealed this genetic structure (Fig. 5d; see Fig. S11d for the fivefold cross-validation). The PCA and ADMIXTURE analyses revealed that the AK2 individuals were admixed between AK1 individuals and those from SR and CL (Fig. 5b and d). The variance associated with the PC3 and PC4 axes was governed by the individual noise at all sites (Fig. 5c).

The nucleotide diversity tended to be greater at AK1 and AK2 than at the other three sites (Fig. 5e). However, the differences were not significant (the 95% CIs overlapped

between the pairs). Although the differences in population-specific F_{ST} were also not statistically significant, the population-specific F_{ST} tended to increase from AK1 to AI. The population-specific F_{IS} was negative at all sites.

Discussion

The genome-wide SNPs distinguished the genetic structure of the eelgrass populations within each of the three local areas, which were far apart genetically. Isolation by geographic distance was found along the 2000-km coastline of Japan as well as in an area with linear dimensions of 200 km. Conversely, IBR was not significant on the scale of Japan but was better explained statistically than that provided by IBD in the local area with linear dimensions of 200 km. The genetic structure

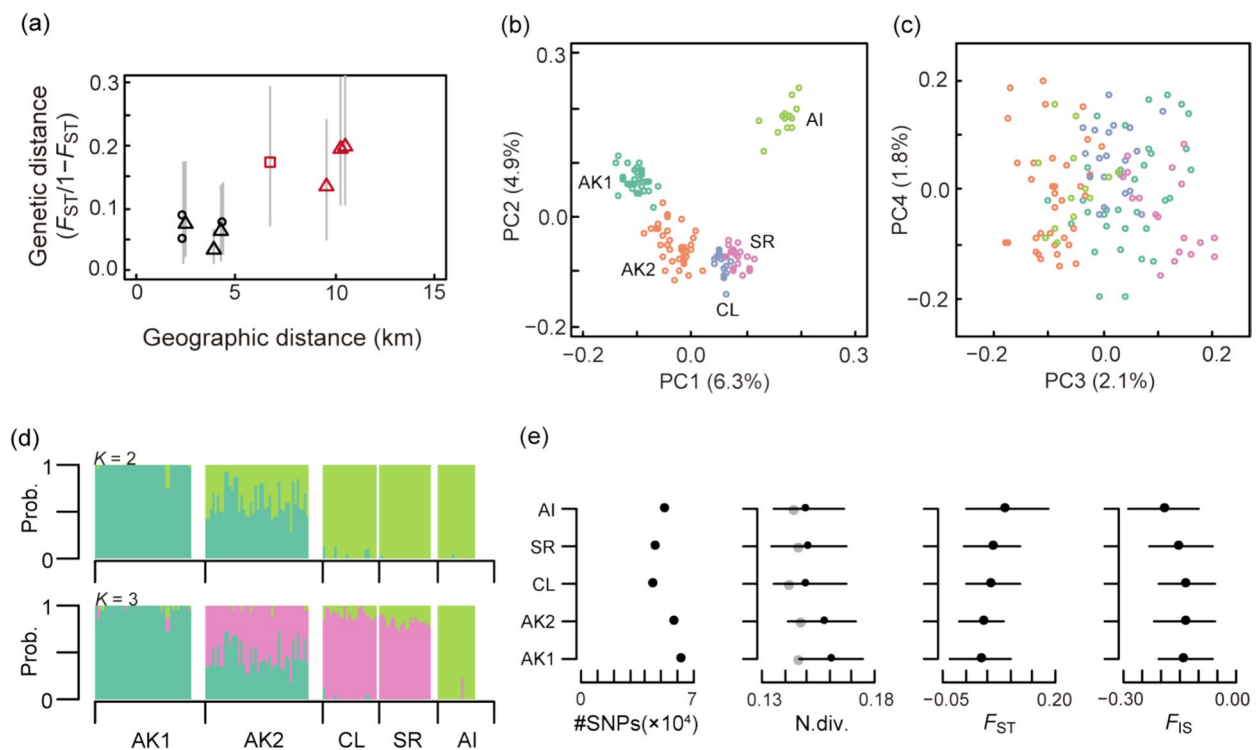


Fig. 5 Genetic structure of the eelgrass populations in Akkeshi. **a** Genetic distance versus geographic distance (red is the pairs between site AI and the other sites); **b**, **c** the results of principal component

analysis; **d** ADMIXTURE analysis; and **(e)** the associated statistics (gray circles were calculated for 1045 SNPs shared among the individuals/sites). See Fig. 2 for detailed explanations

differed between the two sites at a distance of 17 km and varied among individuals within a site. The genetic structure was heterogeneous at a distance of 10 km, where neither IBD nor IBR was statistically significant. These results revealed that heterogeneity can be a governing pattern of genetic structure rather than IBD and IBR on spatial scales less than 20 km.

Isolation by distance was found over a wide range of spatial scales within the whole area and within the local area of the SIS; IBD has previously been reported at these spatial scales in eelgrass (Hori & Sato, 2021; Kim et al., 2017; Martínez-García et al., 2021; Tanaka et al., 2011). Conversely, the complex genetic structures in each of the smaller areas meant that genetic drift and adaptations to the environment governed the genetic structure of eelgrass at each site, not just genetic differentiations through dispersal limitation (Blount et al., 2018). The genetic structure formed in the inner portion of the SIS (Fig. 4c) could be accounted for by genetic drift and adaptations and by genetic exchange driven by currents in the complex topography (Kosako et al., 2025) and through multigenerational scales (Legrand et al., 2022).

The significant difference in genetic distance based on IBR versus IBD in the SIS (Fig. 4a) may be a new finding within seagrass communities. The significant IBR may have resulted from the regulation of current connectivity between

the inner and outer waters in the Hoyo Strait (Kosako et al., 2025). Conversely, the absence of a statistically significant IBR between the estuary and bay (Fig. 5a) may have been caused by the one-way connectivity from the estuary to the bay through estuarine circulation in Akkeshi (Hasegawa et al., 2018). Although these two areas are similar in that they include a narrow strait and channel, their effects on ocean currents, the dispersal of rafting seed, and the gene flow of eelgrass may differ.

Site AI in Akkeshi was distinguished from the other areas genetically (Fig. 5b and d), and its genetic distance from the other sites was significantly greater than the genetic distances between other sites. The difference between site AI and the other sites may have resulted from selective differentiation and adaptation to the relatively high salinity at AI (Abe et al., 2024; Momota & Nakaoka, 2018). Conversely, although site SR was hypothesized to be isolated geographically from the sites in the Akkeshi-ko estuary, this site was genetically close to the sites in the Akkeshi-ko estuary. This similarity may have occurred because of its location close to the outlet of a narrow channel (Fig. 1c) where the salinity decreases (Abe et al., 2024). In addition to the genetic structure of sites AI and SR, a mismatch between the geographic location and the genetic structure was found at the three sites within the Akkeshi-ko estuary. The environmental gradients

at the three sites are known to be complex (Abe et al., 2024; Momota & Nakaoka, 2018). Such environmental conditions can cause local adaptation and a heterogeneous genetic structure on a local scale (Nguyen et al., 2023; Oetjen et al., 2010; Schiebelhut et al., 2023). In addition, because the canopy of eelgrass leaves regulates the horizontal transport of water (Abdelrhman, 2003), a complex current pattern may have formed in the meadow of eelgrass that is widely distributed in the estuary. As a result, environmental selection and/or pollen and seed dispersal via complex currents may create a heterogeneous genetic structure within the estuary.

In Tokyo Bay, the genetic structure was apparent across and within sites in the YSP site (Fig. 3). The high intra-site variability was an exceptional feature of this site compared to the SIS and Akkeshi sites. Intra-site variability may not be caused by environmental selection because there is less of a gradient in water quality among the subsites within the YSP (Watanabe et al., 2024). The heterogeneity of the eelgrass population in the YSP has been caused primarily by transplantation during the 10 years since the construction of the shallow flat (https://www.spf.org/opri/newsletter/120_3.html). Individuals from different donor sites were transplanted at different times for 10 years but probably coexisted within an area with linear dimensions of 20–40 m while they spread spatially through clonal growth (Procaccini et al., 2007). If mating occurs between individuals, the genetic structure would be homogenized among individuals. However, the admixture analysis indicated genetic heterogeneity among individuals within a site, which means that mating was limited after immigration.

The nucleotide diversity and the population-specific F_{ST} tended to be higher and lower at the inner sites than at the outer sites, respectively, in the SIS (Fig. 4e) and Akkeshi (Fig. 5e). These results indicated that the populations at the inner sites could be ancestral. As discussed above, the connectivity from outside is limited by currents driven by the complex topography at the Hoyo Strait in the SIS and by the estuarine circulation in Akkeshi. It is likely that immigration from the outer to inner bay by the rafting of seed is regulated by these currents, especially around the outer sites. The ancestral structure and patterns of transport by the currents suggest that the origins of eelgrass populations are inland seas, semi-enclosed bays, and estuaries. In addition, we note the usual gradients of water temperature, salinity, and nutrients between the inner and outer sites in the estuary. The suggested gene flow from the inner to outer populations may be formed by genetic drift and environmental selection in these areas. However, our data were insufficient to verify this scenario. Future studies are needed to test this hypothesis.

Our successful discrimination between genetic structures was enabled by using a genome-wide analysis and a large number of markers in local areas. We were thus able to detect statistically significant genetic differences. However,

we fear that this ability to discriminate will cause extreme regulation of seagrass transplantations from other populations. Extreme regulation would hinder the recovery of seagrass beds and their ecosystem services. Such conservation actions may have benefits such as reducing the further loss of genetic diversity (Shaw et al., 2025) and increasing resilience to future global climate change (DuBois et al., 2021, 2022). To avoid extreme regulation, it is important to know that statistically significant differences and the strength of genetic differences facilitate distinguishing between genetic structures (Palsbøll et al., 2007).

The case of the SIS revealed that assessing relative genetic differences can make delineating conservation units a reality. The genetic structure of the SIS implies that careful consideration should be given to transplantations between populations across the Hoyo Strait by comparing the populations within the inner portion of the SIS and those exterior to the Hoyo Strait because there are clear genetic differences across the strait. Although in the case of Akkeshi, it was possible to discriminate among sites genetically, it is difficult to envision the delineation of conservation units in these areas. Because the patterns we can refer to for relative assessments may occur on a scale larger than local areas, identifying only the genetic structure of seagrasses at these local scales may be insufficient. How do they differ evolutionarily in ancestral structure, and how much do they differ in evolutionary meaning? The answers to these questions are directly related to genetic differences and will be required for assessing conservation units where the scale of assessment is local.

In conclusion, genome-wide SNPs revealed that IBD was the common pattern in the genetic structure of eelgrass populations on scales of 200–2000 km, as known previously, and that IBR and the heterogeneity associated with spatial complexity and intra-site variability became governing patterns on scales with linear dimensions less than 200 km. This study was the first to reveal the spatial scale of the transition wherein the relative importance of local heterogeneity increases within the genetic structures of seagrasses that are governed by IBD and IBR. The results of this study also imply that our perspective on seagrass conservation should be more local than was the case before genomic tools were developed. The next challenge for delineating seagrass conservation units would be identifying the adaptive and functional meaning of genetic distance and developing methods to assess genetic differences.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12237-025-01547-8>.

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Authors Contribution SH and KM conceived the ideas and designed the methodology; SH, KM, MS, KW, YW, SH, and SO collected samples; SH analyzed the data; SH, KM, MS, SU, TK, and YU guided their interpretation; SH wrote the first draft of the manuscript. All authors contributed critically to the drafts.

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Data Availability GRAS-Di sequence data are available from DDBJ under accession numbers DRR571563–DRR571582, DRR575036–DRR575439, and DRR575503–DRR575551.

Declarations

Conflict of Interest The authors declare no competing interests.

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