ICES Journal of Marine Science, 2022, **0**, 1–12
DOI: 10.1093/icesjms/fsac163
Original Article

High levels of genetic diversity and population structure in the Mediterranean seagrass *Posidonia oceanica* at its easternmost distribution limit

Ozge Tutar[1,2,‡], Miriam Ruocco[1,‡], Emanuela Dattolo[1], Guglielmo Lacorata[3,4], Raffaele Corrado[3], Romain Watteaux[1,†], Daniele Iudicone[1], Bettina Fach[5] and Gabriele Procaccini[1,*]

[1]Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli NA, Italy
[2]Department of Earth and Environmental Sciences, University Milano-Bicocca, 4-Piazza della Scienza 1 e 4, I-20126 Milano, Italy
[3]Consiglio Nazionale delle Ricerche, Istituto di Scienze Marine (ISMAR), Via del Fosso del Cavaliere 100 00133 - Roma, Italy
[4]Center of Excellence of Telesensing of the Environment and Model Prediction of Severe events (CETEMPS), via Vetoio snc (Fraz. Coppito), 67100 L'Aquila (AQ), Italy
[5]Middle East Technical University, Institute of Marine Sciences, Deniz Bilimleri Enstitüsü, Orta Doğu Teknik Üniversitesi, 33731 Erdemli/Mersin, Turkey

[*] *Corresponding author: tel: +39 0815833363; fax: +39 0817641355;* e-mail: gpro@szn.it
[†]Present address: CEA, DAM, DIF, F-91297 Arpajon Cedex, France
[‡]These authors equally contributed to this work

High levels of genetic diversity and connectivity are crucial for the persistence of local populations, especially at the edge of species' distribution ranges. Here, we assessed the potential and realized connectivity of populations of the Mediterranean seagrass *Posidonia oceanica* at its easternmost distribution using physical modelling and genetic analyses. Genetic assessments of diversity and gene flow among populations were carried out with 18 microsatellite loci, while oceanographic connectivity was assessed via Lagrangian dispersal simulations. Levels of genetic and clonal diversities were prevalent among shallow and deep sites without signs of reproductive isolation. Both approaches identified two main clusters corresponding to "Aegean" populations along the western Turkey coast and "Levantine" populations along the southern Turkey coast. Aegean populations were genetically homogeneous, connected by high levels of gene flow, whereas Levantine populations were genetically heterogeneous. Within-sea patterns of genetic connectivity did not fully overlap with those derived from physical modelling; the realized connectivity was greater than that predicted by ocean-current simulations, especially in the Aegean Sea. Lagrangian dispersion dynamics cannot necessarily explain genetic connectivity patterns among populations, which are shaped over longer temporal scales and can be affected by human activities and local environmental conditions.

Keywords: genetic connectivity, Lagrangian dispersion model, population differentiation, seagrass, Turkish coast.

Introduction

The endemic seagrass *Posidonia oceanica* is a key component of the benthic coastal ecosystems of the Mediterranean Sea, where it provides crucial services and supports diverse and complex ecological communities (Campagne *et al.*, 2015). *Posidonia oceanica* is distributed around the Mediterranean basin, covering about 1225000 ha, along 12000 km of coastline from southern Spain and northern Morocco to the eastern Levantine Sea (Telesca *et al.*, 2015). The Alboran Sea, an area of transition between Atlantic and Mediterranean water, is the westernmost distribution limit of the species (Mateo-Ramírez *et al.*, 2016), whereas the Levantine Basin, extending along the southern coast of Turkey, is considered the easternmost edge (Çelebi, 2007; Akçalı *et al.*, 2019). Local abundance is influenced by the presence of suitable substrates and by low salinity, turbidity, and levels of organic matter that impede species survival (Telesca *et al.*, 2015). Current human impacts, including mechanical removal and eutrophication, as well as global changes, are having a profound effect on the distribution and density of *P. oceanica* meadows, leading to severe habitat loss and fragmentation of populations (Arias-Ortiz *et al.*, 2018).

Posidonia oceanica meadows show various levels of genetic/genotypic diversity, resulting from the interplay of recruitment from seeds and clonal growth of existing genotypes (Arnaud-Haond *et al.*, 2007; Serra *et al.*, 2010). A meta-analysis showed the absence of low and medium levels of genetic variation in *P. oceanica* meadows at localities with strong human-driven impacts, suggesting a sensitivity of the species to environmental alterations that overcome its resilience threshold (Jahnke *et al.*, 2015). Almost uniclonal meadows have been observed in various localities (Ruggiero *et al.*, 2002; Arnaud-Haond *et al.*, 2007), raising concern about their capability to face abrupt environmental changes, though genetically isolated populations of *P. oceanica* can thrive in persistent stressful conditions (Tomasello *et al.*, 2009).

Recent studies provide experimental evidence on the physiological and molecular mechanisms of response to high temperature and light changes (e.g. Marín-Guirao *et al.*, 2017; Jahnke *et al.*, 2019). These studies have collectively revealed an important spatial heterogeneity in the overall response of the species, suggesting local adaptation might play a role in shaping divergence between populations.

Received: March 22, 2022. **Revised:** August 19, 2022. **Accepted:** August 22, 2022

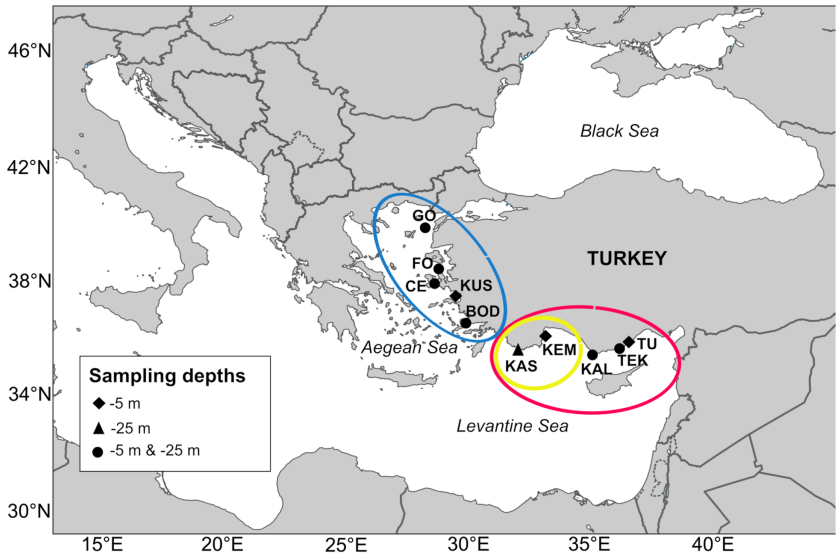

Figure 1. Map of sampling locations of *P. oceanica* along the Turkish coastline. Black diamonds indicate individuals collected only in shallow habitats. Black triangles indicate individuals collected only in deep habitats. Black dots indicate individuals collected at both shallow and deep habitats of the same site. Gokceada—GO; Foca—FO; Cesme—CE; Kusadasi—KUS; Bodrum—BOD; Kas—KAS; Kemer—KEM; Kaledran—KAL; Tekeli—TEK; Turgutlar—TU. Coloured ellipses in the figure reflect population assignment in Figure 2b. TU represents the eastern edge of *P. oceanica* in the Mediterranea Sea.

Posidonia oceanica shows a clear genetic structure across the Mediterranean basin, with the presence of distinct population groups, connected by low levels of gene flow. Two main groups are present, western and eastern Mediterranean populations, connected by a transition zone in the Sicily channel. These genetically distinctive groups reflect the evolutionary history of the species in the basin but also limited contemporary gene flow, constrained by direction of ocean currents, and lifetime of floating fruits (Arnaud-Haond *et al.*, 2007; Serra *et al.*, 2010; Chefaoui *et al.*, 2017).

Little is known about the genetic diversity and structure of *P. oceanica* and the factors limiting its distribution at its eastern boundary. The only study of Turkish populations points to the isolation of meadows in the Marmara Sea from Aegean Turkish populations, probably reflecting the geomorphological history of the enclosed basin and the relatively recent changes in water flow direction through the Dardanelles Strait (Meinesz *et al.*, 2009). No data presently exist on the genetic diversity of *P. oceanica* populations distributed along the southern coasts of Turkey. Turkish coasts encompass two temperature zones, whose boundaries are defined by the 15 and the 18°C SST winter isotherms (Pastor *et al.*, 2019). Most of the Aegean Turkey coast display winter temperatures between 15 and 16°C, whereas the temperatures along the southern coast of Turkey are generally between 16 and 18°C. The 17°C isotherm coincides with the easternmost population of *P. oceanica* in Turgutlar cove in Mersin (Çelebi, 2007; Akçalı *et al.*, 2019).

To assess the demographic status of seagrass populations and predict threats from climate change, ecological, geographical, and genetic processes should be considered together to provide sound management actions for conservation and restoration projects (Jahnke *et al.*, 2020; Pazzaglia *et al.*, 2021). Effective gene flow, measured by diverse genetic approaches, can be complemented by estimates of potential connectivity via (bio)physical dispersal with Lagrangian trajectory models (Rossi *et al.*, 2014; Jahnke *et al.*, 2020). These approaches model directional dispersal based on biologically realistic assumptions of seed release, drift duration, and depth (Ruiz-Montoya *et al.*, 2015; Sinclair *et al.*, 2018). In *P. oceanica*, Lagrangian approaches have been coupled with genetic assessments to establish patterns of connectivity among populations (Serra *et al.*, 2010), giving insights into management strategies, such as establishing networks of marine protected areas (MPAs) (Jahnke *et al.*, 2017). At a larger scale, biophysical simulations and meta-population modelling have recently been applied to quantitatively analyze basin-wide patterns of connectivity and to identify connectivity hotspots requiring conservation (Mari *et al.*, 2021).

The goal of this study was to assess the genetic diversity, population structure, and connectivity of *P. oceanica* populations at the easternmost distribution limit of the species in the Mediterranean Sea. We sampled 16 *P. oceanica* sites along the Turkish coastline, including plants at different depths, where possible. Resulting patterns of genetic connectivity estimated with 14 neutral and 4 outlier microsatellite loci were related to patterns of current-driven dispersal of sexual propagules based on Lagrangian simulations to integrate potential and realized connectivity of *P. oceanica* meadows. Our findings integrate current knowledge on the genetic structure and connectivity of *P. oceanica* in the Mediterranean basin and provide a basis for conservation and management of this foundation species at the edge of its biogeographical distribution.

Material and methods

Sampling

Plants of *P. oceanica* were collected from ten localities (Figure 1 and Table 1) between 29 May 2015 and 14 June 2015. Populations among the western coast of Turkey (Gokceada—GO, Foca—FO, Cesme—CE, Kusadasi—KUS, and Bodrum—BOD) are defined as "Aegean", whereas populations along the southern coast of Turkey (Kas—KAS, Kemer—KEM, Kaledran—KAL, Tekeli—TEK, and Turgutlar—TU) are defined as "Levantine". Both shallow and deep-water plants

Table 1. Genetic and genotypic diversity indices.

Sea	Population		Latitude	Longitude	Depth (m)	N	MLG	R	N_A	N_{PA}	A_R(SE)	H_O (SE)	H_E(SE)	$F_{IS}(p)$	%p
Aegean	Gokceada	GO-S	40.22 280 556	25.86 508 333	5	30	22	0.7	2.4	2	2.3(0.28)	0.31 (0.10)	0.34 (0.06)	0.092 (0.14)	77.78
		GO-D	40.22 280 556	25.86 508 333	25	23	22	1.0	2.4	1	2.4(0.41)	0.45 (0.09)	0.48 (0.06)	0.057 (0.22)	61.11
	Foca	FO-S	38.74 363 889	26.76 922 222	5–9	24	23	1.0	2.7	4	2.6(0.28)	0.32 (0.09)	0.37 (0.06)	0.132 (0.03)*	88.89
		FO-D	38.74 300 000	26.77 141 667	25–27	25	19	0.8	2.2	1	2.2(0.29)	0.47 (0.13)	0.41 (0.07)	− 0.142 (0.91)	66.67
	Cesme	CE-S	38.42 230 556	26.33 361 667	5–7	26	24	0.9	2.4	0	2.3(0.40)	0.48 (0.11)	0.42 (0.06)	− 0.135 (0.97)	61.11
		CE-D	38.42 230 556	26.33 361 667	25–27	26	23	0.9	2.4	2	2.3(0.28)	0.40 (0.11)	0.43 (0.05)	0.074 (0.19)	72.22
	Kusadasi	KUS-S	37.85 952 778	27.24 355 556	5	28	25	0.9	2.6	2	2.5(0.38)	0.49 (0.11)	0.50 (0.05)	0.019 (0.40)	66.67
	Bodrum	BOD-S	36.96 305 556	27.32 316 667	7–9	28	20	0.7	2.6	6	2.6(0.40)	0.42 (0.10)	0.42 (0.07)	0.016 (0.48)	77.78
		BOD-D	36.95 111 944	27.30 647 222	25	22	19	0.9	2.6	1	2.6(0.35)	0.41 (0.09)	0.47 (0.05)	0.139 (0.04)*	77.78
Levantine	Kas	KAS-D	36.16 083 333	29.62 858 333	22	24	24	1.0	2.8	6	2.7(0.41)	0.54 (0.12)	0.49 (0.06)	− 0.109 (0.93)	72.22
	Kemer	KEM-S	36.59 922 222	30.59 019 444	7–12	27	24	0.9	2.2	2	2.1(0.29)	0.45 (0.11)	0.41 (0.06)	− 0.098 (0.84)	72.22
	Kaledran	KAL-S	36.09 741 667	32.55 722 222	6	29	27	0.9	2.9	2	2.7(0.46)	0.39 (0.09)	0.42 (0.07)	0.077 (0.09)	77.78
		KAL-D	36.09 222 222	32.55 132 222	20–22	29	28	1.0	3.1	1	2.8(0.36)	0.37 (0.08)	0.39 (0.06)	0.048 (0.22)	83.33
	Tekeli	TEK-S	36.12 725 000	33.12 541 667	6	29	24	0.8	1.9	0	1.9(0.17)	0.46 (0.11)	0.41 (0.04)	− 0.127 (0.92)	72.22
		TEK-D	36.12 477 778	33.12 752 778	20	27	22	0.8	2.1	1	2.0(0.15)	0.49 (0.11)	0.41 (0.05)	− 0.221 (0.99)	83.33
	Turgutlar	TU-S	36.15 408 333	33.44 461 111	5	27	19	0.7	2.0	2	2.0(0.20)	0.42 (0.13)	0.43 (0.04)	0.018 (0.48)	66.67

Populations are grouped in two regions: Aegean and Levantine. For each population, the following information are shown: acronym, geographical coordinates (latitude and longitude), depth, number of samples (N), number of multilocus genotypes (MLG), genotypic diversity (R), number of different alleles (N_A), number of private alleles (N_{PA}), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}), and percentage of polymorphic loci (%P). -S indicates shallow-water stations and -D indicates deep-water stations; * p<0.05.

could be collected at 6 localities, while the 4 remaining stations could be sampled at only one depth, for a total of 16 sites (Figure 1 and Table 1). Suitable dive sites were primarily determined through the literature and selected according to the information and logistical facilities from dive centres. At each site, *ca.* 30 plants were collected following a zigzag route at least 5 m apart to minimize the risk of sampling the same genotype. After diving, samples were gently cleaned from epiphytes and dried with silica gel.

DNA extraction and microsatellite analysis

About 60 mg leaf tissue was powdered in TissueLyser (Qiagen), and genomic DNA isolated with the Macherey-Nagel NucleoSpin 96 Plant II kit. After isolation, DNA quality was checked by 1% agarose gel electrophoresis. Thirty plants from each population were selected for subsequent analyses (480 in total). All samples were genotyped at 18 microsatellite loci (Procaccini and Waycott, 1998; Alberto *et al.*, 2003; Arranz *et al.*, 2013), assembled in two separate multiplexes and amplified by PCR using QIAGEN Multiplex PCR Kit. Selected microsatellite regions, multiplex assembly, and details of PCR reactions are reported in Supplementary Tables S1 and S2. Genotyping was performed using an ABI Prism 3730 automated DNA sequencer (Applied Biosystems) with the following PCR conditions: 95°C for 15 min, 35 cycles of 94°C for 30 s, 60°C for 90 s, and 72°C for 60 s, followed by 30 min at 60°C. Peak identification and scoring was performed using the Peak Scanner Software 2 (Applied Biosystems). Specimens showing <10% missing data were included in the analysis.

Clonal and genetic diversity, outlier detection

The presence of identical MLGs was assessed by the software RClone (Bailleul *et al.*, 2016), and the following analyses were performed only on different MLGs. For each population, genotypic diversity was assessed as the R ratio: $R = (G-1)/(N-1)$, where G is the number of genotypes and N is the number of individuals (Dorken and Eckert, 2001). We assessed the presence of null alleles using MicroDrop (Wang and Rosenberg, 2012). Linkage disequilibrium (LD) and deviations from Hardy–Weinberg expectations (HWE) at each locus and across loci in each population were tested with Genepop 4.7.5 (Rousset, 2008), using 10000 dememorizations, 1000 batches, and 10000 iterations per batch. The statistical significances of LD pairwise comparisons were determined by applying the Bonferroni correction for multiple comparisons [α (0.05) divided by the number of tests]. Finally, we calculated the probability of identity (PI) in GenAlEx (Peakall and Smouse, 2012) to get an indication of the minimum number of loci needed for genetic tagging at each location. The mean number of alleles per locus (N_A), private alleles (N_{PA}), and percentage of polymorphic loci (%p) per population were estimated with GenAlEx. To compare genetic variation across populations, observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding (F_{IS}) were calculated with Arlequin 3.5.2 (Excoffier and Lischer, 2010). Mean allelic richness (A_R) was calculated using the R package diveRsity 1.9.90 (Keenan *et al.*, 2013), using the rarefaction method to correct for variation in sample size. To identify putative outlier loci within the microsatellite set, a neutrality test was performed using two F_{ST}-based approaches, implemented in LOSITAN (Antao *et al.*, 2008) and BayeScan (Foll and Gaggiotti, 2008). LOSITAN was run with the following set-

tings: 50000 simulations under neutral mean F_{ST} and forced mean F_{ST} options, confidence interval of 0.95, and infinite allele models. BayeScan was used with default settings, resulting in the same probability threshold as used for LOSITAN. We considered as real outliers only those shared between the two methods.

Population differentiation and genetic structure

Genetic differentiation among sites was calculated using Weir and Cockerham's F_{ST}, in Arlequin, and D_{est} (Jost's D, Jost, 2008), in GenAlEx. Significance of D_{est} population's pairwise comparison was based on 999 permutations. A Principal Coordinates Analysis (PCoA) and an Analysis of Molecular Variance (AMOVA) were performed with GenAlEx. AMOVA was performed with 999 permutations, with two groupings: "Sea" including Aegean and Levantine, and "Depth" including shallow and deep sites. A bayesian clustering analysis was performed with STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) for *K*2–*K*8 to identify population structure with the options admixture model, run length 100000, and 100000 MCMC iterations, and correlated allele frequencies. Each *K* consisted of ten independent runs. STRUCTURE output was estimated with Evanno ΔK (Evanno *et al.*, 2005) in STRUCTURE HARVESTER (Earl and von Holdt, 2012), and visualized with CLUMPAK (Kopelman *et al.*, 2015). Analyses were conducted considering (1) all 18 loci, (2) 14 neutral loci, or (3) 4 outlier loci. EDENetworks (Kivelä *et al.*, 2014) was used to measure and visualize genetic distance across sites. Networks were created using an individual-centred genotype matrix and allele sharing as for distance index and automatic network thresholding. The betweenness centrality was calculated for each node, describing the number of shortest paths between other nodes passing through a node.

Genetic connectivity

GENECLASS2 was used to estimate first-generation migrants and to compute the genetic assignment (Piry *et al.*, 2004). These analyses were based on neutral loci, as dispersal should make the biggest contribution to the observed allele frequencies. The Rannala and Mountain (1997) criterion was selected for detecting the likelihood that an individual belonged to the location where it was sampled. For the estimation of first-generation migrants, exclusion probabilities from the reference location were calculated using Monte Carlo resampling with 10000 permutations and a threshold probability of 0.01. For the assignment test, exclusion probabilities from the reference location were calculated using the Paetkau *et al.* (2004) simulation algorithm with 1000000 simulation steps and a type 1 error of 0.05. A probability over 95% was required to exclude an individual. Identified migrants were assigned to another location when there was a probability of over 10%.

Magnitude and patterns of gene flow between locations and directional migration based on D_{est} genetic differentiation of neutral loci were inferred with the function "DivMigrate" in diveRsity with 10000 bootstrap replications. We tested isolation by distance (IBD) via Mantel test with GenAlEx. Pairwise F_{ST} and D_{est} values were correlated to sea distances between locations with 9999 permutations.

Oceanographic connectivity

We used Lagrangian oceanographic analysis to understand the physical connectivity via current transport along the coasts of Turkey. This analysis was possible for nine locations (GO, FO, CE, KUS, KAS, KEM, KAL, TEK, and TU), except for Bodrum (BOD), because of resolution issues of the model, particularly local geographic conditions (the site was too close to the coastline and surrounded by small islands). Lagrangian simulations were computed as described in Supplementary S2.

Large sets of numerical particles were released and tracked over time in the research area domain. These particles, representing floating *P. oceanica* fruits, were assumed to be passively advected by currents. All sets of numerical tracers were released in the surface layer (−3 m) around each sampling site in a disk-like area with a radius of $R_{site} = 4$ km. Starting in March, the beginning of the flowering period in *P. oceanica* (Buia and Mazzella, 1991) and during each of the following 90 d for the years 2010–2013, a batch of 51200 particles was released from each sampling site and their positions were recorded over 28 d, which is considered the life of floating fruits (Serra *et al.*, 2010).

From the numerical trajectories, and for each sampling site, we computed the fine-grained Lagrangian probability density functions (PDFs) to estimate the probability per unit area that a particle coming from a given source was found near a target point after a simulation time (Mitarai *et al.*, 2009). The physical (potential) connectivity matrix, defined as the transition probability matrix between sites over a given interval, was therefore computed from the Lagrangian PDFs. Each site could then be characterized by its retention strength, source strength, and destination (or sink) strength. A "retainer" of Lagrangian particles is defined as a place where released propagules successfully remain *in situ*; a "source" is a place from where released propagules successfully reach other sites; a "sink" is a place to where propagules released from other sites tend to successfully settle (Jahnke *et al.*, 2017).

Results

Genotypic and genetic diversity

A total of 424 individuals were genotyped at 18 microsatellite loci. After genotyping assessment, 365 unique MLGs were used for analysis. The number of distinct MLGs ranged from 19 in FO-D, BOD-D, and TU-S to 28 in KAL-D. Clonal diversity (R) ranged from 0.7 in GO-S, BOD-S, and TU-S to 1.0 in GO-D, FO-S, KAS-D, and KAL-D (Table 1). There was no evidence of allelic dropout. Significant deviations from HWE ($p<0.05$) were observed for 16 loci across all populations (83 of 288 tests [29%]). We found significant LD in 23 of 153 tests across all populations (15%) after applying Bonferroni corrections. Po-5–10, Poc-35, Po-5–39, and Poc-5 drove most of these significant deviations. The PI was low, ranging from 3.5×10^{-5} in GO-S to 2×10^{-7} in KAS-D. The PIsibs were larger, ranging from 6.9×10^{-3} in TU-S to 8.2×10^{-4} in GO-S, which are still sufficient for discerning siblings, considering the number of MLGs.

The population with the largest number of alleles per locus was KAL-D ($N_A = 3.1$), followed by KAL-S, KAS-D, and FO-S, the latter displaying the largest percentage of polymorphic loci (88.89%) (Table 1). At the other extreme, TEK-S had $N_A = 1.9$. The maximal number of private alleles was observed in BOD-S and KAS-D ($N_{PA} = 6$) (Table 1). Levels of allelic richness were generally low, ranging from 1.9 in TEK-S to 2.8 in KAL-D (Table 1). Observed heterozygosity (H_O)

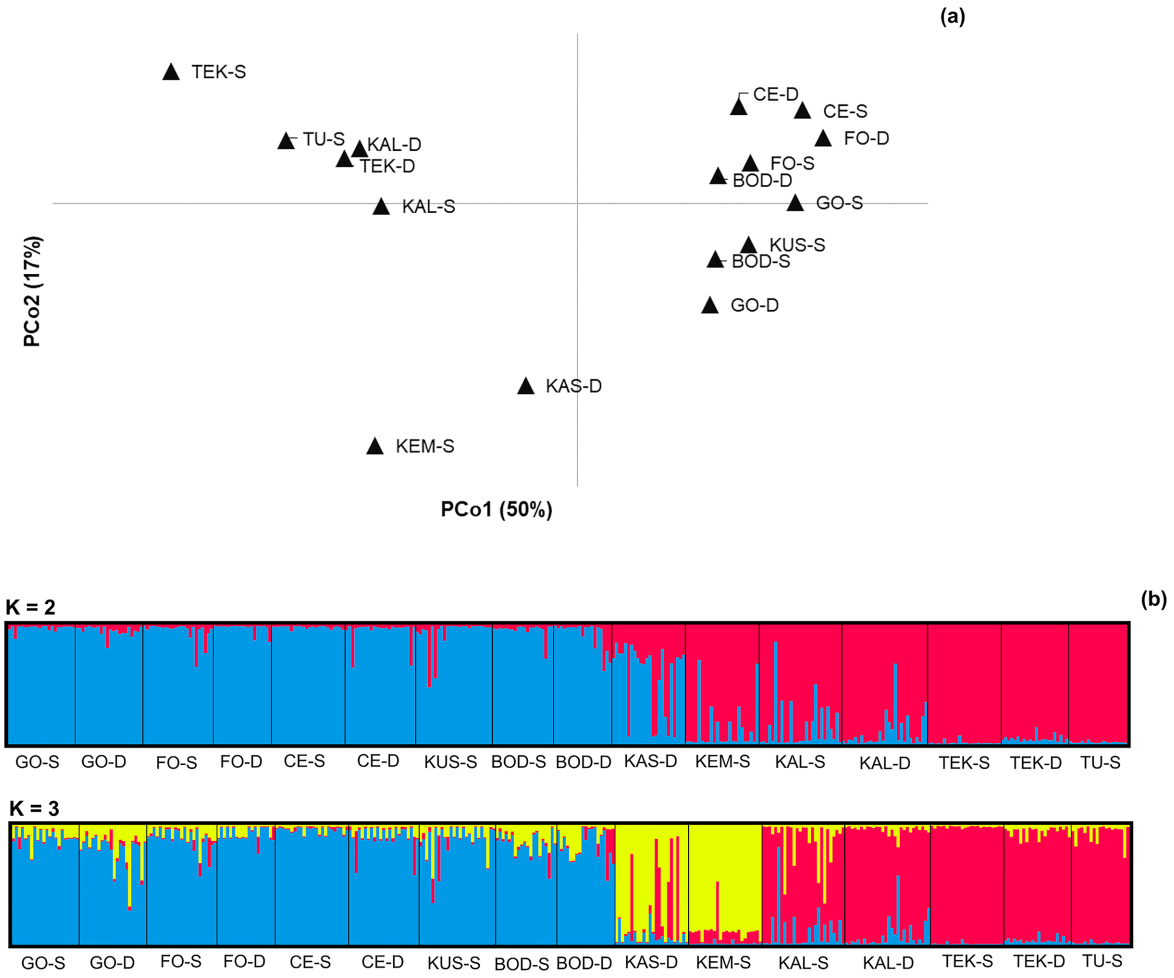

Figure 2. Population differentiation and structure analysis for the 16 *P. oceanica* sites along the Turkish coastline. (a) PCoA for all 18 loci; and (b) STRUCTURE analysis performed for all 18 loci with $K = 2$ and $K = 3$. Plots at higher Ks and for other sets of loci can be found in Supplementary Figure S5. Each individual sampled is represented on the x-axis, while the y-axis denotes the PI to each genetic cluster. Population acronyms as in Table 1.

per population ranged from a minimum of 0.31 in GO-S to a maximum of 0.54 in KAS-D (Table 1).

An excess of heterozygotes ($H_O > H_E$) was observed in some populations and was greatest in TEK-D. On the other hand, an excess of homozygotes ($H_E > H_O$) was particularly evident in BOD-D. F_{IS} ranged from −0.22 in TEK-D to 0.14 ($p < 0.05$) in BOD-D (Table 1). Overall, genotypic richness was similar between Aegean and Levantine locations, though there were some regional (non-significant) differences in genetic diversity indices. Generally, Aegean populations had larger A_R and F_{IS}, whereas Levantine ones had larger H_O and $\%p$. There were no distinct patterns of genotypic nor genetic diversity between shallow and deep sites across populations.

Outlier identification, genetic differentiation, and structure

The outlier analysis of all 16 populations with LOSITAN identified Poc-5 and Pooc-229 as loci under positive selection, while Pooc-PC047G07, Po-5–39, and Pooc-330 were in balancing selection (Supplementary Figure S1). BayeScan gave the same result, except Pooc-330, which was not identified as a putative outlier (Supplementary Figure S2 and Table S3) and was not considered as such in our analysis.

Both F_{ST} and D_{est} detected significant differentiation among all sets of loci with significant comparisons shared between the two coefficients. Values ranged from 0.438 to 0.032 for F_{ST} (Supplementary Table S4), and from 0.304 to 0.017 for D_{est} (Supplementary Table S7). Overall, the greatest genetic differentiation was observed between KEM-S, TEK-S, and TU-S (Supplementary Figure S3). Considering only neutral loci, KAS-D, KEM-S, and TEK-S showed the greatest differentiation. Pairwise differences were smaller between Aegean populations than between Levantine populations (see Supplementary Tables S4–S9 for F_{ST} and D_{est} pairwise comparisons with the three sets of loci). Shallow and deep stands at the same locations exhibited weak, but significant, differentiation considering both F_{ST} and D_{est} (Supplementary Tables S4–S9).

A PCoA of all loci identified two main population groups along PCo1, which explained 50% of the total variance. KAS-D located between the Aegean and Levantine groups (Figure 2a). Aegean populations were more homogeneous and clustered close to each other on the positive x-axis. KEM-S and KAS-D were separated from other Levantine populations (Figure 2a). In most cases, shallow and deep samples from the same locality fell close to each other (Figure 2a). In agreement with the PCoA, the Bayesian grouping in STRUCTURE identified $K = 2$ as the most likely number of clusters with all

Table 2. AMOVA considering 18 loci, neutral loci, and only outlier loci, performed with two groupings (Sea and Depth).

Source	df	SS	MS	Est. Var.	%
		All loci			
Sea					
Among regions	1	483.827	483.827	2.404	25
Among pops	14	657.645	46.975	1.819	19
Within pops	349	1944.331	5.571	5.571	57
Total	364	3085.803		9.794	100
Depth					
Among regions	1	37.940	37.940	0.000	0
Among pops	14	1103.531	78.824	3.216	37
Within pops	349	1944.331	5.571	5.571	63
Total	364	3085.803		8.787	100
		Neutral loci			
Sea					
Among regions	1	183.154	183.154	0.795	12
Among pops	14	534.489	38.178	1.487	22
Within pops	349	1516.113	4.344	4.344	66
Total	364	2233.756		6.626	100
Depth					
Among regions	1	27.786	27.786	0.000	0
Among pops	14	689.857	49.275	1.973	31
Within pops	349	1516.113	4.344	4.344	69
Total	364	2233.756		6.317	100
		Outlier loci			
Sea					
Among regions	1	300.673	300.673	1.609	51
Among pops	14	123.155	8.797	0.333	10
Within pops	349	428.218	1.227	1.227	39
Total	364	852.047		3.168	100
Depth					
Among regions	1	10.154	10.154	0.000	0
Among pops	14	413.674	29.548	1.243	50
Within pops	349	428.218	1.227	1.227	50
Total	364	852.047		2.470	100

"Sea" indicates Aegean and Levantine and "Depth" indicates shallow and deep habitats.

sets of loci (Figure 2b, Supplementary Figure S4). In the 18-locus dataset, KAS-D appeared between the clearly identified Aegean and Levantine groups (Figure 2b). Although less significant, the analysis with $K = 3$ showed that both KAS-D and KEM-S represented a separate genetic cluster between the two main population groups (Figure 2b). In the analysis of neutral loci with $K = 2$, KAS and KEM were included in the Levantine cluster (Supplementary Figure S5). $K = 4$ still had a large ΔK, and separated KAS and KEM from the rest, as well as TEK (both shallow and deep) and TU-S (Supplementary Figure S5).

The separation between Aegean and Levantine populations was also evident in the EDENetwork analysis (Supplementary Figure S6). The Aegean group appeared more homogeneous, with a much higher level of gene flow based on allele sharing. Levantine individuals were more scattered and exhibited fewer links among populations, while having larger intra-population relationships. Most of the connections between Aegean and Levantine samples occurred through the shallow and deep populations of KAL and KEM-S (Supplementary Figure S6).

The largest variance in the AMOVAs occurred between Aegean and Levantine populations, using only outlier loci (variance = 51%) (Table 2), but dropped to 25 and 12%, with all loci or only neutral loci, respectively. Variance among populations was 19% with all loci, and 22 and 10% for neutral and outlier loci, respectively (Table 2). The variance observed between depths was 0% with all sets of loci (Table 2).

To identify possible local adaptation between the two regional genetic clusters, we repeated the LOSITAN and BayeScan outlier analyses for the Aegean-vs-Levantine grouping. KAS-D and KEM-S were included in the Levantine group. The EST-linked locus, Pooc-PC045G11, which was identified as possibly under selection by both methods (Supplementary Table S10), showed a significant similarity to the conserved eukaryotic initiation factor 5A (UniProtKB/Swiss-Prot: P69039.1; Jahnke *et al.*, 2019).

Genetic (realized) connectivity

A total of 286 out of 365 (78%) individuals were correctly assigned to population of origin. The population with the least number of correctly assigned individuals was GO-S (45%), and populations with correct assignments generally increased from north to south. The best correctly assigned populations were KAS-D, KEM-S, and TEK-S (all 96%). Among Levantine populations, all but KAL-D showed values above 80%. GENECLASS2 identified 12 of 365 individuals as significant first-generation migrants (3%, $p<0.01$) (Table 3). The greatest genotype dispersals occurred between northern Aegean populations, whereas Levantine populations showed restricted dispersal. GO-D, FO-D, and CE-S were the most important source populations, providing at least two individuals to other sites (Table 3). In the southern region, TEK-D and TU-S provided one migrant each (Table 3).

Table 3. Results of the assignment test and data for the 12 detected first-generation migrants ($p<0.01$) of *P. oceanica* among the 16 sites along the Turkish coast using GENECLASS2.

	Home location	% Correctly assigned individuals	No. of recent migrants	Exclusion probability	Assigned location
Aegean	GO-S	45	1	0.0003	GO-D
	GO-D	55	1	0.0077	FO-D
	FO-S	52	3	0.0057	CE-S
				0.0070	BOD-D
				0.0011	GO-D
	FO-D	68	1	0.0085	CE-S
	CE-S	67	1	0.0001	FO-D
	CE-D	61	–	–	–
	KUS-S	80	1	0.0002	GO-D
	BOD-S	90	–	–	–
	BOD-D	95	–	–	–
Levantine	KAS-D	96	–	–	–
	KEM-S	96	–	–	–
	KAL-S	89	2	0.0078	TEK-D
				0.0026	GO-D
	KAL-D	79	1	0.0004	KAL-S
	TEK-S	96	1	0.0005	TU-S
	TEK-D	95	–	–	–
	TU-S	89	–	–	–
	Total		12		

Only neutral loci are considered in the analysis.

Gene flow between populations, based on "DivMigrate", D_{est}, and neutral loci also showed that the greatest amount of gene flow occurred between Aegean populations (Supplementary Figure S7). Stronger, but non-significant, network connections were visible among GO-S, KUS-S, FO-S, FO-D, and CE-D (Supplementary Figure S7).

Tests of IBD showed a significant relationship between pairwise F_{ST} and D_{est} (Supplementary Tables S5–S8), and shoreline distances between all sites (F_{ST}: $r = 0.439$, $p = 0.001$; D_{est}: $r = 0.424$, $p = 0.001$). No significant correlation was observed among Aegean samples with F_{ST} ($p = 0.2$) or D_{est} ($p = 0.1$) and geographical distance, whereas Levantine samples showed a weak, but significant, correlation (F_{ST}: $r = 0.480$, $p = 0.037$; D_{est}: $r = 0.487$, $p = 0.032$).

Oceanographic connectivity

Dispersal simulations were made using ocean-current data from 2010 to 2013 (Figure 3). Further information on particle trajectories for 10, 14, and 20 d of passive dispersal appears in Supplementary Figures S8–S10. Patterns of particle dispersal agree with genetic patterns, in identifying two main areas of dispersal corresponding to the Aegean and the Levantine coasts. After 28 d, CE, TU, KAL, TEK, and FO had the greatest source strengths, respectively (Supplementary Figure S11). KUS had the largest retention probability (Supplementary Figure S11). FO, KEM, and KAL had the greatest sink strength (Supplementary Figure S11). Particles released from the Aegean populations never reached southern localities, remaining trapped within the system of islands along the Turkish Aegean coast. Particles originating from GO drifted only northward (Figure 3a). Particles originating in FO and CE were trapped in the island system and, due to their proximity, overlapped in the dispersal area (Figures 3b and c). Particles released from the Levantine populations moved toward the west but never reached Aegean populations in 28 d. TU- and TEK-sourced particles drifted to both the west and east and possibly reached the Cyprus coast (Figure 3h and i). In contrast, particles originating at KAL drifted to Antalya Bay (Figure 3g), which connected the KEM population to KAL.

Discussion

Major features of genetic population structure of the foundation species *P. oceanica* in the Mediterranean basin has been previously characterized using microsatellite markers (Arnaud-Haond *et al.*, 2007; Serra *et al.*, 2010; Jahnke *et al.*, 2017). However, few data are available for populations at the easternmost distribution limit of the species in the Aegean and Levantine Seas (Meinesz *et al.*, 2009). Our assessment of population structure, genetic diversity, and patterns of gene flow for 16 Anatolian *P. oceanica* meadows, including shallow and deep sites, showed that *P. oceanica* meadows at the edge of the species' distribution range in the Eastern Mediterranean were not genetically isolated. A genetic break occurs between Aegean and Levantine populations, which was also supported by physical modelling. In contrast, within-sea patterns of realized and genetic connectivity were not in complete agreement, especially in the north Aegean Sea, where genetic connectivity was stronger than that predicted by oceanographic transport.

Overall, Turkish populations exhibited high levels of clonal diversity, as indicated by a large mean R value (0.9 ± 0.03 SE), and high levels of mean genetic diversity ($H_O = 0.43 \pm 0.02$ SE). The smallest values of R and H_O were observed in the shallow population of Gokceada (GO-S) ($R = 0.7$ and $H_O = 0.31$), though the same values were larger for the deep stand of the same location (GO-D, $R = 1$ and $H_O = 0.45$). However, the meadows possessed low levels of allelic richness ($A_R = 2.38$). Clonal and genetic diversity are similar to those found for *P. oceanica* meadows in the Central Mediterranean, such as in the transition zone of the Strait of Sicily ($R = 0.7$ and $H_O = 0.5$; Arnaud-Haond *et al.*, 2007; Serra *et al.*, 2010).

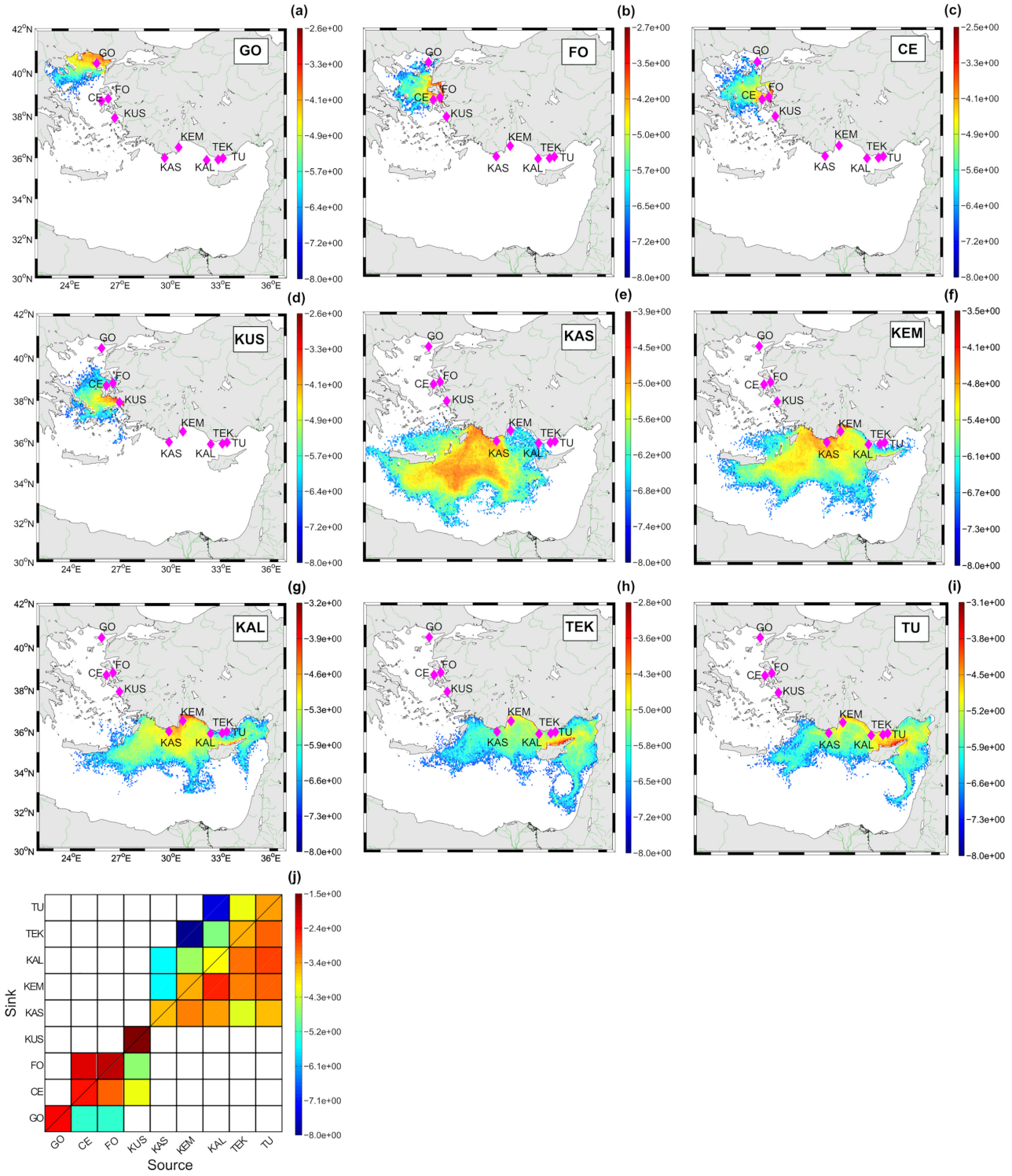

Figure 3. Oceanographic connectivity of *P. oceanica* along the Turkish coastline. (a–i) Mean Lagrangian PDFs of particles after an advection time of 28 d for nine locations along the Turkish coastline (GO, FO, CE, KUS, KAS, KEM, KAL, TEK, and TU) considering all the years of simulations. (j) Matrix showing potential connectivity between locations estimated via Lagrangian simulations. Each box gives the probability that a particle released in a region in X-axis (source) reaches a region in Y-axis (sink). PDFs produced considering other advection times can be found in Supplementary Figures S8–S10.

The high level of genotypic richness suggests that sexual recruitment is considerable at these sites, even sites close to the south-eastern distribution limit of the species. This is in contrast with findings in other plant species, where the contribution of sexual reproduction to population maintenance was generally seen to be less at the geographical margins of species' ranges (Eckert, 2002). For example, the seagrass *Zostera marina* showed a greater incidence of clonal growth and low levels of genetic diversity and gene flow at its south-eastern Atlantic distribution limit in the Ria Formosa lagoon, compared to central populations (Billingham *et al.*, 2003). However, reproductive strategies in this species vary across its distribu-

tion range in response to environmental variability influencing seedling recruitment, producing annual and perennial populations (Phillips *et al*.,1983; Jarvis and Moore, 2015). The investment in sexual reproduction of *P. oceanica* at its marginal distribution in the eastern Mediterranean could also be linked to long-term warm sea surface temperature (SST) patterns (Nykjaer, 2009; Pastor *et al*., 2019). Yet, the Aegean Sea shows greater variability in SST during summer months (Nykjaer, 2009), in part due to upwelling. Warmer climates can affect the reproductive behaviour of plant species, hence modifying the relative importance of sexual vs clonal reproduction (Johansson *et al*., 2013). Warming-induced flowering has been experimentally demonstrated in *P. oceanica*, and flowering intensity was positively correlated with genetic diversity (Ruiz *et al*., 2018).

None of the analyzed populations shows signs of genetic isolation, contrary to other *P. oceanica* meadows in the Eastern Mediterranean, such as in the North-Adriatic Sea (Ruggiero *et al*., 2002; Arnaud-Haond *et al*., 2007) and in the Marmara Sea (Meinesz *et al*., 2009). Five *P. oceanica* meadows in the Marmara Sea and Dardanelles Strait, as well as in the Aegean Sea adjacent to the Dardanelles Strait, showed little clonal diversity, significant excesses of heterozygosity, and small allelic diversities (Meinesz *et al*., 2009). This was especially true for the population in the Marmara Sea, where a bottleneck in population size was hypothesized from the predominance of clonal growth and promoted the long-term adaptation of *P. oceanica* to extreme low salinities of brackish waters (Meinesz *et al*., 2009).

Strong genetic structuring was present, as we found significant pairwise population differentiations between sites with both all and neutral loci, for F_{ST} and D_{est}. Assignment tests also revealed a limited number of recent migrants and a global large percentage of correctly assigned individuals across populations. Two main genetic clusters, corresponding to Aegean populations along the western Turkey coast and Levantine populations along the southern Turkey coast, were clearly identified by multiple analyses of the three sets of loci. The *P. oceanica* populations of KAS and KEM were considered closer to the Levantine group by most analyses, although they may represent a genetic sub-group, as evident from STRUCTURE at larger values of *K* (Supplementary Figure S5). Levantine populations generally appeared more genetically heterogeneous than homogeneous Aegean populations, which were connected by a higher level of gene flow. This pattern has also been observed for other species in this area (*Mytilus galloprovincialis*, Giantsis *et al*., 2014; *Diplodus sargus*, Exadactylos *et al*., 2019). In further support, we found a significant pattern of IBD across populations of the southern Turkey coast, but not among Aegean populations.

The irregular Aegean coastline produces a complex surface circulation system (Olson *et al*., 2007), whereas the Mediterranean coast of Anatolia is influenced by a dominant westward current (Bianchi *et al*., 2012). The complex coastal profile and small islands along the Aegean coast may act as barriers to long-distance dispersal, isolating Aegean populations from populations on the southern coast of Turkey and resulting in little directional gene flow.

Genetic assignments indicated that GO-D, FO-D, and CE-S were the most important source populations in the northern area, and FO was the most important sink location. The deep population of Gokceada (GO-D) provided the largest number of individuals to other populations in the Aegean area and was apparently the only population whose propagules could reach southern locations. The physical connectivity analysis indicated CE as the largest source strength and confirmed FO as the greatest destination strength after 28 d of passive dispersal. Yet, oceanographic modelling indicated particles released from the Aegean sites never reached southern localities (and *vice versa*), confirming the strong separation between the two population groups. Amongst Levantine localities, genetic assignment tests indicated that TU and KAL have the greatest source strength, confirming the physical simulations, and are expected to provide propagules to other populations.

Interestingly, within-sea patterns of realized and potential connectivity were not always in complete agreement. For instance, northern *P. oceanica* populations (KUS, GO, FO, and CE), possess the greatest retention strength across localities, as clearly shown by the oceanographic connectivity matrix (Figure 3J, Supplementary Figure S11). Gokceada and Kusadasi appear to be strong retainers, while having no source nor sink strength, contrary to the genetic assignments. Hence, realized connectivity may be greater than potential connectivity, especially within the northern Turkey coast. Importantly, the ocean circulation fields included in the Lagrangian trajectory model may not provide sufficient spatio-temporal resolution to accurately describe the dynamics at sites close to the coast or surrounded by numerous islands. Furthermore, as a first approximation, the Lagrangian simulations were carried out at fixed constant depth near the sea surface, whereas genetic connectivity was assessed for both deep and shallow stands. Alternatively, the considerable maritime traffic, mainly tourist activities among Aegean localities, may transport vegetative propagules. According to recent estimates through AIS (Automatic Identification System) data analysis, Aegean waters have the greatest recreational traffic in the Mediterranean (Maglio *et al*., 2015). This may contribute to connectivity and greater genetic homogeneity among Aegean *P. oceanica* populations than among Levantine populations and may also play a role in the southward dispersal of vegetative propagules.

The contrast between physical modelling and the genetic results may reflect different time frames on which gene-flow is assessed, in contrast to contemporary sea-current dispersal. Genetic connectivity cannot necessarily be explained by contemporary currents alone, as it may also result from past evolutionary events (Serra *et al*., 2010; Chefaoui *et al*., 2017).

As our sampling scheme encompassed a latitudinal gradient of temperature across locations (Supplementary Figure S12), we performed an outlier analysis for the "Aegean vs Levantine" grouping to identify signs of local adaptation between the northern and southern genetic clusters. The outlier locus, Pooc-PC045G11, showed a similarity to the conserved eukaryotic initiation factor 5A (eIF5A) identified as a candidate latitude-associated locus in *P. oceanica* (Jahnke *et al*., 2019). Plant eIF5A proteins are involved in multiple biological processes, including protein synthesis regulation, cell proliferation, leaf and root growth, senescence, programmed cell death, and stress responses to temperature extremes (Wang *et al*., 2012). Our analysis has limited power to identify candidate loci possibly involved in the adaptive response of *P. oceanica* populations to the temperature gradient along the Turkish coastline, as the microsatellite dataset was small. However, these data can be the starting point of further analyses based on genome-wide analysis of polymorphisms across regions.

Conclusions

In this study, we found unexpected high levels of genetic and clonal diversities in *P. oceanica* meadows at the edge of its distribution range in the Eastern Mediterranean, resembling those previously observed in Central Mediterranean populations. In addition, strong genetic differentiation was evident between Aegean populations along the northern Turkey coast and Levantine populations along the southern Turkey coast. This genetic break is also supported by physical modelling and may result from the inhibition of long-range dispersal due to the complex coastal morphology of the Aegean region, and potentially to adaptive differences to distinct temperatures between northern and southern regions.

Genetic and physical connectivity analyses both identified major source and sink populations. However, there was poor agreement for within-sea patterns. In the Aegean Sea, the effective connectivity was significantly greater than that predicted by model simulations, indicating that other factors may affect the genetic patterns of connectivity among populations. Marine traffic density, or other human activities, could affect the dispersal of vegetative propagules in the area.

Data availability statement

The data underlying this article are available in the article and in its online supplementary material.

Supplementary data

Supplementary material is available at the ICESJMS online.

Author contributions

OT and GP conceived the ideas and designed the study. OT conducted the sampling, laboratory work, and microsatellite scoring (under the guidance of ED). OT and MR conducted the genetic data analyses and interpretation (under the guidance of GP). GL, RC, RW, and DI conducted the Lagrangian modelling analysis. MR and OT led the writing of the manuscript. All authors contributed in drafting the manuscript and approved the final version.

Funding

The work has been partially supported by the projects Marine Hazard, PON03PE_00203_1, funded by the Italian Ministry of Education, University and Research (MIUR), and DEKOSIM (BAP-08-11-DPT2012K120880) funded by the Turkish Ministry of Development.

Conflict of interest

Authors declare no conflict of interest.

Acknowledgements

O.E. was the recipient of a Ph.D. grant from the Italian flagship project RITMARE (MIUR) to the University Milano Bicocca (Italy). The sampling was performed in collaboration with METU-IMS under the permission of Republic of Turkey Ministry of Food, Agriculture and Livestock, Ministry of Forestry and Water Management, and Ministry of Environment and Urbanization. We thank Ahmet Kideys and Arzu Karahan for their guidance for legal regularities.

References

Akçalı, B., Kaboğlu, G., and Güçlüsoy, H. 2019. A review on *Posidonia oceanica* (Linnaeus) Delile coverage along the Turkish coasts until 2019. Journal of the Black Sea/Mediterranean Environment, 25: 115–124.

Alberto, F., Correia, L., Arnaud-Haond, S., Billot, C., Duarte, C. M., and Serrão, E. 2003. New microsatellite markers for the endemic Mediterranean seagrass *Posidonia oceanica*. Molecular Ecology Notes, 3: 253–255.

Antao, T., Lopes, A., Lopes, R. J., Beja-Pereira, A., and Luikart, G. 2008. LOSITAN: a workbench to detect molecular adaptation based on a FST-outlier method. Bmc Bioinformatics [Electronic Resource], 9: 1–5.

Arias-Ortiz, A., Serrano, O., Masqué, P., Lavery, P. S., Mueller, U., Kendrick, G. A., Rozaimi, M. *et al.* 2018. A marine heatwave drives massive losses from the world's largest seagrass carbon stocks. Nature Climate Change, 8: 338–344.

Arnaud-Haond, S., Migliaccio, M., Diaz-Almela, E., Teixeira, S., van de Vliet, M. S., Alberto, F., Procaccini, G. *et al.* 2007. Vicariance patterns in the Mediterranean Sea: east-west cleavage and low dispersal in the endemic seagrass *Posidonia oceanica*. Journal of Biogeography, 34: 963–976.

Arranz, S. E., Avarre, J.-C., Balasundaram, C., Bouza, C., Calcaterra, N. B., Cezilly, F., Chen, S.-L. *et al.* 2013. Permanent genetic resources added to molecular ecology resources database 1 December 2012–31 January 2013. Molecular Ecology Resources, 13: 546–549.

Bailleul, D., Stoeckel, S., and Arnaud-Haond, S. 2016. RClone: a package to identify Multilocus Clonal Lineages and handle clonal data sets in r. Methods in Ecology and Evolution, 7: 966–970.

Bianchi, C. N., Morri, C., Chiantore, M., Montefalcone, M., Parravicini, V., and Rovere, A. 2012. Mediterranean Sea biodiversity between the legacy from the past and a future of change. InLife in the Mediterranean Sea: A Look at Habitat Changes, pp.1–15. Ed. byN. Stambler Nova Science Publishers, New York, NY.

Billingham, M. R., Reusch, T. B., Alberto, F., and Serrão, E. A. 2003. Is asexual reproduction more important at geographical limits? A genetic study of the seagrass *Zostera marina* in the Ria Formosa, Portugal. Marine Ecology Progress Series, 265: 77–83.

Buia, M. C., and Mazzella, L. 1991. Reproductive phenology of the Mediterranean seagrasses *Posidonia oceanica* (L.) Delile, *Cymodocea nodosa* (Ucria) Aschers., and *Zostera noltii* Hornem. Aquatic Botany, 40: 343–362.

Campagne, C. S., Salles, J.-M., Boissery, P., and Deter, J. 2015. The seagrass *Posidonia oceanica*: ecosystem services identification and economic evaluation of goods and benefits. Marine Pollution Bulletin, 97: 391–400.

Çelebi, B. 2007. A study on *Posidonia oceanica* (L.) delile, 1813 seagrass meadows in the levant sea. MS thesis, Middle East Technical University, Erdemli, Turkey.

Chefaoui, R. M., Duarte, C. M., and Serrão, E. A. 2017. Palaeoclimatic conditions in the Mediterranean explain genetic diversity of *Posidonia oceanica* seagrass meadows. Scientific Reports, 7: 1–8.

Dorken, M. E., and Eckert, C. G. 2001. Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). Journal of Ecology, 89: 339–350.

Earl, D. A., and von Holdt, B. M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4: 359–361.

Eckert, C. G. 2002. The loss of sex in clonal plants. InEcology and evolutionary biology of clonal plants, pp. 279–298. Ed. by H. Huber. Springer, Dordrecht.

Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14: 2611–2620.

Exadactylos, A., Vafidis, D., Tsigenopoulos, C. S., and Gkafas, G. A. 2019. High connectivity of the white seabream (*Diplodus sargus*, L. 1758) in the Aegean Sea, Eastern Mediterranean Basin. Animals, 9: 979.

Excoffier, L., and Lischer, H. E. L. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10: 564–567.

Foll, M., and Gaggiotti, O. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics, 180: 977–993.

Giantsis, I. A., Mucci, N., Randi, E., Abatzopoulos, T. J., and Apostolidis, A. P. 2014. Microsatellite variation of mussels (*Mytilus galloprovincialis*) in central and eastern Mediterranean: genetic panmixia in the Aegean and the Ionian Seas. Journal of the Marine Biological Association of the United Kingdom, 94: 797–809.

Jahnke, M., Casagrandi, R., Melià, P., Schiavina, M., Schultz, S. T., Zane, L., and Procaccini, G. 2017. Potential and realized connectivity of the seagrass *Posidonia oceanica* and their implication for conservation. Diversity and Distributions, 23: 1423–1434.

Jahnke, M., D'Esposito, D., Orrù, L., Lamontanara, A., Dattolo, E., Badalamenti, F., Mazzuca, S. *et al.* 2019. Adaptive responses along a depth and a latitudinal gradient in the endemic seagrass *Posidonia oceanica*. Heredity, 122: 233–243.

Jahnke, M., Moksnes, P. O., Olsen, J. L., Serra Serra, N., Nilsson Jacobi, M., Kuusemäe, K., Corell, H. *et al.* 2020. Integrating genetics, biophysical, and demographic insights identifies critical sites for seagrass conservation. Ecological Applications, 30: e02121.

Jahnke, M., Olsen, J. L., and Procaccini, G. 2015. A meta-analysis reveals a positive correlation between genetic diversity metrics and environmental status in the long-lived seagrass *Posidonia oceanica*. Molecular Ecology, 24: 2336–2348.

Jarvis, J. C., and Moore, K. A. 2015. Effects of seed source, sediment type, and burial depth on mixed-annual and perennial *Zostera marina* L. seed germination and seedling establishment. Estuaries and Coasts, 38: 964–978.

Johansson, J., Bolmgren, K., and Jonzén, N. 2013. Climate change and the optimal flowering time of annual plants in seasonal environments. Global Change Biology, 19: 197–207.

Jost, L. 2008. GST and its relatives do not measure differentiation. Molecular Ecology, 17: 4015–4026.

Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W., and Prodöhl, P. A. 2013. diveRsity: an R package for the estimation and exploration of population genetics parameters and their associated errors. Methods in Ecology and Evolution, 4: 782–788.

Kivelä, M., Arnaud-Haond, S., and Samarki, J. 2014. EDENetwork: ecological and evolutionary networks. Molecular Ecology Resources, http://becs.aalto.fi/edenetworks/. doi: 10.1111/1755-0998.12290.

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., and Mayrose, I. 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Molecular Ecology Resources, 15: 1179–1191.

Maglio, A., Pavan, G., Castellote, M., and Frey, S. 2015. Overview of the noise hotspots in the ACCOBAMS area. Final Report to the ACCOBAMS Secretariat.

Mari, L., Melià, P., Gatto, M., and Casagrandi, R. 2021. Identification of ecological hotspots for the seagrass *Posidonia oceanica* via metapopulation modeling. Frontiers in Marine Science, 8: 456.

Marín-Guirao, L., Entrambasaguas, L., Dattolo, E., Ruiz, J. M., and Procaccini, G. 2017. Molecular mechanisms behind the physiological resistance to intense transient warming in an iconic marine plant. Frontiers in Plant Science, 8: 1142.

Mateo-Ramírez, Á., Urra, J., Rueda, J., Marina, P., Bañares-España, E., and García Raso, E. 2016. *Posidonia oceanica* (L.) Delile in its westernmost biogeographical limit (northwestern Alboran Sea): meadows characterisation, phenology and flowering events. *In* Frontiers of Marine Science Conference Abstract: XIX Iberian Symposium on Marine Biology Studies. doi:10.3389/conf. FMARS.

Meinesz, A., Cirik, Ş., Akcali, B., Javel, F., Migliaccio, M., Thibaut, T., Yüksek, A. *et al.* 2009. *Posidonia oceanica* in the Marmara Sea. Aquatic Botany, 90: 18–22.

Mitarai, S., Siegel, D., Watson, J., Dong, C., and McWilliams, J. 2009. Quantifying connectivity in the coastal ocean with application to the Southern California Bight. Journal of Geophysical Research: Oceans, 114: C10026.

Nykjaer, L. 2009. Mediterranean Sea surface warming 1985–2006. Climate Research, 39: 11–17.

Olson, D. B., Kourafalou, V. H., Johns, W. E., Samuels, G., and Veneziani, M. 2007. Aegean surface circulation from a satellite-tracked drifter array. Journal of Physical Oceanography, 37: 1898–1917.

Paetkau, D., Slade, R., Burden, M., and Estoup, A. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Molecular ecology, 13: 55–65.

Pastor, F., Valiente, J. A., and Palau, J. L. 2019. Sea surface temperature in the Mediterranean: trends and spatial patterns (1982–2016). In Meteorology and climatology of the Mediterranean and Black Seas, pp. 297–309. Ed. by I. Vilibić, K. Horvath, and J. L. Palau. Pageoph Topical Volumes. Birkhäuser, Cham.

Pazzaglia, J., Nguyen, H. M., Santillán-Sarmiento, A., Ruocco, M., Dattolo, E., Marín-Guirao, L., and Procaccini, G. 2021. The genetic component of seagrass restoration: what we know and the way forwards. Water, 13: 829.

Peakall, R., and Smouse, P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. Bioinformatics, 28: 2537–2539.

Phillips, R. C., Grant, W. S., and McCroy, P. 1983. Reproductive strategies in eelgrass (*Zostera marina* L.). Aquatic Botany 16:1–20.

Piry, S., Alapetite, A., Cornuet, J. M., Paetkau, D., Baudouin, L., and Estoup, A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity, 95: 536–539.

Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945–959.

Procaccini, G., and Waycott, M. 1998. Brief communication. Microsatellite loci identified in the seagrass *Posidonia oceanica* (L.) Delile. Journal of Heredity, 89: 562–568.

Rannala, B., and Mountain, J. L. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences, USA, 94: 9197–9201.

Rossi, V., Ser-Giacomi, E., López, C., and Hernández-García, E. 2014. Hydrodynamic provinces and oceanic connectivity from a transport network help designing marine reserves. Geophysical Research Letters, 41: 2883–2891.

Rousset, F. 2008. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources, 8: 103–106.

Ruggiero, M. V., Turk, R., and Procaccini, G. 2002. Genetic identity and homozygosity in North-Adriatic populations of *Posidonia oceanica*: an ancient, post-glacial clone? Conservation Genetics, 3: 69–72.

Ruiz-Montoya, L., Lowe, R. J., and Kendrick, G. A. 2015. Contemporary connectivity is sustained by wind-and current-driven seed dispersal among seagrass meadows. Movement ecology, 3: 1–14.

Ruiz, J., Marín-Guirao, L., García-Muñoz, R., Ramos-Segura, A., Bernardeau-Esteller, J., Pérez, M., Sanmartí, N. *et al.* 2018. Experimental evidence of warming-induced flowering in the Mediterranean seagrass *Posidonia oceanica*. Marine Pollution Bulletin 134: 49–54.

Serra, I. A., Innocenti, A. M., Di Maida, G., Calvo, S., Migliaccio, M., Zambianchi, E., Pizzigalli, C. *et al.* 2010. Genetic structure in the Mediterranean seagrass *Posidonia oceanica*: disentangling past vicariance events from contemporary patterns of gene flow. Molecular Ecology, 19: 557–568.

Sinclair, E. A., Ruiz-Montoya, L., Krauss, S. L., Anthony, J. M., Hovey, R. K., Lowe, R. J., and Kendrick, G. A. 2018. Seeds in motion: genetic assignment and hydrodynamic models demonstrate concordant patterns of seagrass dispersal. Molecular Ecology, 27: 5019–5034.

Telesca, L., Belluscio, A., Criscoli, A., Ardizzone, G., Apostolaki, E. T., Fraschetti, S., Gristina, M. *et al*. 2015. Seagrass meadows (*Posidonia oceanica*) distribution and trajectories of change. Scientific Reports, 5: 12505.

Tomasello, A., Di Maida, G., Calvo, S., Pirrotta, M., Borra, M., and Procaccini, G. 2009. Seagrass meadows at the extreme of environmental tolerance: the case of *Posidonia oceanica* in a semi-enclosed coastal lagoon. Marine Ecology, 30: 288–300.

Wang, C., and Rosenberg, N. A. 2012. MicroDrop: a program for estimating and correcting for allelic dropout in nonreplicated microsatellite genotypes version 1.01. Available: https://web. stanford . edu/group/rosen/berglab/microdrop.html.

Wang, L., Xu, C., Wang, C., and Wang, Y. 2012. Characterization of a eukaryotic translation initiation factor 5A homolog from *Tamarix androssowii* involved in plant abiotic stress tolerance. BMC Plant Biology, 12: 1–17.

Handling Editor: W Stewart Grant