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ORIGINAL PAPER

Genetic evidence for multiple dispersal mechanisms in a marine direct developer, *Leptasterias sp*. (Echinodermata: Asteroidea)

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Abstract

Quantifying patterns of gene flow is critical for predicting the future resilience of local marine communities. Brooding species may be particularly susceptible to extirpation, as the lack of a pelagic larval phase may limit dispersal capacity and increase the risk of local extinction. Here, we investigate the population genetic structure of *Leptasterias sp*., a brooding sea star and important intertidal predator. Dispersal in this genus occurs primarily by crawling, but some have suggested that occasional "rafting" events can move individuals long distances. We used microsatellite markers to estimate genotypic variation across six collection sites of varying distances apart along the Oregon coast. We found evidence of strong population structure at the level of capes, and especially high divergence between two sites within Cape Blanco. Although cape-level genetic structure is broadly consistent with isolation-by-distance, additional mechanisms may be required to explain the elevated magnitude of genetic divergence on a finer scale. We propose that a known offshore water jet off Cape Blanco may act as phylogeographic barrier, hindering transport of rafting individuals from neighboring sites. This pattern suggests, indirectly, that rafting may be more important than previously appreciated in generating long-term genetic isolation between nearby sites. Our findings suggest that even for a sessile brooding species, connectivity and genetic structure can vary on a fine spatial scale and may be subject to environmental and oceanographic forces.

Keywords Population structure · Microsatellites · Connectivity · Isolation-by-distance · Migration rate

Introduction

In the face of increasing threats to coastal systems, understanding the connectivity of a species—the flow of genetic information between geographic locations—is crucial to predict the resilience of local populations. Under pressure from ocean acidification, warming, habitat destruction, disease, and more, the level of connectivity will strongly dictate whether populations must rely on localized alleles to adapt to their regimes (when connectivity is low) or whether they may be "rescued" by migrant alleles from already adapted populations.

Evolutionary theory suggests that the degree of connectivity is related to, and largely predictable from, a species' reproductive strategy (Bohonak 1999). Direct developers (brooders) are expected to display lower connectivity than species whose larvae disperse pelagically (broadcast spawners) (Cowen and Sponaugle 2009). Consequently, direct developers are expected to display higher genetic differentiation among geographic locations than broadcast-spawning species (Barbosa et al. 2012; Untersee and Pechenik 2007). Finally, theory predicts that the genetic connectivity of a brooding species should follow an isolation-by-distance pattern, wherein genetic divergence is expected to increase predictably with distance.

Empirical studies, however, have demonstrated that the relationship between reproductive strategy, connectivity, and genetic structure is not as simple as theory suggests. Many studies indeed report the predicted relationship between dispersal and population structure (Sunday et al. 2014; reviewed in Bohonak 1999). For example, comparisons of congeneric marine gastropods with and without a pelagic larvae phase report an inverse correlation between pelagic larval duration (PLD) and population differentiation (e.g., Kyle and Boulding 2000; reviewed in Marko and Hart 2018).

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In contrast, others have found unexpectedly high levels of genetic structure among species with a long PLD (Keever et al. 2009). Furthermore, across reproductive strategies, the spatial scale of population structure is highly variable (Conover et al. 2006). Genetic differentiation among marine invertebrates has been documented at mesoscales of tens of kilometers (e.g., Sokolova and Boulding 2004; Untersee and Pechenik 2007), site-specific scales of < 1 km (e.g., Kirby et al. 1994; Sotka et al. 2004; Struhsaker 1968), vertical scales within a water column (Serrano et al. 2014), and fine scales at the level of adjacent tide pools (Barbosa et al. 2013).

These findings suggest that the connectivity of marine populations, though significantly influenced by reproductive strategy, is also impacted by a complex interaction of other intrinsic and extrinsic factors. These factors may include larval behavior, historical and contemporary barriers to dispersal, habitat composition, and oceanographic processes (Cowen and Sponaugle 2009; Kamel et al. 2014). It is not sufficient, therefore, to form predictions about the magnitude, scale, or pattern of genetic connectivity based solely on a species' reproductive strategy. Instead, between-site genetic differentiation must be calculated to determine whether, and to what degree, a species conforms to theoretical predictions. Only then can predictions about the potential for resilience, collapse, adaptation, or migration be accurately made. Conversely, evidence for variable levels of connectivity along a species' range may reveal hidden barriers to dispersal and inform us about potential dispersal mechanisms.

Here, we test whether patterns of genetic connectivity in a brooding intertidal species vary across presumed oceanographic breaks. *Leptasterias sp*. is a six-rayed sea star and an important intertidal predator in the California Current Large Marine Ecosystem (CCLME) (Menge and Menge 1974; Gravem and Morgan 2017; Sullivan-Stack and Menge 2020). This genus is thought to contain at least five nominal species along the west coast of North America, between the Aleutian Islands, Alaska, and San Simeon, in central California, with some recent evidence suggesting the existence of cryptic species among them (Flowers and Foltz 2001; Foltz et al. 2008). In Oregon, one nominal species is currently recognized (*L. aequalis*) according to Foltz et al. (2008), who also conclude that closer phylogenetic examination of this taxon is warranted based on levels of DNA sequence variation detected in a few localities. To avoid conflict in an ongoing debate about fine-scale taxonomy, we refer to this species by its genus name only (Melroy et al. 2017). *Leptasterias* reproduce by direct development. A female *Leptasterias* broods over clutches of eggs for a period of several months (Chia 1966). The primary mode of movement for mature *Leptasterias* is crawling over rocky substrate (Chia 1966). Thus, the expected dispersal capacity for both adult and larval *Leptasterias* is low, leading to the prediction that between-site genetic differentiation will be high and linearly correlated with geographic distance. On the other hand, a few studies propose an additional mechanism for *Leptasterias* dispersal: "rafting." *Leptasterias* have been found clinging to algae (washed-up kelp sporophytes, rockweed, sargassum, etc.) in the intertidal zone (Highsmith 1985; Parker and Tunnicliffe 1994). Studies document high rates of movement of these algal masses, which frequently become dislodged from rock during harsh wave conditions, circulate offshore, and wash up many kilometers away (Reimer et al. 2018). Despite these published observations of rafting *Leptasterias*, the rate of migration via this mechanism is thought to be low, but this has not been examined in this species. Such rafting would facilitate higher rates of between-site exchange—and hence, weaker population structure—in *Leptasterias* than would otherwise be predicted for a species with direct development and low adult mobility. In this case, the dispersal of *Leptasterias* could be influenced by phylogeographic barriers (headlands, bays, sandy beaches) and oceanographic patterns (currents, eddies), causing deviation from linear isolation-by-distance patterns on a local scale.

Only recently have a few studies indicated that both hypotheses—generally low connectivity, with some suggestion of oceanographic dispersal—may be warranted in the case of *Leptasterias.* Melroy et al. (2017) described cryptic clades of *Leptasterias* endemic to San Francisco Bay, consistent with expectations of strong genetic differentiation on a fine spatial scale. At the same time, however, the magnitude of genetic differentiation was not consistent across geographic areas, nor was it predictable based solely on the distance between populations. Populations sampled in Marin and San Francisco counties showed a higher degree of genetic differentiation (based on pairwise F_{ST} values) than populations to the north and south. The researchers suggest that Point Reyes and the San Francisco estuary may act as phylogeographic barriers to dispersal, and that "rafting" plays a small but meaningful role in population structure. A separate analysis by Melroy and Cohen (2021) provided similar support, but they still argue that rafting by *Leptasterias* is very infrequent.

In this paper, we used nine microsatellite markers to quantify levels of genetic differentiation among, and migration rates between, populations of *Leptasterias* from six sites across three main Oregon capes (Foulweather, Perpetua, and Blanco). To complement our genetic analysis, we investigated a previously described oceanographic "break" off Cape Blanco. This is based on strong coastal jet documented by Barth et al. (2000), which we hypothesize might act as a barrier to gene flow between two sites within the Cape Blanco region (Cape Blanco proper = CB and Rocky Point = RP) if pelagic rafting is a common means of dispersal in this species. We conducted preliminary analysis of

seasonal current patterns (direction and magnitude) to determine if oceanographic dynamics appear different at Cape Blanco than the other capes, which would justify further oceanographic study on a larger timescale.

Materials and methods

Sample collection and processing

Sea stars were collected from three major capes (Blanco, Foulweather, and Perpetua) representing a wide geographic range of the Oregon coast (Table 1, Fig. 1). Within each Cape, two study sites were chosen based on accessibility, demonstrated abundance of *Leptasterias* (e.g., Sullivan-Stack and Menge 2020), and availability of long-term ecological and environmental monitoring data. This selection process led to a sampling of sites that are differing distances apart. The sites within Cape Foulweather (BB and FC) are 1 km apart, separated by rocky substrata. The sites within Cape Perpetua (SH and YB) and within Cape Blanco (CB and RP) are 7 km and 23 km apart, respectively, and separated mostly by sand with patches of rocky substrata (Fig. 1).

Specimens were collected during twelve low tides from April to May 2019. A minimum of 60 adult *Leptasterias* per site ($n = 384$ total) were opportunistically sampled. All specimens were gathered in the mid- to low-tide zone, on rocky substrate, and tended to be found in crevices. Of note, many (though not all) individuals were collected near

Table 1 Sampling locations of *Leptasterias* specimens

Site	Lat/long	Sample size
Cape Foulweather		
Fogarty Creek (FC)	44° 50′ 20.5224″ N 124° 3′ 14.3856″ W	64
Boiler Bay (BB)	44° 50′ 0.5136″ N 124° 3′ 35.4744″ W	63
Cape Perpetua		
Yachats Beach (YB)	44° 14′ 59.4384″ N 124° 6′ 50.0472″ W	56
Strawberry Hill (SH)	44° 18′ 56.0844″ N 124° 6′ 33.0084″ W	68
Cape Blanco		
Cape Blanco (CB)	42° 50′ 23.028″ N 124° 33′ 55.6092″ W	57
Rocky Point (RP)	42° 40′ 39.3456″ N 124° 27′ 6.1452″ W	57

Sites are abbreviated with initials; capes are written as full names throughout. CB is an individual site within the cape (Blanco) of the same name. Sample size shows the number of individuals successfully genotyped

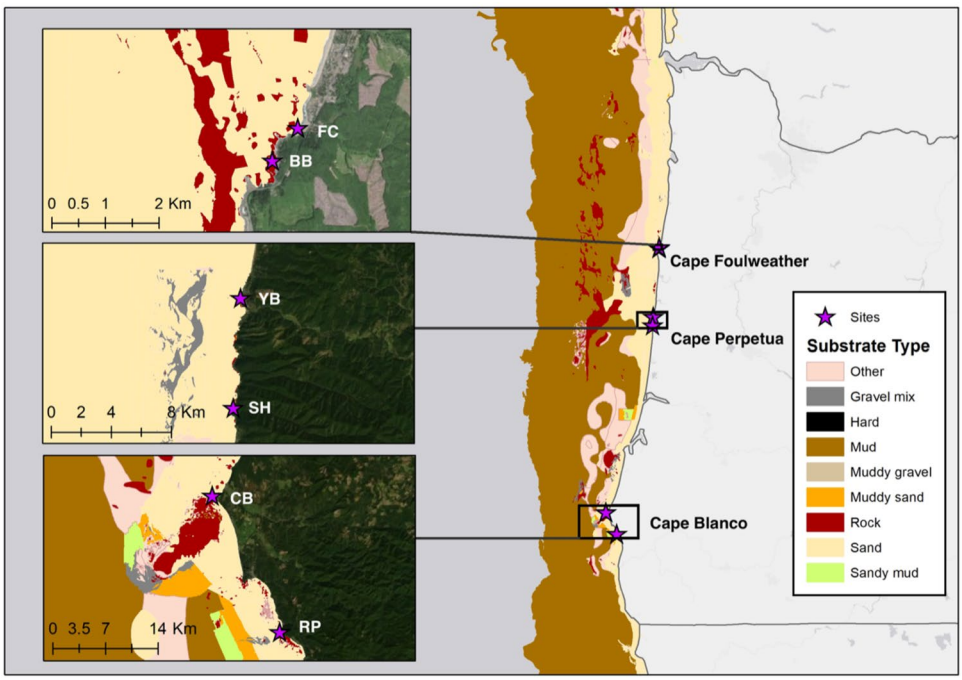

Fig. 1 Map of study locations, showing habitat composition within and between sites. Map data obtained from OSU Active Tectonics Laboratory, Version 4.0 U.S. West Coast Habitat Map, layers V4 Lith1 and Lith2 (http://activetectonics.coas.oregonstate.edu/data.htm#2)

artificial features (wire cages and metal plates) installed for other ecological monitoring studies. A ~ 5 mm arm clip was excised from each adult and stored in 95% ethanol, and individuals were released.

Microsatellite genotyping

Genomic DNA was isolated from tube feet tissue using the E.Z.N.A. Tissue DNA Kit (Omega Bio-tek, Norcross, GA), checked for quality on a 1.5% agarose gel, and quantified using a Qubit Fluorometer (Thermofisher, Waltham, MA). DNA concentrations were in the range of 11–57 ng/µl, and the DNA samples were diluted 1:10 with molecular grade water for PCR and stored in a – 20 °C freezer. Ten microsatellite loci developed by Barreto and Bauer (2019) were selected based on high allelic variation and previously demonstrated Hardy–Weinberg and linkage equilibrium (Table S1, S2). An M13 tail (5′-CAC GAC GTT GTA AAA CGA C-3′) was added to the 5′ end of each locus-specific forward primer. All primer pairs and M13 were synthesized by Integrated DNA Technologies. Additionally, the M13 primer was labeled with one of four different fluorescent dyes (Applied Biosystems) to allow pooling of PCR products for electrophoresis. PCR was performed in 12.5 µl reactions using $MgCl_2$ concentrations specific to each locus (Table S1), 1 µl of diluted genomic DNA, 1X PCR buffer, 0.25 mM dNTP, 0.1 units AmpliTaq DNA Polymerase (Applied Biosystems), 0.05 µM of the M13-tailed locus-specific forward primer, 0.5 µM of locus-specific reverse primer, and 0.5 µM of labeled M13 primer. Each PCR cycle consisted of initial denaturation at 95 ºC for 2 min, 35 cycles of 95 ºC for 15 s, locus-specific annealing temperatures (Table S1) for 30 s, and 72 ºC for 30 s, with a final extension step at 72 ºC for 15 min. From each batch of reactions, 2–4 PCR products were haphazardly checked on a 2% agarose gel to confirm successful reaction.

In preparation for capillary electrophoresis, PCR products were pooled in the following ratios: 4:2:4:8:1 µl for loci Lepta27, 28, 40, 42, and 47 respectively, followed by dilution with 61 µl of deionized water, and 3:3:1.5:3:1 µl for loci Lepta10, 11, 14, 48, and 49, followed by dilution with 48.5 µl water. Dilution factors were determined by Barreto and Bauer (2019) to result in peaks with 300–10,000 relative fluorescent units. Samples were genotyped via capillary electrophoresis in an ABI 3730 DNA Analyzer. Electropherograms were visualized and allele peaks scored using Geneious Prime v.2019.2.3. Only adults successfully genotyped at 5 or more loci were retained for analysis ($n = 365$).

Analysis of microsatellite data

GenePop (Rousset 2008) was used to estimate heterozygosity and test for Hardy–Weinberg and linkage equilibrium. A False Discovery Rate (FDR) adjustment was applied to resulting *P*-values to account for multiple testing. To examine whether deviations from HWE are due to population genetic factors (e.g., inbreeding) or due to null alleles or technical errors (Marshall et al. 1998), the program FreeNA (Chapuis and Estoup 2007) was used to estimate null allele frequencies, and MicroChecker (Van Oosterhout et al. 2004) was used to test for the occurrence of large allele dropout and scoring errors.

Pairwise tests of genetic differentiation were conducted using GenoDive (Meirmans and Van Tienderen 2004). In addition to traditional F_{ST} (Weir and Cockerham 1984), we also calculated $G"_{ST}$, which corrects for the high allelic diversity of microsatellite markers following Meirmans and Hedrick (2011). To test the significance of an isolation-by-distance pattern, a Mantel test was performed using GenoDive. Between-site geographic distance was log-transformed and compared to transformed genetic distance ($G"_{ST}/1\text{-}G"_{ST}$) to produce a regression line and site-pair residuals (Rousset 1997). Finally, the inbreeding coefficient G_{IS} (corrected F_{IS} for microsatellites) was calculated for each site using GenoDive.

Population structure was assessed using STRUCTURE v.2.3.4 (Pritchard et al. 2000), a model-based clustering algorithm that estimates population structure and assigns individuals to ancestral population groups to minimize within-group Hardy–Weinberg and linkage violations. Correlated allele frequencies and population admixture were assumed, and LOCPRIOR was used to account for sampling location (Putnam and Carbone 2014). We tested the number of populations by varying the values of K from 1 to 6, and these were simulated with 10^6 Markov chain Monte Carlo (MCMC) repetitions following 10^3 burn-in iterations. Q-matrix outputs from STRUCTURE were visualized using the "strataG" package (https://CRAN.R-project.org/package=strataG) in R.

To quantify long-term migration rates among locations, we used MIGRATE v4.4.4 (Beerli and Felsenstein 2001). MIGRATE uses a Bayesian approach to estimate the likelihood of demographic models varying in direction of gene flow, and it can estimate mutation-scaled migration rates (M) and mutation-scaled population sizes (θ). Our aim with this analysis was to examine how migration rates between adjacent sites within Cape Blanco (RP and CB) compared to migration rates between other capes, since Cape Blanco is the location of the oceanographic break described by Barth et al. (2000). First, we tested which directionality of gene flow is most prominent along the Oregon coast by comparing three models: northward among capes, southward among capes, or bidirectional among capes. For this MIGRATE run, the pair of sites within each cape were coded as being one population, and 30 random individuals per site were subsampled to reduce computational burden. Run settings

were tested for convergence starting from different random seeds, and settings for the final run included three replicate long chains, 25,000 burn-in samples, and with 25,000,000 samples visited and recorded at intervals of 100. Uniform priors were used for M and θ, and upper bounds for these parameters were set to 100. All runs employed a Brownian motion model that closely approximates a stepwise mutation model, while being more computationally efficient. After these three runs, models were compared using log-Bayes factors (difference between pairs of marginal log-likelihoods) in which the model with highest log-likelihood was set to zero and the likelihood of each subsequent model was subtracted from the first (Beerli and Palczewski 2010).

After determining that the model of northward gene flow was the most likely (see *Results*), we performed another MIGRATE run in which Cape Foulweather and Cape Perpetua were each coded as a population, and each site within Cape Blanco (CB and RP) were coded as a population (for a total of four populations). Migration was then only allowed to be northward between adjacent populations, and M and θ were estimated between them after a run with the following settings: 5 replicate long chains, 100,000 burn-in samples, and 10,000,000 samples visited, with one recorded every 100 samples. Priors and upper bounds for parameter estimates were the same as above. In this run, 50 individuals were randomly sampled per geographic site.

Oceanographic data

To complement our genetic analysis, we utilized data from a long-term oceanographic monitoring program to perform a preliminary analysis of the differences, if any, in current patterns off Cape Blanco versus Capes Foulweather and Perpetua. Measurements of surface current velocity (m/s) were obtained from the Mapping Oregon Coastal Oceans Current project (Kosro 2004–2020; see also https://hfradar.ioos.us/). This long-term project uses a network of shore-based, high-frequency radars (HFR) to measure current direction and strength and produce daily vector maps. For more detailed information on this method, see Paduan and Washburn (2013).

A subset of these pre-existing data (2018–19) was then selected and filtered to include only those measurements closest to the study sites in latitude, and closest to shore in longitude (see Figure S1 for a map of the HFR locations relative to our field sites). The two sites within Cape Foulweather were treated as a single location, as were the two sites within Cape Perpetua, while the two sites within Cape Blanco (RP and CB) were sufficiently distant to be captured by two different radars and treated as separate locations. These data were used to construct time series plots in R showing the direction and magnitude of North/South (N/S, ±v) and East/West (E/W, ±u) vector components for each location. Because oceanographic patterns fluctuate in predictable semi-sinusoidal patterns based on seasonality (Strub et al. 1987), we split our analysis into summer (days 105–295 of 2018) and winter (days 296–365 of 2018 and 1–104 of 2019), following Barth et al. (2007). To compare the overall magnitude and direction of current flow at each location, we calculated cumulative seasonal water displacements (in km) by summing the daily displacements (velocity x time) over all of the months in summer, and all of the months in winter. We then created four plots of cumulative displacement (E/W summer and winter, and N/S summer and winter) to compare patterns of water transport between each location, and to see whether such patterns qualitatively correspond to our observed genetic trends.

Results

Genotypic diversity

Tests of linkage disequilibrium revealed no significant linkage among any pairs of loci after FDR correction (all $P > 0.20$). Tests of HWE for each locus in each population revealed only two locus-population pairs (out of 60) with potential HWE deviations: Lepta10 within CB ($P < 0.0001$), and Lepta48 within RP ($P < 0.0001$) (Table S2). Analysis by FreeNA and MicroChecker found relatively low frequencies of null alleles for Lepta10 across all populations (range: 0.00–0.08, Table S2). This suggests that deviation from HWE at this locus is likely caused by a biological mechanism (e.g., inbreeding) rather than null alleles. Therefore, Lepta10 was retained for analysis. In contrast, the frequency of null alleles at Lepta48 was consistently higher across populations (range: 0.012–0.21; mean: 0.098). We therefore excluded Lepta48 from further analyses. No other technical errors (large allele dropout or scoring errors) were detected by MicroChecker at any locus. Every population had private alleles in at least one locus, and Lepta28 and Lepta48 were the only loci in which no private alleles were found (Table S3). Levels of inbreeding (G_{IS}) were low in Capes Foulweather and Perpetua (range: 0.000–0.045) but increased threefold at Cape Blanco sites (0.124 and 0.126) (Table S4).

Population genetic structure

STRUCTURE analysis revealed strong genetic structuring between *Leptasterias* populations at the level of capes (Foulweather vs. Perpetua vs. Blanco), with further structuring at the level of some individual sites (RP and CB) (Fig. 2). When the model assumed $K = 2$ ancestral populations, specimens from Cape Blanco cluster separately from those at Capes Foulweather and Perpetua. At $K = 3$, individuals

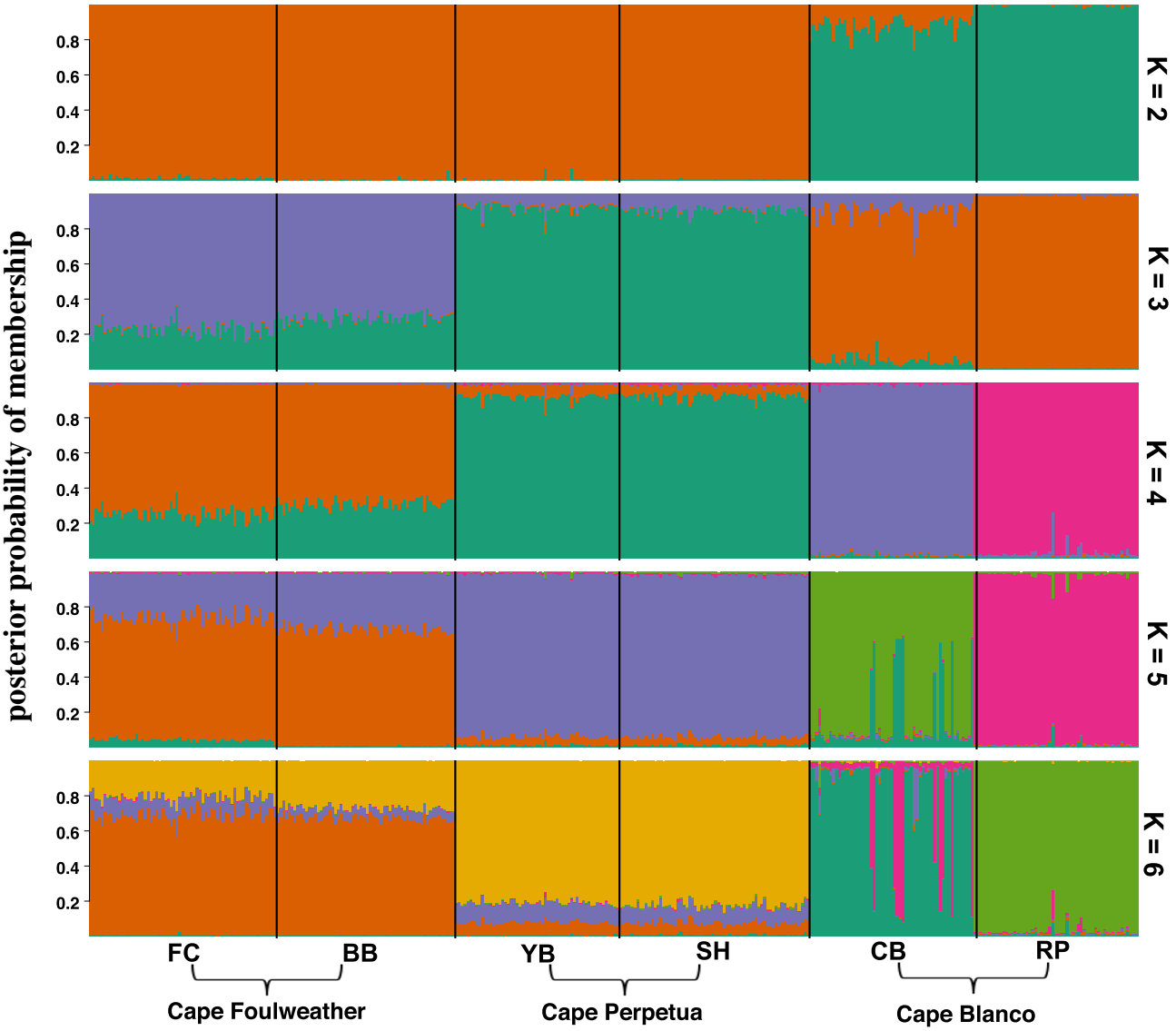

Fig. 2 *Leptasterias* population structure across six sites, arranged left-to-right from North to South. Bars show the average probability of membership of each individual in a population cluster from STRUCTURE runs assuming $K = 2$–6 ancestral populations. Site abbreviations are defined in Table 1

cluster into three distinct populations corresponding to each of the three main capes, with Capes Foulweather and Perpetua sharing more similarities than either site within Cape Blanco. At $K = 4$, the two sites within Cape Blanco, RP and CB, separate into additional distinct populations. This latter pattern is maintained as K is increased to 5 and 6.

Quantitative analysis of genetic divergence (F_{ST} and $G''_{ST;}$ Table 2, Table S5) supports the same pattern as STRUCTURE analysis. Pairs of sites within Cape Foulweather (FC and BB) and Cape Perpetua (SH and YB) display low and non-significant G''_{ST} values (0 and 0.005, respectively), consistent with the finding that these sites do not separate into distinct populations even when $K > 4$ in STRUCTURE. Sites between capes were highly differentiated (G''_{ST} range: 0.111–0.544). All but the two lowest F_{ST}/G''_{ST} estimates were highly significant ($P < 0.001$). Notably, sites within Cape Blanco (RP and CB) are highly differentiated, with a G''_{ST} value (0.47) higher than any value within or between Capes Foulweather and Perpetua. A Mantel test comparing geographic distance (log km) to genetic divergence ($G''_{ST}/1\text{-}G''_{ST}$) supports a statistically significant pattern of isolation-by-distance (Mantel's $r = 0.695$, $P = 0.005$). However, given the high Mantel regression residual for RP-CB (Table S6) and the STRUCTURE patterns, we used MIGRATE to investigate finer scale differences in rates of migration.

Our comparison of MIGRATE models of gene flow among the three Oregon capes suggested that a long-term northward exchange most likely explains genotypic

Table 2 Pairwise F_{ST} and G''_{ST} values for *Leptasterias* populations

	FC	BB	YB	SH	CB	RP
FC	–	0 ^{ns}	0.037	0.047	0.122	0.146
BB	0 ^{ns}	–	0.032	0.042	0.109	0.139
YB	0.131	0.111	–	0.005 ^{ns}	0.115	0.163
SH	0.161	0.142	0.005 ^{ns}	–	0.122	0.173
CB	0.443	0.442	0.45	0.462	–	0.131
RP	0.475	0.451	0.544	0.522	0.47	–

F_{ST} values displayed above the diagonal, and G''_{ST} values shown below the diagonal. All values highly significant ($P < 0.001$) except those marked 'ns'

Table 3 Comparison of gene flow models among *Lepasterias* populations from Oregon capes

Model	Log-probability (marginal likelihood)	Log-Bayes factors	Model rank
Northward	− 43,321.51	0	1
Southward	− 43,622.32	− 300.81	2
Bidirectional	− 71,588.12	− 28,266.61	3

Migrate v4.4.4 was used to models varying in the direction of gene flow among Cape Foulweather, Cape Perpetua, and Cape Blanco

distribution (Table 3). Allowing only northward gene flow, we ran another MIGRATE model to quantify migration rates among capes and between nearby sites of Cape Blanco. This model estimated that migration between RP and CB within Cape Blanco (0.35 migrants per generation) is as low as that between CB and Cape Perpetua (0.30), despite being ~ 7.5 × closer geographically (Table 4). In other words, migration rates between CB and RP are lower than would be expected based on geographic distance alone. In addition, migration between the two northern capes was an order of magnitude higher (4.57 migrants per generation) than that within Cape Blanco, while distances between these pairs of populations differed by ~ 2.5x (Table 4).

Oceanographic current patterns

Analysis of shore-based high-frequency radar data revealed differing patterns of E/W and N/S cumulative seasonal displacement by surface currents at Cape Blanco versus the other two study sites (Fig. 3). In both summer (15 April 22 October 2018) and winter, the N/S current patterns of Cape Foulweather and Perpetua track together (Fig. 3a and e), while Cape Blanco follows a separate trajectory (greater southward displacement than the other capes in summer, and less northward displacement in winter). All sites have roughly similar direction and magnitude of E/W displacement in summer, primarily westward (offshore) (Fig. 3c). However, in winter, offshore displacement off Cape Blanco is consistently stronger than the other two capes, and falls off sharply from February to April, reaching a maximum of 1,000 km cumulative westward displacement in April (Fig. 3g). Comparison between the radars at CB and RP revealed that these sites roughly track one another in terms of N/S and E/W displacement across the seasons. However, CB shows a slightly higher magnitude of westward (offshore) flow in both summer (Fig. 3d) and winter (Fig. 3h).

Discussion

In this study, we found that *Leptasterias* in Oregon exhibit strong population structure at the level of capes (Foulweather vs. Perpetua vs. Blanco), and further structure between two sites (CB and RP) within Cape Blanco. Genetic structure is

Table 4 Parameter estimates for mutation-scaled population sizes (θ), mutation-scaled migration rates (M) and number of migrants per generation ($Nm = \theta M/4$) between source and receiving populations

Source population	Receiving population	Geographic distance (km)	Parameter (95% C.I.)		
			θ	M	Nm
Cape Perpetua	Cape Foulweather	58	0.90 (0.0–2.2)	20.30 (13.4–27.6)	4.57
CB	Cape Perpetua	172	0.91 (0.0–2.1)	1.32 (0.0–2.4)	0.30
RP	CB	23	1.10 (0.0–2.4)	1.30 (0.0–2.6)	0.35

Estimates were obtained using the best-supported model of northward gene flow between adjacent populations. Populations included two capes (Cape Perpetua, Cape Foulweather) and two sites within Cape Blanco *CB* Cape Blanco, *RP* Rocky Point

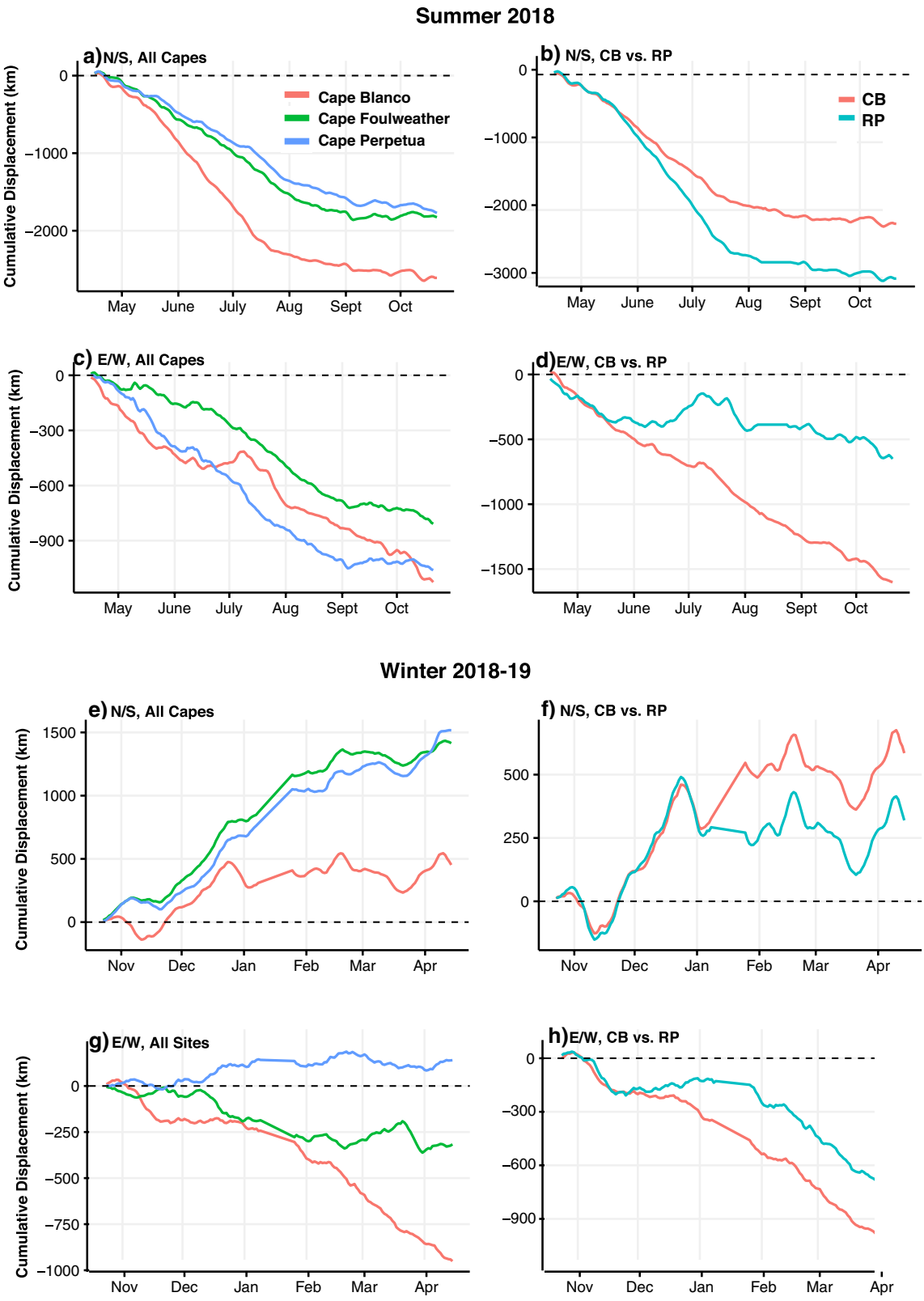

Fig. 3 Cumulative displacement, calculated from shore-based high-frequency radar velocity measurements for summer (days 105–295 of 2018, panels **a**–**d**) and winter (days 296–365 of 2018 and 1–104 of 2019, panels **e**–**h**). Positive values indicate northward and eastward (retentive) flow; negative values indicate southward and westward (offshore) flow

broadly consistent with an isolation-by-distance pattern, as expected for a brooding species that migrates primarily by crawling. However, unexpectedly high genetic divergence between CP and RP two adjacent sites within Cape Blanco, separated by only 23 km suggests that additional oceanographic dispersal methods may be relevant to explain finer scale patterns of genetic structure.

Genetic structure

Both STRUCTURE and genetic divergence analyses (F_{ST} and G''_{ST}) revealed that *Leptasterias* populations exhibit strong genetic structure on two spatial scales. First, assuming three ancestral populations, individuals sort into genetic clusters consistent with the geographic divisions between Capes Foulweather, Perpetua, and Blanco. When four ancestral populations are assumed, Foulweather and Perpetua remain distinct cape-level populations, while individuals within Cape Blanco separate out at the site level. CB forms a separate cluster from RP, despite the sites' adjacent position within the cape. This pattern of four distinct clusters (Cape Foulweather, Cape Perpetua, CB site, and RP site) remains strong even when more potential ancestral populations are tested (Fig. 2). Values beyond $K = 4$ do not increase the clarity of structural patterns nor reveal genetic separation of site-pairs within Cape Perpetua or Cape Foulweather. This structural finding suggests high rates of gene flow within Capes Foulweather and Perpetua (between FC and BB, and between YB and SH, respectively), but low gene flow between the three capes and between sites with Cape Blanco.

The simplest explanation for this observed pattern—strong between-cape genetic structure, with site-level genetic structure at only one out of three capes—is isolation-by-distance. Theory predicts that individuals from neighboring locations should share more alleles than individuals from distant regions; genetic divergence should increase as a function of geographic distance (Wright 1943; reviewed in Sanford and Kelly 2011). Indeed, our $K = 4$ STRUCTURE result supports this trend. As expected, the first population break occurs between the most geographically distinct regions—the three capes—with the next separation occurring between CB and RP, the most geographically distinct sites within any single cape (Fig. 1). These sites are separated by 23 km, in contrast to 7 km distance between SH and YB, and 1 km between BB and RP. A Mantel test confirms a statistically significant isolation-by-distance trend.

Despite this overall pattern of isolation-by-distance, our quantitative analysis of genetic divergence (G''_{ST} values) revealed unexpectedly high divergence between CB and RP, which alerted us that additional explanations may be needed to account for fine-scale structure. Based on G''_{ST} values, RP and CB are not only more genetically distinct from one another other than any other site-pair, but these sites are also more divergent than the two northern capes compared to one another (e.g., BB to YB). The G''_{ST} value between CB and RP (0.47) is roughly equal to the divergence between Capes Perpetua and Blanco (0.462; SH to CB) (Table 2). These findings are striking considering that the geographic distance between RP and CB (23 km) is less than half the distance between Capes Foulweather and Perpetua (54 km), and eight times less than the distance between Capes Perpetua and Blanco (157 km). If differentiation followed a strict pattern of isolation-by-distance alone, the G''_{ST} value between RP and CB should be less than—not more than or equal to—the G''_{ST} values between Capes Foulweather and Perpetua, and between Capes Perpetua and Blanco.

Having observed that CB and RP may be more genetically distinct than distance alone can account for, we used MIGRATE to quantify whether rates of gene flow differ significantly across *Leptasterias*'s range. Our results support the finding that connectivity is quantitatively lower between CB and RP than between any other site-pairs or capes. Migration rates between Capes Foulweather and Perpetua were an order of magnitude higher (4.57 migrants per generation) than within Cape Blanco, while distances between these pairs of populations differed by only ~ 2.5x. Migration rates between RP and CB ($M = 1.32$, $Nm = 0.30$) are comparable to migration rates between Capes Perpetua and Blanco ($M = 1.30$, $Nm = 0.35$), despite the fact that CB and RP are 134 km closer to one another than Capes Perpetua and Blanco. In other words, the same number of migrants per generation cover a distance 7.5 × greater between Capes Perpetua and Blanco than between CB and RP.

This finding supports the hypothesis that onshore crawling may not be the only mechanism of dispersal for *Leptasterias*. The substrate composition between Capes Perpetua and Blanco, and between CB and RP, is roughly equal—a mixture of rock and sand (Goldfinger 2018). If *Leptasterias* disperse solely by crawling, then rates of migration should approximately scale with distance and remain constant over these two stretches of coastline. Instead, the variability of migration rates and genetic diversity over the span of *Leptasterias*'s Oregon range suggests another potential dispersal mechanism: "rafting" on mobile substrata such as kelp, rockweed, and sargassum. Rafting is common among marine species, especially sessile species from intertidal habitats (reviewed in Grantham et al. 2003). Studies document high rates of dispersal of these algal masses (Highsmith 1985; Reimer et al. 2018). Melroy et al. (2017) report genetic patterns consistent with oceanographic transport of *Leptasterias* in the San Francisco Bay area. This rafting mechanism may dramatically increase the dispersal potential of direct-developing organisms who are otherwise confined by lack of pelagic larval transport (Martel and Chia 1991), and it may account for differences in the rates of migration across different regions of *Leptasterias*'s range.

Oceanographic patterns: enhancing the story

If rafting is a mechanism of *Leptasterias* dispersal, then local oceanographic patterns should be roughly consistent with patterns of migration and genetic structure. Using data from a shore-based high-frequency radar system, we asked, do oceanographic current regimes differ markedly between Cape Blanco and the other capes? If so, then can these differing ocean dynamics help account for the much lower rates of migration between CB and RP than between other sites?

Overall, our preliminary analysis suggests a qualitative difference in oceanographic regimes off Cape Blanco vs. Capes Foulweather and Perpetua. All year, the N/S current patterns of Cape Foulweather and Perpetua follow roughly the same pattern in direction and magnitude (Fig. 3a, e). Cape Blanco follows a separate trajectory—greater southward displacement in summer, and less northward displacement in winter—suggesting that a different set of oceanographic conditions may be present at this southernmost cape. Most notably, in winter, offshore displacement off Cape Blanco is consistently stronger than the other two capes. It turns sharply westward from February to April, reaching a maximum of 1,000 km cumulative offshore displacement in April. This finding is consistent with a previously noted oceanographic feature documented by Barth et al. (2000). Just south of Cape Blanco, an interaction between surface equatorward upwelling and a deep poleward undercurrent causes a strong separating jet to veer offshore. We propose that this separating jet may act as a "phylogeographic barrier" between CB and RP. Whereas sea stars on the northern two capes can overcome comparatively lower magnitudes of offshore transport in order to wash up between sites, the 1,000 km westward displacement created by the separating jet at Cape Blanco may pull specimens offshore, preventing survival of individuals and hence lowering gene flow between CB and RP.

This hypothesis of a "phylogenetic break" influenced by California Current dynamics is consistent with the conclusions drawn about *Leptasterias* structure by Melroy et al. (2017). However, our hypothesis comes with two important qualifications. First, our oceanographic analysis is preliminary, intended to visualize the dynamics described in Barth et al. (2000) and extend their analysis northward to the other two capes of our study. Future studies should extend this analysis over a greater timescale (we surveyed only 2018–19) and examine whether currents have been constant relative to the timescale of *Leptasterias* divergence. Second, it should be noted that for a species with direct development, oceanographic transport is likely far less influential for genetic structure than simpler explanations such as isolation-by-distance, onshore adult dispersal (over multiple generations), or historical vicariance. However, the "one-migrant-per-generation" rule posits that even a single individual per generation may contribute to the homogenizing of populations and exert a large influence on genetic structure (Wright 1931). Future studies should attempt to document and quantify instances of *Leptasterias* observed in the act of rafting to determine the rate at which this dispersal mechanism does or does not occur.

Finally, our findings situate *Leptasterias* within a growing body of literature about the resilience of local marine communities. In the face of climate-related threats to coastal environments—disease, rising temperature, acidification, habitat destruction, and more—can we expect that Oregon *Leptasterias* populations will migrate, adapt, or collapse? On one hand, brooding behavior and lack of a pelagic dispersal phase may hinder *Leptasterias* resilience by (a) preventing migration to new areas in the face of local stressors, and/or (b) preventing the exchange of fitness-enhancing adaptations between populations (Bernhardt and Leslie 2013). On the other hand, our finding of cape-level genetic structure with some finer scale genetic divergence suggests that *Leptasterias* may be prime candidates for local adaptation; their genetic divergence may occur on a smaller spatial scale than important selective gradients (Sanford and Kelly 2011). For example, Chan et al. (2017) showed that sites in northern Oregon (FC and BB) have historically been "hotspots" of low pH. *Leptasterias* from these sites may already have been exposed to, and developed genetic defenses against, the climate extremes expected to affect the larger coastline in the future. If such defense has a genetic basis, then understanding the rate and direction of gene flow between site- and cape-level populations is crucial to predict the possible spread of adaptive traits and survival rates across a wide region. Although our study of microsatellites did not identify or quantify markers of local adaptation, it sets the stage for future explorations of "pheno-geography": the distribution of functional traits among geographic regions.

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Author contributions ELB, FSB, and BAM designed the research. ELB carried out field sampling and performed lab work with the assistance of FSB. ELB and FSB analyzed the data. ELB, FSB, and BAM wrote the manuscript.

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Data availability Microsatellite genotypes are available in the supplementary information. Oceanographic current vector data and sediment map data are cited as [datasets] in bibliography.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

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