



# Diversity, divergence and density: How habitat and hybrid zone dynamics maintain a genomic cline in an intertidal barnacle

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## Funding information

National Science Foundation, Grant/Award Number: OCE-1924599; UGA Department of Genetics

Handling Editor: Giacomo Bernardi

## Abstract

**Aim:** As within-species genomic data have been shown useful in interpreting broader biogeographic trends, we analysed the mode of population genomic isolation involved in a well-studied intertidal genomic cline to better understand the mechanisms maintaining it. These results were interpreted in the context of spatial variation in habitat use and availability as well as likely fitness consequences for hybridization between the two lineages.

**Location:** Pacific coast of North America.

**Taxon:** Arthropods (Class Maxillopoda, Order Sessilia, Family Balanidae; *Balanus glandula*).

**Methods:** Genotype-by-sequencing approaches were used to generate single-nucleotide polymorphism markers across sites sampled between southern Alaska and Southern California. Inference using standard population genomic methods, including analysis of population structure, inbreeding and linkage disequilibrium, was used to identify the steepest transitions across the largest number of loci examined. These data were put in the context of observed population density and habitat availability.

**Results:** We show that the majority of markers analysed show strong clinal transitions in a very narrow portion of the California coast. Patterns of linkage disequilibrium among markers, along with prior evidence of variation in reproductive potential by latitude and by mitochondrial lineage, suggest some reproductive isolation among the northern and southern lineages of *B. glandula* that are concordant with the drop in population density and habitat availability in central California.

**Main Conclusions:** A significant clinal transition in genomic diversity is stronger and more localized than previously recognized and exhibits statistical patterns suggesting that the lineages are reproductively and phenotypically distinct in ways that may be ecologically important. As this species has been used to infer process in coastal biogeography, further study of concordant patterns will be important for advancing our understanding of this region.

## KEY WORDS

*Balanus glandula*, barnacles, biological oceanography, California, demography, genomic clines, habitat, Pacific coast, seascapes genetics

Authors are presented in reverse alphabetical order to represent shared effort on this paper as well as the strength of their respective rock-scissors-paper prowess.



## 1 | INTRODUCTION

Landscape genomics, and the deeper-time efforts of 'phylogeography', provide a complementary perspective on biodiversity within communities and ecosystems relative to approaches based on species biogeography (Avise, 2000; Wares et al., 2001). In many cases, evolutionary differences among distinct populations of organisms are comparable to divergence found among morphologically distinct species (Hickerson et al., 2006; Pfenninger & Schwenk, 2007; Wares, 2020). Spatially isolated populations may carry functional or physiological distinctions that are reflective of local selection pressures (e.g., Kelly et al., 2012; Sotka et al., 2003) and that yield ecological consequences. Such signs of local adaptation may be more common across species ranges than once expected (Sanford et al., 2003; Sanford & Kelly, 2011; Sotka, 2012) and will emerge more frequently as biologists complement experimental manipulation, high-throughput phenotyping and higher resolution genomic data that have become more readily available (Cornwell, 2020; Gagnaire & Gaggiotti, 2016; de Villemereuil et al., 2016). Of key interest is the extent to which broad-scale biogeographic and community transition patterns are reinforced by these inferences of dispersal and evolutionary dynamics (Wares et al., 2001).

Many examples of cryptic evolutionary differences across a landscape are explained by historical separation of populations and their subsequent non-equilibrium hybridization via secondary contact (Grosberg & Cunningham, 2001; Wares & Cunningham, 2005). Fitness consequences of gene flow across these hybrid zones can lead to genomic conflict (Barreto et al., 2018; Willett & Burton, 2003) which itself can lower population densities and yield cascading ecological effects (Szymura & Barton, 1986). That is, the effects of hybridization between clearly divergent lineages may lead to mismatched phenology or resource use—a form of outbreeding depression—and the lower mean fitness of these spatially intermediate populations is then associated with lower density or survival. However, if hybrids of divergent evolutionary lineages persist, hybrids may also influence community-level diversity, as when increased insect species richness and diversity occur within hybrid populations of cottonwood trees (Jarvis et al., 2017; Mitton, 2003; Urban et al., 2020).

There is a long-standing effort to delineate the forces that maintain a boundary, or cline or tension zone, between historically separated populations. Clines maintained by the environment are termed exogenous selection and models indicate that as the environment changes, the boundary will move with it. Alternatively, clines can be maintained by genomic conflict (also termed endogenous selection, a form of outbreeding depression) or an interaction between environment and genomic conflict. Frequently, both mechanisms will have empirical support (Bierne et al., 2011; Bronson et al., 2003; Rolán-Alvarez et al., 1997; Sotka & Palumbi, 2006).

Delineating between these mechanisms depends on the spatial context of this tension zone, its mobility and the relative fitness of hybrid offspring itself (Buggs, 2007). For example, there are fewer offspring in 'hybrid' nests of chickadees (*Poecile*) in the tension zone

between *P. atricapillus* and *P. carolinensis* (Bronson et al., 2003). Additionally, this hybrid zone is moving northward with minimum winter temperatures (Taylor et al., 2014), and so it exhibits characteristics of both endogenous and exogenous selection. In cases where the extrinsic or environmental factor itself is stable in time, the location of the cline between parental types tends to be drawn to these interruptions in habitat quality or lower population densities because these exogenous factors themselves limit the homogenizing influence of gene flow (Bartob & Gale, 1993; Pringle & Wares, 2007; Rosser et al., 2014; Sotka & Palumbi, 2006).

Because marine coastal species must physiologically respond to large environmental changes at multiple timescales, most studies of apparent phenotypic adaptation focus on clear geographic features or strong transitions in the environment (El Ayari et al., 2019; Sanford et al., 2003; Sotka et al., 2003), and studies of marine clines often focus on paleoenvironmental (Fenberg et al., 2014; Strand et al., 2012) and/or persistent physical forcing such as current-driven boundaries (Ewers-Saucedo et al., 2016; Hare et al., 2005) to describe likely mechanisms for persistent clines. However, there are many species that harbour transitional genomic diversity in regions with less apparent mechanisms for maintenance (Dawson, 2001; Kelly & Palumbi, 2010; Wares, 2002), and it is possible that greater understanding of these mechanisms will provide insight into species-level biodiversity transitions (Small & Wares, 2010; Wares et al., 2001).

The acorn barnacle *Balanus glandula* is a common mid-intertidal species of rocky shorelines from Alaska to Baja California. Previous studies demonstrate a surprisingly low density (per unit area of rocky shore) in a particular region of central California between San Francisco and Monterey Bays (i.e., 37–38°N; Blanchette et al., 2008; Wares & Skoczen, 2019) that is not associated with distributional limits (Barry et al., 1995). Other barnacles and many rocky substrate obligate species do not exhibit similar drops in abundance in this region despite some suitable habitat (Blanchette et al., 2008; see Supporting Information). This coastal region, between San Francisco and Monterey Bays, is also associated with the centre of clines in *B. glandula* at one mitochondrial locus (cytochrome oxidase I) and one nuclear marker (elongation factor 1-alpha; Sotka et al., 2004; Wares & Cunningham, 2005), which is in weak mitonuclear disequilibrium, suggesting the influence of both selection and gene flow (Galindo et al., 2010; Pringle & Wares, 2007; Sotka et al., 2004; Wares & Skoczen, 2019). However, there remains little evidence for the mechanism or strength of selection that maintains this 'seascape' genomic pattern.

Sotka and Palumbi (2006) and Sotka et al. (2004) predicted that climatic or other environmental variables help to maintain the cline between locally adapted lineages (i.e., exogeneous mechanism). It has been also proposed that the warmer waters of El Niño events could indicate such an association (see Wares et al., 2018). Such climate-related clinal shifts are known in other marine taxa (Stanley et al., 2018; Strand et al., 2012), and in many instances, these shifts are detectable even in recent history (Dawson et al., 2010; Sunday et al., 2012), but identifying such a change from population genomic



frequency data can be difficult relative to observing a novel distribution for a species (Bell et al., 2014; Bugs, 2007) and may also be counterintuitive (Fuchs et al., 2020; Hilbush et al., 2010). Wares and Skoczen (2019) re-evaluated mitochondrial data from barnacles collected in the late 1990s and early 2000s with new data from individuals collected nearly 20 years later and saw no apparent shift in this boundary despite recognizable increases in sea surface temperature.

Assuming these clines reflect secondary contact of historically allopatric lineages, another mechanism for maintenance of the cline and its coastal position would be an endogenous mechanism such as outbreeding depression (Sotka & Palumbi, 2006; Willett & Burton, 2003). This model would suggest that where the lineages interact, they are able to hybridize—but with important fitness consequences. As a preliminary test of this hypothesis, Wares and Skoczen (2019) found that there are latitudinally associated reproductive consequences in *B. glandula* that are correlated with the individual mitotype. Though more data are warranted, it appears that northern-mitotype individuals exhibit fewer signs of successful ovigery and fertilization when sampled at lower latitudes, and vice versa (Wares & Skoczen, 2019). Collectively, this suggests that the genomic cline in *B. glandula* has an endogenous mechanism—likely in interaction with the environment—and would be predicted to persist where there is an interruption in habitat availability (Pringle & Wares, 2007; Sotka & Palumbi, 2006) or some other environmental mechanisms that limit gene flow in combination with hybrid interactions (Brannock et al., 2009; Hare et al., 2005; Pringle et al., 2017).

Selection in response to purely environmental factors likely affects only a minority of genomic regions, assuming the lineages are otherwise reproductively compatible. A combination of exogenous and endogenous mechanisms, however, should lead to a larger proportion of genomic regions associated with the cline than narrow selection processes would predict—depending on the origin and strength of hybrid fitness costs. Here, we apply reduced representation genomic sequencing (RAD-seq; methods from Bayona-Vásquez et al., 2019) to develop a deeper understanding of spatial heterogeneity in *B. glandula* in its native distributional range. We hypothesize that as is often seen when there are clear mitochondrial spatial patterns of diversity (e.g., Ewers-Saucedo et al., 2016), there are likely to be strong coincident genomic and demographic signatures despite the broad dispersal potential of barnacle larvae. Our focus on the clinal region of *Balanus* allows us to ask whether there are signatures of endogenous selection, as well as exogenous habitat features that may lead to long-term maintenance of this cline in the face of climate change.

## 2 | MATERIALS AND METHODS

Genomic DNA from *B. glandula* was sampled and isolated using the Puregene (Gentra) protocol as indicated in Wares and Skoczen (2019). We selected a subset of locations from that study that span most of the native range of *B. glandula*, with increased density in the region previously identified as clinal. Sites (hereafter listed and identified

only by latitude on the west coast of North America) include Juneau AK (58.3°N), Friday Harbour WA (48.5°N), Cape Meares OR (45.5°N), Point St. George CA (41.7°N), Cape Mendocino CA (40.5°N), Bodega Marine Lab CA (38.3°N), Linda Mar CA (37.15°N), Terrace Point CA (36.95°N), Hopkins Marine Lab CA (36.6°N), Shell Beach CA (35.2°N) and Goleta CA (34.4°N). These locations span from where the southern mitochondrial lineage is fixed (Sotka et al., 2004) to where the southern lineage is consistently found in fewer than 5% of individuals (Wares & Skoczen, 2019). From each location, 16 individuals were haphazardly selected based on DNA quality.

The genome of Balanid barnacles is roughly 0.6–0.7 Gbp (Kim et al., 2019); we estimated need for roughly 1.5 million reads per library based on this genome size (N. Bayona-Vasquez, pers. comm.). DNA was diluted to ~20 ng/μl using a Qubit 2 fluorometer for initial estimation. The reduced representation genomic sequencing (RAD-seq) protocol followed Bayona-Vasquez et al. (2019), using the restriction enzymes *Clal*, *BamHI* and *MspI*. Size selection of resultant fragments using a Pippin prep (Sage Science, Inc.) targeted 300–450 nt. Libraries were arrayed across three PCR replicates of each of two PCR plates, with adapters designed for subsequent demultiplexing by plate replicate and by well. Pooled reactions were sequenced on a single lane of an Illumina HiSeq 4000 (PE150) by Novogene.

Upon return, we pooled the technical replicates for each plate and removed adapter sequences and repetitive sequence data using Trimmomatic (Bolger et al., 2014), with default parameters. Unpaired reads were excluded from subsequent steps of demultiplexing using process\_radtags (Catchen et al., 2013), and sequences were further trimmed to 139 bp. Only paired reads were included in the Stacks v2 assembly (Catchen et al., 2013); the value M was evaluated with a subset of data and appeared to be of low consequence so the default value of M = 3 was chosen. Following Paris et al. (2017), we selected homologous regions represented in 80% or more of all individuals in the data set, and from each homologous region, a random SNP was selected for analysis. Loci with a minor allele frequency <0.05 were excluded.

As an additional quality control step for determining how read variation maps to loci, we attempted to reconstruct the known intraspecific variation at the elongation factor 1-alpha locus (Sotka et al., 2004) as well as mitochondrial reads from this species (summarized in Wares & Skoczen, 2019) and the mitochondrial genome for *B. Balanus* (GenBank NC\_026466.1). These assemblies were attempted using the high sensitivity settings of Geneious Mapper in Geneious Prime 2020.0.4. All other major computational and bioinformatic works were performed at the Georgia Computational Research Center (GACRC) on the Sapelo2 cluster.

### 2.1 | Discriminant analysis of principal components

We performed subsequent analyses in the R 4.0.0 statistical environment (R Core Team, 2020). Analysis of data proceeded after excluding loci which were missing genotypes in more than half of individuals and individuals that were missing more than half of those remaining genotypes. At that point, the data were analysed using hierfstat (Goudet



& Jombart, 2015) to calculate values of  $F_{IS}$  by locus and by sampled location. These data were inspected for mean value across locations and the standard deviation, allowing for deviation at some locations in case of Wahlund effects or other biological causes; loci for which the average  $F_{IS} > 0.25$  were excluded from subsequent analysis.

Remaining data were first evaluated using discriminant analysis of principal components (Jombart et al., 2010) in the adegenet package (Jombart & Ahmed, 2011). We retained 50 principal components for subsequent analyses after noting that would capture >50% of the cumulative variance and that results were insensitive to changes above this. Data were grouped by sample location. In addition to plotting the DAPC results, we evaluated the latitudinal shift in frequency of those loci with the strongest contribution (loadings) to the overall pattern to see how they co-vary with the observed mitochondrial and nuclear EF1a sequence data from previous studies.

## 2.2 | Co-Co plots of clines

We visualized the distribution of cline location (coincidence) and slope (concordance) for large numbers of loci. We call the resulting plot a genomic Co-Co plot (sensu Strand et al., 2012 following Szymura & Barton, 1986). We fit a three-segment 'twice-broken-stick' model to the relationship between collection site latitude and focal allele frequency. This model assumes that allele frequencies do not vary with space outside of the cline and that they vary linearly within the cline. Four parameters are fit: the allele frequencies North and South of the cline and the locations of the transitions from non-clinal to clinal portions of the range. These parameters correspond to the coordinates of the 'breaks' in latitude/allele frequency space and were estimated using the R function *optim*. From these four parameters, we calculated the midpoint and slope of each cline.

Modelling clinal variation as three straight line segments represents a significant departure from traditional analyses. It is more common for single-locus clines to be described by a four-parameter logistic model (e.g., Szymura & Barton, 1986) whose parameters describe the frequencies of the focal allele at either end of the geographic transect, the steepness of the cline, and the location of the cline. However, numerical estimation of the logistic model can be difficult when applied in the unsupervised manner required to screen large numbers of loci. In some cases when no clinal pattern exists, failure to converge on the parameter estimates is expected. Unfortunately, in cases in which visual inspection indicates an obvious cline but the difference in allele frequency at the ends of the cline is moderate or the ends of the cline are poorly defined, non-linear regression packages that implement logistic models (SAS; R-package *nlme*) also fail to converge. Broken-stick models are much less sensitive to these effects. A previous effort that directly compared broken-stick and logistic models revealed similar conclusions about coincidence and concordance of loci in the killifish *Fundulus heteroclitus* hybrid zone (Strand et al., 2012).

## 2.3 | Population inbreeding coefficients

Following DAPC, we complemented the exploration of the loci that support the strong spatial pattern recovered using a conservative SNP outlier analysis (OutFLANK; Whitlock & Lotterhos, 2015). This approach evaluates the behaviour of each locus relative to mean  $F_{ST}$  in the system and is intended to be less sensitive to the underlying population model than other methods; we used a default  $q$  value (false discovery rate) of 0.05. Additionally, values of  $F_{IS}$  and  $F_{ST}$  were calculated across the system using adegenet to explore how prior knowledge of clinal variation in *B. glandula* would appear with multilocus SNP data.

Diversity was evaluated with the remaining data calculating the Hamming distances among individuals at each site and estimates of linkage disequilibrium using  $\bar{r}_d$  as calculated in poppr (Kamvar et al., 2014), with 999 permutations for statistical evaluation.

## 2.4 | Spatial structure analysis

We next analysed the data using the sparse non-negative matrix factorization (*snmf*) function in package LEA (Frichot & François, 2015) following data transformation with a script from Clark (2019). This approach estimates individual admixture coefficients and generates results similar to those generated in Bayesian clustering programmes (Kim & Park, 2007). We evaluated possible cluster values  $K$  between 1 and 10, choosing an optimal set for analysis based on minimizing the cross-entropy criterion as recommended. These same optimal values of  $K$  were selected for analysis in conStruct (Bradburd, 2019), with initial runs for each value of  $K$  employing 500,000 iterations and 2 chains with the delta parameter at 0.95. Following identification of the most applicable value of  $K$ , a final run used the same parameters but  $10^7$  iterations.

## 2.5 | Habitat assessment

To estimate the proportion of the outer coast of California that is rocky or boulder habitat, we utilized the Environmental Sensitivity Index (ESI) database ([https://response.restoration.noaa.gov/esi\\_download](https://response.restoration.noaa.gov/esi_download)). These GIS databases characterize shoreline habitat types at small scales (1:24,000) for their risk to oil spills. We removed all units that were clearly estuarine (i.e., ESI indices 8–10; e.g., 10A: Salt and Brackish Water Marshes). For the remaining open coast shorelines, we characterized rocky and boulder habitats (i.e., 'rocky') available for *Balanus glandula* as ESI indices (1A: Exposed rocky shores, Exposed wave-cut platforms in bedrock) and the remaining habitats as non-rocky, unavailable for *B. glandula*. We then generated an estimate of the proportion of rocky habitat for each 0.2° latitude along the California Coast.



## 3 | RESULTS

### 3.1 | Sequencing data

Raw data were removed if >10% N or >50% low-quality sequencing scores. A proportion of data (11.59%) represented PhiX and/or chimeric iTru barcodes and was discarded. A remainder of 934,546,484 reads remained at  $Q_{20} > 95\%$ ; based on samples per plate, and coverage of each individual is approximately 5.9 million reads per individual on Plate 1 and 4.5 million reads per individual on Plate 2. All reads are deposited at NCBI under BioProject PRJNA628613, with individual locations under SRA entries SAMN14734899-14735073. Following data assembly and filtering in STACKS, 1519 loci were retained with a mean coverage of 30.8× (standard deviation 21.5). Subsequent filtering for data missing in more than 50% of individuals reduced the data to 1500 SNP loci in 142 individuals across 10 of the sequenced locations; the libraries from Point St. George, California were too sparse for subsequent analysis. Additional filtering based on mean elevated  $F_{IS}$  reduced the data to 876 SNP loci.

In Figure 1, we present the density histogram per population of first axis of a DAPC scatter plot when 50 principal components are included. Similar results are retained when other data filtering is applied (analyses not shown). There appears to be clear delineation between populations from three regions Washington and Alaska (blue), Oregon south to San Francisco Bay in California (grey) and Monterey Bay to southern California (red). Across a broad range of inclusion of principal components, the *find.clusters* function of adegenet generated minimal BIC scores for  $K = 3$  clusters.

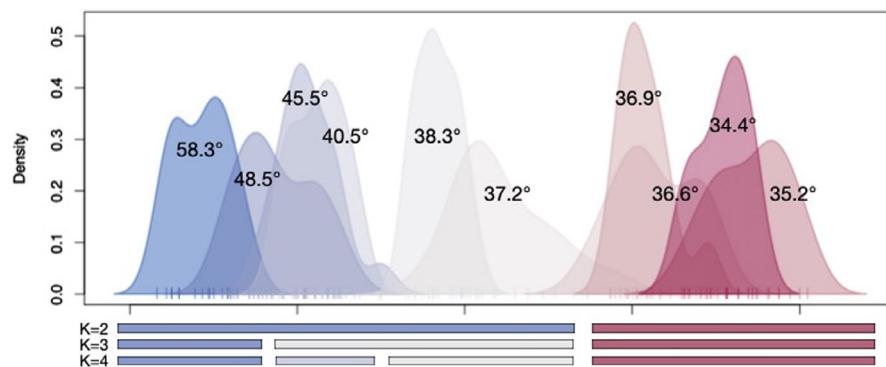
These regional shifts were reflected in spatial structure analysis using *snmf*. Using five iterations for  $K$  values ranging from 1 to 10, there was uncertainty between the ‘best’ models of  $K = 2$  or  $K = 3$  (note: we display location of these regional admixture shifts in Figure 1). The individual proportional assignments are shown in Figure 2 for  $K = 2$ ; the primary distinction with  $K = 3$  was separation of the Juneau and Friday Harbour sites from the rest of the diversity (when  $K = 4$ , the Meares and Mendocino sites set apart, another

suggestion that isolation by distance could be a component of fitting  $K$ ). Analysis of these data using *conStruct* indicated clear improvement from  $K = 1$  to  $K = 2$ , and almost no contribution from a third layer of diversity ( $K = 3$ ), indicating that the most appropriate representation of these data is for  $K = 2 + IBD$ .

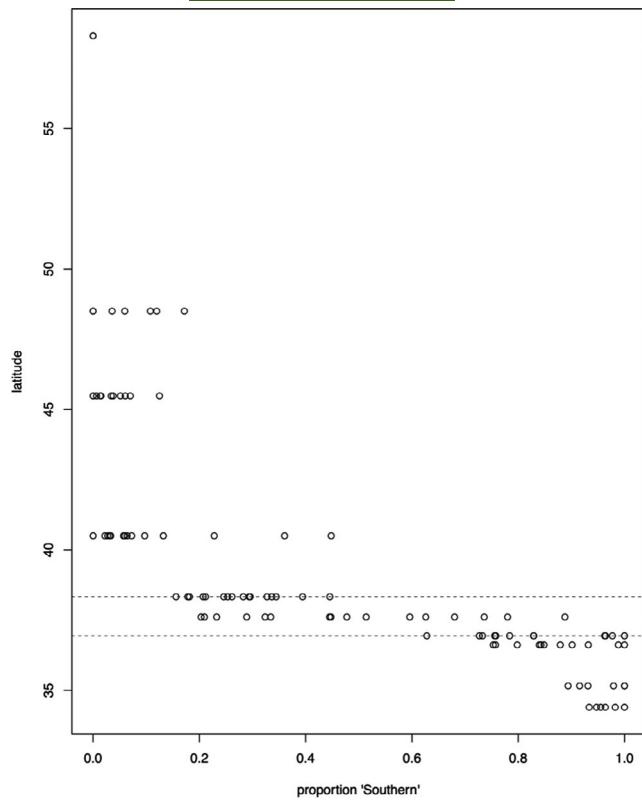
We found that 310 of the 876 loci (35%) were well fit by a broken-stick model. An analysis of cline location (coincidence) and slope (concordance) for these 310 SNPs is shown in Figure 3 (Co-Co plot). Previously published data for mtCOI and EF1a (see red points of Figure 3) are wholly concordant with plots of the latitudinal clines of all loci (black points of Figure 3) with DAPC loading contributions  $>0.0025$  (Supporting Information, part B) and suggests an overall genome-wide signature of clinal variation. There is a cluster of clinal midpoints at approximately 37–40°N, including the mtCOI and EF1a clines. Clines with the steepest slope occurred at approximately 37°N.

None of the 876 loci examined exhibited ‘outlier’ behaviour following analysis with *outFLANK* (using two different methods for evaluating  $q$  values; data not shown). Overall mean  $F_{ST} = 0.118$ , (without correcting for variation in sample size,  $F_{ST} = 0.156$ , with a range from 0 to 0.658). We thus retain all remaining loci for subsequent analysis. The most obvious shift of genomic differentiation per unit distance (km) is between our sites at Linda Mar (37.61°) and Terrace Point (36.94°) sites, with pairwise  $F_{ST}$  of 0.038 (Table 1). This represents a change in  $F_{ST}$  per degree latitude (0.19), more than an order of magnitude greater than anywhere else in our sample range.

The inbreeding coefficients ( $F_{IS}$ ) at each sampled location from remaining loci do not reveal any clear latitudinal signal (Figure 4); the same is true for the within-sample Hamming distance among individuals. However, estimates of linkage disequilibrium using  $\bar{r}_d$  suggest significant elevation at sites between latitudes 35 and 38 (Figure 4, plotted as 10 $\times$  observed value of  $\bar{r}_d$ ). While it is also elevated for our Friday Harbour site ( $\bar{r}_d = 0.0259$ ,  $p = 0.048$ ) and Bodega Bay, CA ( $\bar{r}_d = 0.00503$ ,  $p = 0.031$ ), the most striking deviations are at Linda Mar ( $\bar{r}_d = 0.0227$ ,  $p = 0.002$ ), Terrace Point ( $\bar{r}_d = 0.0108$ ,  $p = 0.002$ ), Hopkins ( $\bar{r}_d = 0.0301$ ,  $p = 0.001$ ), and Shell



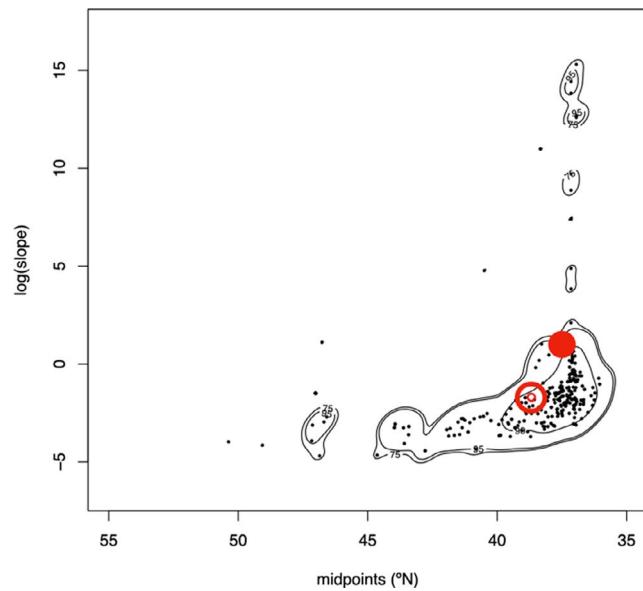
**FIGURE 1** DAPC density plot of individual genotypes of *Balanus glandula* along the primary discriminant axis, pooled by latitudinal sample location (indicated in plot). Colour spectrum chosen subjectively to represent cooler-to-warmer waters. Along the horizontal axis, the results of *snmf* population structure analysis is shown; for analysis with  $K = 2$  genomic groupings, the division is between 36.95°N and 37.15°N (top horizontal bars); results for  $K = 3$  and  $K = 4$  are additionally displayed below the plot



**FIGURE 2** Assignment probabilities by sample location for each individual *Balanus glandula* to the 'southern' of the  $K = 2 + \text{IBD}$  populations inferred in combination with *snmf* and *construct*. Boundaries at sites near  $37^{\circ}$  and  $38^{\circ}\text{N}$ , chosen as individuals transition from primarily northern to primarily southern, are indicated with dashed lines

Beach ( $\bar{r}_d = 0.0162$ ,  $p = 0.001$ ; permutation plots in Supporting Information).

We displayed the geographic context of clinal midpoints, habitat type and population density in Figure 5. This region between San Francisco and Monterey Bays ( $37\text{--}38^{\circ}\text{N}$ ) has the peak of clinal midpoints, a relatively low frequency of rocky habitat along the shore, and generally low *B. glandula* density on the few rocky shorelines that are present (Blanchette et al., 2008). This region also has statistically significant elevated linkage disequilibrium (Figure 4) and



**FIGURE 3** Co-Co plot of the latitudinal midpoint of clines exhibited by the 310 statistically significant clinal loci in these data. Mitochondrial cline shown in red circle, EF1a in red dot. For visualization of the strongest loading loci supporting this pattern, see Supporting Information part B

represents a major break between genetic clusters in our *snmf* analysis (Figures 1 and 2).

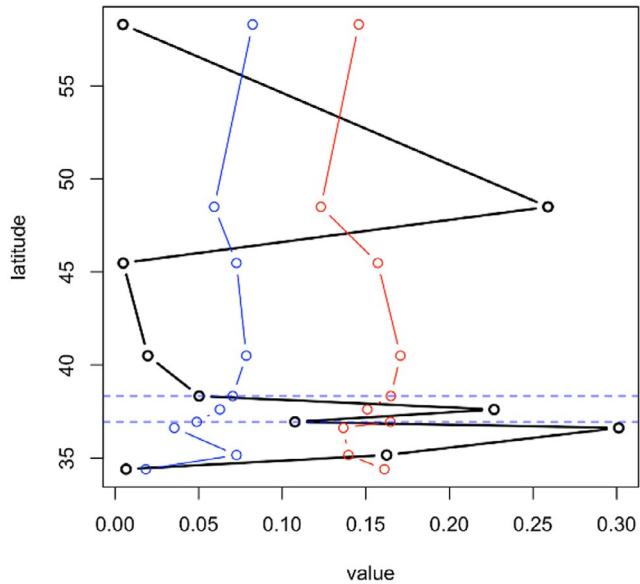
## 4 | DISCUSSION

The barnacle *B. glandula* is abundant (as high as  $70,000 \text{ m}^{-2}$  across a range of over 4000 km; Ricketts & Calvin, 1971), genetically diverse (Figure 4 and unpublished data by both JPW and EES related to high estimates of  $N_e$ ), and has high potential for larval dispersal (Galindo et al., 2010; Sotka et al., 2004; Strathmann, 1987). Yet, abundance in this species also varies by orders of magnitude across spatial reaches  $<100 \text{ km}$  (Connolly et al., 2001; Woodson et al., 2012; Shanks et al., 2017), and genomic diversity transitions rapidly across similarly small spatial scales (Sotka et al., 2004; Wares & Cunningham,

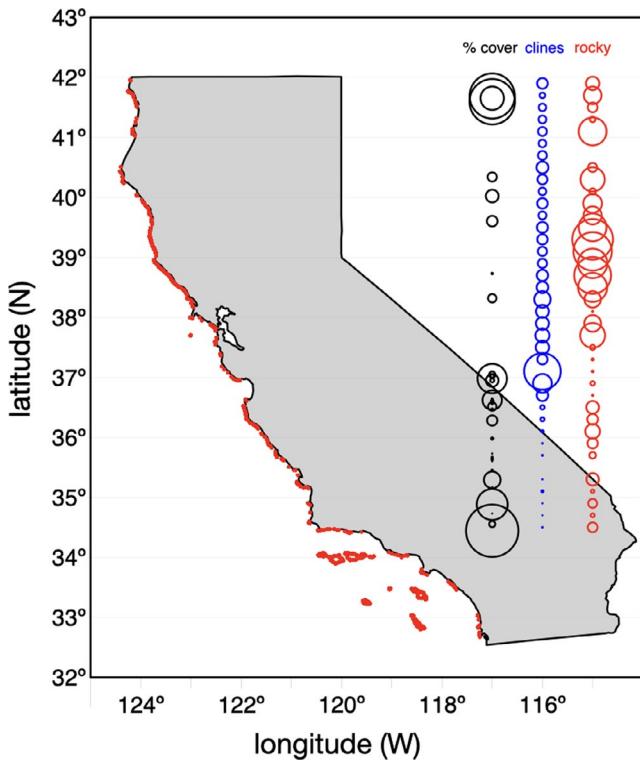
**TABLE 1** Pairwise  $F_{ST}$  among sampled locations, indicated by latitude

58.3°	—								
48.5°	0.0105	—							
45.5°	0.0570	0.0412	—						
40.2°	0.0624	0.0470	0.0054	—					
38.3°	0.0660	0.0525	0.0283	0.0229	—				
37.1°	0.1069	0.0873	0.0609	0.039	0.0103	—			
36.9°	0.2127	0.1935	0.1571	0.1271	0.0837	<b>0.0381</b>	—		
36.6°	0.2436	0.2244	0.1925	0.1546	0.1018	0.0541	0.0038	—	
35.2°	0.2959	0.2804	0.2085	0.2085	0.1526	0.0968	0.0199	0.0128	—
34.4°	0.3403	0.3183	0.2917	0.2490	0.1912	0.1170	0.0378	0.0265	0.0082

Note: The greatest  $F_{ST}$  value per spatial separation, between Linda Mar CA ( $37.15^{\circ}\text{N}$ ) and Terrace Point CA ( $36.95^{\circ}\text{N}$ ), is indicated in bold.



**FIGURE 4** Values for  $F_{IS}$  (blue), within-population Hamming distances (red), and  $\bar{r}_d$  (multiplied by 10, shown in black) at each location sampled for *Balanus glandula*. Dashed horizontal lines indicate primary transitional region as in Figure 2



**FIGURE 5** Red points on the coastal map of California indicate intervals that are 'rocky' by defined thresholds. The vertical plots indicate the % cover of habitat by *Balanus glandula* per interval (black), density of clinal midpoints from the 310 significant loci as in Figure 2 (blue), and the proportion of rocky coastline in that interval (red, reflecting same distribution as coastal points)

2005); thus, successful larval dispersal and gene flow appear to have limits below what can be estimated as dispersal potential via currents and other ocean dynamics (Barshis et al., 2011). Several lines of evidence indicate that the *B. glandula* cline is maintained by both endogenous and exogenous factors. We address each of these and suggest that patterns of marine diversity transitioning within and between species in this region are useful for understanding broader biogeographic patterns as well as how global marine introductions are sourced. We also address the somewhat surprising finding that other rocky-intertidal species with similar abundances and life histories and subject to similar spatial heterogeneity in habitat availability exhibit weak to no clinal patterns across this same region.

Direct evidence of endogenous selection is suggested by Wares and Skoczen (2019), which show a statistical signature for seasonal ovigery that is determined by interaction between their lineage and latitude. This is consistent with the notion that reproductive potential depends on a physiological match to the local environment. Additionally, our genomic data and previous studies on this barnacle indicate a rapid and persistent spatial transition in genomic diversity in central California, with evidence of elevated linkage disequilibrium; this suggests hybridization happens but with limited progress towards further introgression of the distinct genomic backgrounds. What is of key interest here is that, as predicted by theory (Szymura & Barton, 1986) and modelling approaches (Pringle & Wares, 2007), the clinal region with lower fitness is attracted to, and anchored at, a region of poor habitat availability. In this way, climate and habitat and evolutionary dynamics interact to maintain this cline, perhaps with more stability (Wares & Skoczen, 2019) than the distribution of otherwise similar species (Dawson et al., 2010).

The massive population size and genomic diversity, coupled with strong regional selection, is also evident in the statistically distinct diversity at each sampled location across a coastal transect of >2000 km (Figure 1). The large population efficiency of selection, as in *Drosophila* and other organisms of comparable population size (Filipović et al., 2020), then results in a transition between the two dominant regional lineages of *B. glandula* that is quite narrow and consistent across our sample of the genome (Figures 2 and 3; Supporting Information). As predicted (Sotka & Palumbi, 2006; Sotka et al., 2004), the transitional cline region exhibits elevated linkage disequilibrium among genomic markers, even though the dispersal of *B. glandula* has resulted in sufficient admixture of populations across sites that nucleotide diversity is stable throughout the range. The elevated linkage disequilibrium is one signature predicted in areas of admixture with low hybridization (Barton & Gale, 1993); however, we do not see elevated inbreeding coefficients, which is another signature. It seems likely that a combination of selection and lower gene flow is maintaining these clines (Galindo et al., 2010; Pringle & Wares, 2007; Sotka et al., 2004). As it is not trivial to perform controlled cross-fertilizations in barnacles, it may be difficult to determine the degree to which reproductive success or offspring fitness is reduced when the two lineages cross.

The genomic discontinuity in *B. glandula* lies on a part of the coast (between San Francisco and Monterey Bay; Figures 2 and 3,



Supporting Information part B) where there is (1) lower density of these barnacles on a shore when rocks are present and (2) generally sparser rocky intertidal habitat (Figure 5; Supporting Information). As predicted by cline theory and seen in examples such as a rain-driven population density trough in *Heliconius* butterflies (Rosser et al., 2014), areas where exogenous features are fixed in place and serve to limit gene flow will tend to attract and retain genomic clines. These environmental features may also interact with biased coastal dispersal (Ewers-Saucedo et al., 2016; Galindo et al., 2010; Pringle & Wares, 2007; Small & Wares, 2010) and an increased probability of oceanographic fronts—the boundaries marking strong temperature or salinity gradients—near the northern edge of Monterey Bay (Woodson et al., 2012). High oceanographic front probability is associated with transitions in biodiversity and increased recruitment in organisms like *Balanus* (Woodson et al., 2012).

Other rocky-intertidal species of similar life history or habitat on the California coast either have no clinal structure (e.g., *Cthamalus dalli*; Wares & Castañeda, 2005) or have clines that do not coincide with those of *B. glandula* (Dawson, 2001; Kelly & Palumbi, 2010; Small & Wares, 2010). For example, while the mussel *Mytilus* is very well-studied for clinal transitions globally (Wenne et al., 2020), it seems that the hybrid transition between *M. trossulus* and the introduced *M. galloprovincialis* has a transition that takes place farther to the north (Rawson et al., 1999); it is also more labile, following short-term temperature transitions (Hilbish et al., 2010). Brannock and Hilbish (2010) note that hybridization is common in *Mytilus*, but F1 gametogenesis is often incomplete, limiting the potential for introgression.

Cornwell (2020) indicates that the most robust genomic differentiation in the anemone *Anthopleura elegantissima* is associated to the south of the *Balanus* cline, across Point Conception, CA (34.5°). Intriguingly, the mean location of a locus cline in this study was around 37°; however, Cornwell (2020) argues that cline steepness in anemones is higher at latitudes to the south of our focal region. He proposed, following Dawson (2001), that this is associated with the biogeographic turnover between Monterey Bay and Point Conception (Montereyan province in Blanchette et al., 2008). Our results suggest a sharp transition, maintained by lack of habitat and hybrid fitness deficits, more likely related to the biogeographic transition between the Mendocinian and Montereyan provinces (sensu Blanchette et al., 2008). As additional high-resolution genomic data sets become available, it will be valuable to re-assess the spatial concordance of transitional data to contrast with earlier work based on a small number of markers (Dawson, 2001; Small & Wares, 2010). It is also key to keep these transitions in the context of ocean-driven dispersal, which should drive a genomic cline ‘downstream’ of the specific cause of the cline (Pringle & Wares, 2007).

The hybrid zone is apparent across much of the genome; the Co-Co analysis (Figure 3) suggests 35% of polymorphic SNPs fit a broken-stick clinal model, and a majority of these SNPs had midpoints along the California coast around ~37°N. The overall transition is strong enough across all markers (Figure 3, Supporting Information part B) to make it difficult to identify any as exhibiting

outlier behaviour using a conservative test (Whitlock & Lotterhos, 2015; Strand et al., 2012); this would suggest likely secondary contact between the two lineages following an ancient isolation event. Wares and Cunningham (2005), based on mitochondrial data alone, inferred that the northern and southern lineages likely diverged approximately 0.1–0.5 mya. Pringle and Wares (2007), Szymura and Barton (1986) and Bierne et al. (2011) note that when there are low quality or low density portions of the habitat, then a genomic transition zone will tend to move towards that area—indicating that the two types may be divergent enough that there begin to be fitness consequences for hybridization.

It is tempting to pair the timing of divergence in *B. glandula* with the sediment load and geological shifts associated with the formation of San Francisco Bay itself, as the massive Central Valley of California was suddenly drained through the Carquinez Strait roughly 0.6 mya (Wong, 2006). If true, then the cline in *B. glandula* forms ‘downstream’ (following the offshore California Current) from this site, and we see evidence of transient allopatry and secondary contact in this case. This mechanism is supported by other analyses, in part. There is not elevated homozygosity in the cline region as might be expected with assortative mating; instead, there is elevated linkage disequilibrium (Figure 4) indicating a fitness boundary to introgression. The region the genomic cline is attracted to have high habitat heterogeneity, but enough habitat for obligate rocky intertidal species like *Balanus*. There are apparent fitness differences between the two lineages as indicated by mitotype alone (Wares & Skoczen, 2019), though that result warrants additional attention. Of other abiotic data available for this region (MARINe, 2020), there are no suggestive physical, environmental or community characteristics—and the idiosyncratic location of the cline in *B. glandula* itself may indicate that the original mechanism of divergence is, after all, unclear.

Our study has three broader implications. The first relates to the warming ocean along the California coast, where others have noted poleward shifts in the ranges of rocky-intertidal species and lineages in the last few decades (e.g. Barry et al. 1995; Dawson et al., 2010; Sanford et al., 2019). There is no evidence that the mtCOI cline in *B. glandula* has shifted similarly (Wares & Skoczen, 2019); although no exploration of shifts in nuclear clines has been attempted. The lack of a poleward shift in southern mitochondrial lineages in *B. glandula* is consistent with endogenous selection and its association with low density on the coast, rather than a solely exogenous factor like temperature. The apparent stability of the clinal location also suggests that any warming that is occurring may not be generating selection pressures that are strong enough to shift clinal midpoint for surviving adults (see Bell et al., 2014 for a similar stasis in the face of climate change in the eurythermal fish *F. heteroclitus*). Note too, that genomic shifts within California rocky intertidal species are not always sensitive to mean climatic shifts in seawater temperature, but instead to decadal shifts associated with El Niño cycles (see Hilbish et al., 2010). It will be of interest to watch for further study of how selection-driven clines respond to climate change and decadal cycles, as the interactions between adaptive genomic diversity, larval dispersal and ocean currents can



complicate our predictions (Coyle et al., 2019; Pringle et al., 2011, 2017; Wares & Pringle, 2008).

Second, the cline persists despite the high likelihood of anthropogenic movement of *B. glandula* along the coastline. The divergence has persisted since long before ships and other anthropogenic mechanisms might be expected to homogenize this diversity, and *B. glandula* has been introduced to almost every other continent globally—southeastern South America and Japan (Geller et al., 2008), west coast of Africa (Simon-Blecher et al., 2010) and Europe (Kerkhof et al., 2018). As noted earlier, the parallels of how diversity can be introduced globally, yet maintains a persistent pattern on the western North American coast, is another cause to note how the strength of local adaptation (Sanford & Kelly, 2011) can supersede anthropogenic movement, a persistent inquiry about gene flow in barnacles.

Finally, our results have implications for biogeographic patterns among species along shores of western North America north of California. For example, we note some statistical indication of diversity transition associated with the Friday Harbor sample, and certainly there is tremendous environmental transition in comparing the Salish Sea and Puget Sound with the outer Pacific coast (Lamont et al., 1996; Harley et al., 2006). Already there is an abundant evidence for distinct evolutionary ecology of marine diversity in this region (Marko et al., 2010), but this transitional area begs for its own focused examination of how communities respond to the history and environmental gradients of the Salish Sea.

Certainly, there is “increasing appreciation for the ecological effects of [genomic] variation within species... intraspecific effects and species effects have roughly similar impacts on most ecological responses.” (Des Roches et al., 2018; also see Jarvis et al., 2017). How the diversity recovered in *B. glandula* maps to functional and taxonomic distinctions (Vellend, 2018) seems approachable with respect to the different performance of diversity of the two types at distinct latitudes (Wares & Skoczen, 2019). Similary strong mitochondrial signatures of overall physiological and adaptive performance occur in green crabs (Coyle et al., 2019), ribbed mussels (Fields et al., 2012), and numerous other marine organisms (Sanford & Kelly, 2011). Here, we strengthen support for what the mitochondrial diversity of *B. glandula* has long informed (Barshis et al., 2011; Galindo et al., 2010; Sotka et al., 2004; Wares & Cunningham, 2005; Wares et al., 2001), but these data give broader insight into the formation and maintenance of this evolutionary transition zone in central California and possible ecological ramifications.

## ACKNOWLEDGEMENTS

This work was made possible by funding from the UGA Department of Genetics thanks to A.J. Moore and the National Science Foundation (OCE-1924599). Assistance from Natalia Bayona-Vasquez, Rachel Toczydlowski, Travis Glenn, Bud Freeman, Karen Bobier, Paige Duffin, Shan-ho Tsai, Gideon Bradburd, Katie Lotterhos and Katie Skoczen was key to this paper. We thank Jennifer Dare for help with the ESI dataset. The manuscript was significantly improved following comments by Jamie Pringle and Rick Grosberg. This study utilized data

collected by the Multi-Agency Rocky Intertidal Network (MARINe): a long-term ecological consortium funded and supported by many groups. Please visit [pacificrockyintertidal.org](http://pacificrockyintertidal.org) for a complete list of the MARINe partners responsible for monitoring and funding these data. No collecting permits were needed for this research. Submitted with gratitude to Dr. Bill Newman, who passed in 2020 and informed much of JPW's exploration of barnacle diversity through the years.

## DATA AVAILABILITY STATEMENT

All reads are deposited at NCBI under BioProject PRJNA628613, with individual locations under SRA entries SAMN14734899-14735073.

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Author contributions: J.W. and E.S. conceived the ideas; J.W. and E.S. collected the data; J.W. and E.S. and A.S. analysed the data; and all authors contributed to the writing.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Wares JP, Strand AE, Sotka EE. Diversity, divergence and density: How habitat and hybrid zone dynamics maintain a genomic cline in an intertidal barnacle. *J Biogeogr*. 2021;00:1–12. <https://doi.org/10.1111/jbi.14142>