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COMPLEX SPATIAL GENETIC CONNECTIVITY OF MUSSELS *MYTILUS CHILENSIS* ALONG THE SOUTHEASTERN PACIFIC COAST AND ITS IMPORTANCE FOR RESOURCE MANAGEMENT

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ABSTRACT To ensure the maintenance of natural mussel beds along the southeastern Pacific coast of Chile, it is important to understand their population dynamics. This means evaluating their genetic population structure and gene flow, and the degree of connectivity among natural beds. To do this, the spatial genetic population structure of seven natural *Mytilus chilensis* beds within the mussels' present distribution range along the Chilean coast was evaluated. Genetic differences were established between populations with cytochrome oxidase I (COI) gene sequences (Fst = 0.099) and microsatellites (Fst = 0.048), showing that locations that consistently presented greater differentiation were those at the extremes of the geographical distribution. An "isolation by distance" pattern was not observed in the COI and microsatellite data. We suggest that because of the high resolution of these markers, the differences between locations may be explained by high reproductive variance, which determines local changes in each reproductive cycle of the species. These changes would account for the differences between the natural beds. Furthermore, differentiated genetic types were observed in some locations, demonstrating the presence of local processes in some cases, perhaps caused by gene flow restrictions resulting from the local geomorphological and oceanographic conditions. The gene structure and connectivity of natural beds in sessile species with larval dispersion are strongly determined by local retention characteristics. For this reason, the data generated in this study can be used to improve population management. These data can also be used to support and motivate the creation of a marine protected area containing natural beds of this species with sufficient levels of genetic diversity.

KEY WORDS: *Mytilus chilensis*, mussels, COI, southeastern Pacific, microsatellites

INTRODUCTION

In general, marine invertebrate species are expected to have high dispersal capacity because of the presence of larval stages and the influence of ocean currents, which increase their ability to travel relatively long distances, generating large, panmictic populations (Waples 1987). Moreover, restrictions or barriers (i.e., currents and physical breaks) might effectively limit dispersal in structured populations of marine invertebrates, resulting in relatively little gene flow. These factors of the marine environment have been extensively discussed with varying results (Scheltema 1986, Hedgecock 1986, Palumbi 1992, Cowen & Sponaugle 2009). High genetic homogeneity and absence of population structure over wide geographical distributions have been observed in certain cases for most marine bivalves, such as clams (Benzie & Williams 1992, Vadopalas et al. 2004) and mussels (Levinton & Koehn 1976, Skibinski et al. 1983). Genetic differentiation over small geographical scales has also been observed in other molluscs (Ridgway 2001, Luttikhuizen et al. 2003, Zhan et al. 2009).

There are three potential explanations for the genetic differences observed between marine animal populations. First, they could be driven by the dispersal capacity associated with the larval stages (e.g., Bohonak 1999, Riginos & Nachman 2001), which is expected to produce greater homogenisation through the dispersal of larvae in the planktonic phase. Dispersal ability is highly determined by the life history of a given species; for example, sessile and benthic species lacking larval stages usually show higher population genetic differentiation than species with long planktonic larval stages (Hunt 1993, Kyle & Boulding 2000, Collin 2001, Grosberg & Cunningham 2001). Second, genetic differences among populations could be influenced by ocean currents (e.g., Lundy et al. 1999, Baus et al. 2005, Kenchington et al. 2006), which on the one hand may cause greater dispersal and reduced genetic structure (Riginos & Nachman 2001, Luttikhuizen et al. 2003, Kenchington et al. 2006), but alternatively could result in larval retention because of closed current systems (e.g., for Chile: Hinojosa et al. 2010, Yannicelli et al. 2012; for South Africa: Teske et al. 2008, Porri et al. 2014). These oceanographic conditions may change depending on the geomorphological conditions present throughout the distribution range of a species (e.g., coral reefs, Cowen et al. 2006; gulf, Riginos & Nachman 2001), and may be more evident for species residing in benthic or coastal environments. For example, the geomorphological variations may be greater in canals and fjords, which have been observed to generate genetic and biogeographic breaks in various species (Cárdenas et al. 2009, Macaya & Zuccarello 2010). Finally, high reproductive variance resulting from high auto-recruitment, differential recruitment between locations, or differentiated cohorts within locations could lead to sharp genetic differences between individuals of different ages or cohorts (Hedgecock 1994, Hedgecock & Pudovkin 2011, Broquet et al. 2013).

Further studies have been carried out to understand the processes that determine population structure because of their

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implications for species conservation and response to evolutionary processes over time. For most marine bivalves, including clams, scallops, mussels, and oysters, however, the mechanisms by which geographical differentiation is driven and the genetic population structure is created are still somewhat unknown. Understanding the causes of the genetic population structure of a resource becomes particularly important when these natural populations are used to support culture-based fisheries [as defined by FAO (1997) and Garcia (2009)] or extensive aquaculture, as occurs in the case of the aquaculture of mytilids in the world (*Mytilus chilensis* and *Mytilus galloprovincialis*) (Uriarte 2008, FAO 2009). This is of great importance because if natural beds or populations of mussels are highly structured genetically, that is, highly differentiated from one another or present low genetic diversity, they may not be able to sustain the whole aquaculture of this species (mytilids), given that aquaculture of all mytilid species is based on obtaining seed from these natural beds (FAO 2009). Knowledge of the genetic diversity and population structure of these natural beds provides information that can ensure a thriving aquaculture industry. This is because natural beds with low genetic diversity, or which are highly structured, may present greater fragility because of their lesser ability to respond to environmental changes, or to selection and genetic drift processes, which may lead to local extinctions (Hughes et al. 2008).

An important mussel species, *Mytilus chilensis*, is found along the southeastern Pacific coast and supports a growing aquaculture sector in Chile (Bagnara & Maltrain 2008). This species is produced in large quantities by aquaculture, with production reaching a total of 340,000 tons in 2017 (Sernapesca 2017). Production depends completely on seed obtained from natural beds, and therefore on the healthy state of these beds (Molinet et al. 2015). Historically, this species has been called *M. chilensis*; however, because of the significant genetic similarity between the species of *Mytilus* complex (Daguin & Borsa 2000, Hilbish et al. 2000), its identification has been the subject of considerable debate (Borsa et al. 2012). Its identification as a distinct species has finally been corroborated by DNA sequencing (Astorga et al. 2015) and by sequencing the complete mitochondrial genome (Śmietanka & Burzyński 2017). As it has been confirmed as a distinct species—although its name has not been defined with certainty—and to avoid confusion with samples from other locations, here, its traditional name, *M. chilensis*, will be used henceforth.

Previous studies have examined the population structure of this species in different locations over a range of 1,900 km, using RAPD markers (Toro et al. 2004) and allozymes (Toro et al. 2006); panmictic populations with no genetic differentiation were observed, except at the southernmost study site in the range. Ouagajjou et al. (2011) developed hypervariable microsatellite markers for this species, which have been applied in studies of restricted local areas such as fjords (Astorga et al. 2018). Other microsatellites set have also been developed for this species, which have been used for analysis principally in samples from cultivation systems (Larraín et al. 2012, Larraín et al. 2014, Larraín et al. 2015). An alternative technique using SNP was developed by Araneda et al. (2016), to evaluate neutral and adaptive genetic variation in this species. No studies have investigated the spatial pattern of genetic structure using DNA sequencing and microsatellites throughout their distribution range. Consistent with the population genetics literature for marine invertebrates and previous studies of *Mytilus chilensis*, low genetic structuring can be expected for this species because of its extensive larval dispersion stage; however, local oceanographic processes and the currents of the South Pacific suggest that greater population genetic structure or breaks in the gene flow may be encountered as a result of these conditions (Toro et al. 2004, Zakas et al. 2009, Ibáñez et al. 2011). The objective of this work was therefore to evaluate the spatial distribution of genetic diversity and the population genetic structure of this native species in natural beds along the Chilean coast. This was accomplished by using two types of molecular markers: the mtDNA cytochrome oxidase I (COI) gene and analysis of nine microsatellite loci of nuclear DNA. The purpose of this evaluation was to study the degree of connectivity and gene flow in the species and to understand the causes of genetic differentiation in sessile species with high larval dispersal capability. Finally, it is hoped that this information will enable the resource to be maintained over time through the implementation of resource management strategies, such as defining administrative measures for the protection of areas with natural beds, which may be acting as sources of genetic variability, or by defining protected areas. Mytilids are the dominant aquaculture molluscs in this region, supporting not only artisanal fishermen (who have diversified into aquaculture) but also small and medium-scale aquaculture companies.

MATERIALS AND METHODS

Samples of *Mytilus chilensis* were collected from seven study sites along the Chilean coast. From north to south, these sites were as follows: Puerto Saavedra (PSA), Queule (QUE), Chaihuin (CHA), Huinay (HUI), Yaldad (YAL), Puerto Raúl Marín (PRM), and Punta Arenas (PTA) (Table 1, Fig. 1). T1 F1

DNA was extracted from the mantle of the samples using a mollusc extraction kit (E.Z.N.A.) following the manufacturer's instructions. A 644-bp DNA fragment of the mitochondrial DNA COI gene was amplified using LCO1490 and HCO2198 universal primers (Folmer et al. 1994). The sequences were edited and aligned using the BIOEDIT 5.0.9 software (Hall 1999). Sequences were aligned using CLUSTALW (Thompson et al. 1994) as implemented in BIOEDIT (Hall 1999). A total of 100 sequences were deposited in GenBank under accession no. KR066657–756.

Nine microsatellite loci were amplified using the primers defined by Ouagajjou et al. (2011): Mch1, Mch2, Mch3, Mch4, Mch5, Mch6, Mch7, Mch9, and Mch10. The size of the amplified alleles was determined using an automatic DNA sequencer (ABI Prism 377; Applied Biosystems). Fragment analysis was carried

TABLE 1.

Locations where *Mytilus chilensis* was studied, indicating the coordinates and the number of samples (*N*) by type of marker used.

Locality	Abbreviation	Lat/long	COI	Msat
Pto. Saavedra	PSA	38° 47′ S/73° 23′ W	9	26
Queule	QUE	39° 23′ S/73° 13′ W	12	27
Chaihuín	CHA	39° 56′ S/73° 35′ W	10	23
Huinay	HUI	42° 22′ S/72° 24′ W	13	10
Yaldad	YAL	43° 06′ S/73° 42′ W	16	23
Pto. Marín	PRM	43° 45′ S/72° 58′ W	11	20
Punta Arenas	PTA	53° 25′ S/69° 23′ W	29	20
Total			100	149

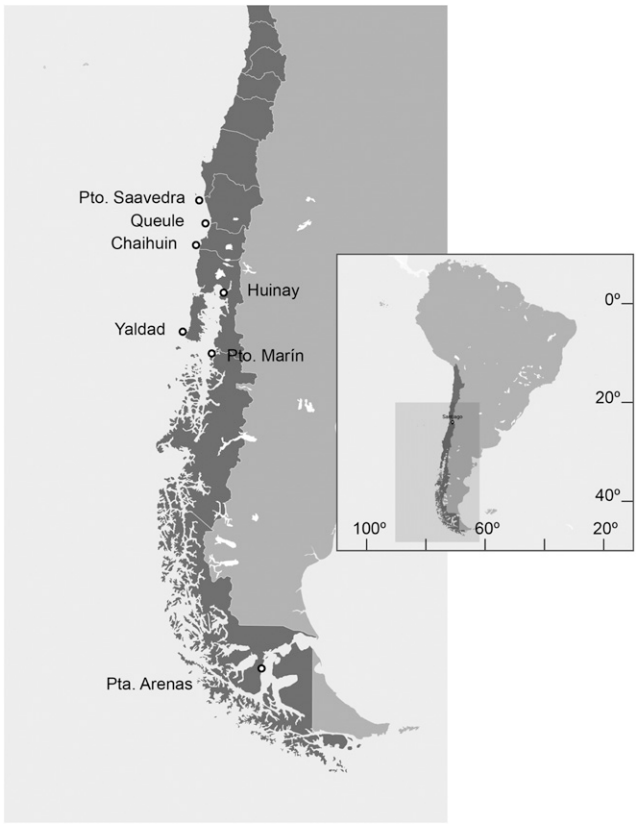

Figure 1. Map of the southeastern Pacific coast, showing the location of the study sites.

out using Peak Scanner software from Applied Biosystems. Allele sizes were assigned bins using FLEXIBIN (Amos et al. 2007), and the evaluation of null alleles was reviewed and corrected using the MICRO-CHECKER software (Van Oosterhout et al. 2004). The nine loci analyzed were studied in 149 *Mytilus* individuals, corresponding to the seven populations described earlier (Table 1).

The statistical independence between loci was assessed using GENEPOP 4.0 (Rousset 2008). Genotypic linkage disequilibrium between each pair of loci within populations and between each pair of loci over the whole data set was tested using Fisher's exact tests with a Markov chain. Tests for deviation from the genotypic proportions expected under the Hardy–Weinberg equilibrium for each locus and population were estimated with exact *P* values by the Markov chain method using GENEPOP 4.0. The observed heterozygosity (Ho) and expected heterozygosity (He) were estimated with GenAlex 6.5 software (Peakall & Smouse 2012), and the allelic richness (Rs) standardized for the number of data was calculated with FSAT 2.9.4 (Goudet 2003). To adjust for multiple comparisons, Bonferroni-adjusted *P* values were determined (Rice 1989).

To analyze the mitochondrial genetic diversity, standard diversity indices were estimated for each group and gene, including the number of segregating sites (*S*), the number of haplotypes (Hk), haplotype diversity (Hd), nucleotide diversity (πPi), and the mean number of pairwise differences (Πk). The genetic divergence between locations was estimated using Fst values, based on Hudson et al. (1992a) (Eq. 3). The statistical differentiation value was estimated by the permutation (randomization) test and the Chi-square test (haplotype data) (Hudson et al. 1992b, Eq. 1). The genetic data were analyzed using DnaSP v6.11.01 (Rozas et al. 2009).

The relationships among the haplotypes observed in this study were assessed by constructing median joining networks (Bandelt et al. 1999), and the haplotype genealogies were determined using HapView (Salzburger et al. 2011).

To assess the spatial distribution of the genetic diversity in *Mytilus*, two approaches, for microsatellite data, were used. First, the differentiation between sampling locations was established by multivariate principal component analysis (PCA) using GenAlex 6.5 software (Peakall & Smouse 2012). Second, the most probable number of genetic groups (*K*) was estimated based on a Bayesian analysis implemented in the STRUCTURE v.2.3.2 software (Pritchard et al. 2000). This program uses individual multilocus genotype data to cluster individuals into *K* groups while minimizing the Hardy–Weinberg and linkage disequilibrium. The estimation procedure consists of running trial values of the number of populations, *K*, and then comparing the estimated log probability of data for each value of *K*, Ln[Pr($X|K$)]. We conducted a series of independent runs with different values of *K* using a maximum equal to the total number of locations sampled in the data set. Each run used 10^6 iterations after a burn-in of length 4×10^4, using an admixture model and the option of correlated allele frequencies between populations. Cluster assignment was based on the individuals' sampling location, following Hubisz et al. (2009). To check for the convergence of the Markov chain Monte Carlo, we performed five replicates for each value of *K*. The analysis defined the most probable *K* value with a peak likelihood of the logarithm of *K*; however, this value varied between different runs of Markov chain Monte Carlo. To eliminate variation, the correction proposed by Evanno et al. (2005) was implemented in the STRUCTURE HARVESTER 0.6.7 software (Earl 2012). To generate graphical representations for specific *K*, we used the web-based STRUCTURE SELECTOR software (Li & Liu 2018).

Genetic diversity in the microsatellite data was measured by the number of alleles per location, the observed and expected heterozygosities, and the fixation index (Fis). The differences between these indices were estimated with *P* values using Ho and He and their confidence intervals. The population genetic differentiation for microsatellite data was assessed using pairwise Fst based on all polymorphic loci (Weir & Cockerham 1984), and Nei's average number of pairwise differences within and between populations (Nei & Li 1979). The sum of the squared differences (Rst-like) was estimated as a distance method using ARLEQUIN v3.5.1.2 software (Excoffier & Lischer 2010). The significance of the genetic distances was tested by permuting the individuals between the populations. The genetic differentiation between locations and the global differentiation test by genotype frequencies were estimated with ARLEQUIN v3.5.1.2 software (Excoffier & Lischer 2010). The multilocus estimate of the effective number of migrants (Nm) was estimated according to Slatkin (1985). Three estimates of Nm were provided, using the three regression lines published in Barton and Slatkin (1986); a corrected estimate was provided using the values from the closest regression line (see Barton & Slatkin 1986).

Finally, isolation by distance was assessed by the correlation between the geographical distance between locations and the corrected value of Fst = (Fst/1 – Fst), for both the microsatellite and the COI data set, using the Mantel test as implemented in the GenAlex v 6.5 software (Peakall & Smouse 2012).

RESULTS

Mitochondrial DNA-COI

The COI data for genetic diversity showed a total of 44 haplotypes among the 100 individuals analyzed, which represented seven locations (Table 2). The number of haplotypes (Hk) within populations ranged from six in PSA (north end) to 13 in PTA (south end), whereas the haplotype diversity (Hd) showed lower variation, ranging from 0.80 in PTA to 1.00 in CHA. The average number of pairwise differences also presented differences between locations, with a range between 2.136 and 5.222; the lowest values were found in QUE and PRM, and the highest in CHA and HUI.

Overall, genetic differentiation for the COI locus based on Fst estimation was 0.0988. Pairwise Fst showed that HUI (intermediate location) and PTA (south end) present the greatest differentiation (Table 3, above the diagonal).

The network analysis using the COI data showed three central haplotypes, with H1 present in six locations, H2 in five locations, and H3 in four locations. In addition, a high number of haplotypes with low diversity arose from the central haplotypes (Fig. 2). In general, no geographical pattern was observed in the genealogical relationship among haplotypes (with some exceptions). The presence of several unique haplotypes present only in PTA is remarkable. Haplotypes from PSA and CHA were the most distant from the central haplotype.

Nuclear DNA-Microsatellites

The analysis of null alleles revealed that only two of the nine loci showed the presence of null alleles (locus MCH4 and locus MCH5); however, the analysis results of genetic diversity and population structure did not change when working with just seven loci, so all loci were retained in the analysis. The adjustment to HWE was evaluated through the fixation index (Fis), which showed significant differences from HWE in three locations, PSA (north end) and two other locations further south (YAL and PRM). The highest Fis values were found at YAL (0.50) and the lowest at HUI (–0.05) (Table 2). The microsatellite data showed that the allelic richness per location varied significantly from 3.333 ± 0.624 in HUI to 4.871 ± 0.899 in PSA. The Ho presented less variation, ranging from 0.33 in YAL to 0.44 in PTA (Table 2).

Multivariate PCA using the microsatellite data showed no separation pattern associated with geographical distribution (i.e., clines); however, differences were observed between two nearby sites, HUI and CHA, followed by PSA (north end) and YAL (Fig. 3).

Structure analysis revealed that the most probable number of genetic clusters in the study zone is $K = 5$ (Fig. 4). It was observed that CHA presented greater differentiation than the other populations and appears to be a more distinct genetic group. In the remaining locations, no evidence of any spatial structure pattern was observed, indicating high admixture along the Chilean Coast in general.

Genetic differentiation was significant (Fst = 0.048) across all locations, and was obtained by calculating the Fst from microsatellite data. The estimated number of migrants (Nm = 4.691), however, suggests gene flow. Nevertheless, when the locations were compared pairwise (Table 3, below the diagonal), significant differences were observed, principally between the site at the far north (PSA) and five other locations, and between the site at the far south (PTA) and three other locations. The sites that presented the smallest differentiation from the rest are the intermediate locations of QUE, HUI, and YAL.

Isolation by distance was not observed with the microsatellite data ($r = 0.438$; $P = 0.120$) or with the COI gene sequencing data ($r = 0.328$; $P = 0.140$).

DISCUSSION

Genetic diversity in natural populations provides the basis for their ability to respond to changes (e.g., climate change), and their fluctuations should be monitored over time, especially in species that are extracted by fisheries or for aquaculture. Thus, in this work, different indicators of genetic diversity were estimated and their relationship with data from the literature considered.

The COI gene sequencing data presented slightly higher values for haplotype diversity (Hd = 0.917) than those observed by Gérard et al. (2008) (Hd = 0.51). When the COI gene sequencing data obtained in this work were compared with other

TABLE 2.

Indices of genetic variability based on sequences of the COI gene and nine microsatellite loci indicated for the seven locations studied.

	COI gene					Microsatellites				
Locations	***H*(*k*)**	**Hd**	***S***	**Π(*k*)**	**π(Pi)**	**Na**	**Rs**	**Ho**	**He**	**Fis**
PSA	6	0.833 ± 0.127	15	3.500 ± 1.966	0.006	6.556	**4.871 ± 1.994**	0.431 ± 0.082	0.592	**0.301 ± 0.104**
QUE	8	0.879 ± 0.078	9	2.136 ± 1.276	0.003	4.889	4.209 ± 2.024	0.400 ± 0.088	0.546	0.208 ± 0.109
CHA	10	1.000 ± 0.052	28	5.222 ± 2.788	0.011	5.222	3.903 ± 2.066	0.349 ± 0.106	0.467	0.272 ± 0.135
HUI	9	0.936 ± 0.051	16	4.513 ± 2.374	0.007	3.333	**3.333 ± 1.871**	0.389 ± 0.111	0.393	0.046 ± 0.114
YAL	11	0.908 ± 0.063	15	2.200 ± 1.282	0.004	6.556	4.853 ± 2.021	0.334 ± 0.091	0.607	**0.501 ± 0.113**
PRM	7	0.909 ± 0.066	8	2.182 ± 1.306	0.004	5.667	4.540 ± 2.199	0.356 ± 0.081	0.520	**0.341 ± 0.126**
PTA	13	0.800 ± 0.065	19	2.645 ± 1.453	0.004	5.333	4.155 ± 2.012	0.442 ± 0.109	0.485	0.146 ± 0.140
TOTAL	44	0.917 ± 0.071	66	3.471 ± 1.250	0.006	5.365	4.896 ± 2.028	0.376 ± 0.035	0.511	0.324 ± 0.097

H(*k*), number of haplotypes; Hd, haplotype diversity means with SD; *S*, number of polymorphic sites; Π(*k*), average number of pairwise differences with SD; π(Pi), nucleotide diversity; Na, number of alleles; Rs, allelic richness; Ho, observed heterozygosity; He, expected heterozygosity; Fis, fixation index with SD. Rs values in bold indicate pair of locations with significant differences. Fis values in bold indicate values that do not adjust to the Hardy–Weimberg equilibrium.

TABLE 3.

Pairwise genetic divergence in locations, estimated by pairwise Fst's for all population pairs; above the diagonal are the Fst values given by COI gene sequencing data, and below the diagonal are the Fst values with allele identity (Rst stats), using the nine microsatellite loci, and significant values are indicated in bold.

Pop	PSA	QUE	CHA	HUI	YAL	PRM	PTA
PSA		**0.1018***	0.0357	**0.1126*****	–0.0215	0.0406	–0.0042
QUE	**0.1542***		0.0088	**0.0608***	0.0543	–0.0084	**0.1273*****
CHA	**0.1029****	0.0126		0.0250	0.0069	–0.0152	**0.0601***
HUI	0.0124	0.0554	0.0132		**0.0692****	0.0298	**0.1381*****
YAL	**0.1517*****	0.0071	0.0050	0.0555		0.0004	0.0184
PRM	**0.2620*****	0.0090	0.0501	0.1451	0.0043		**0.0746***
PTA	**0.2647*****	0.0043	0.0671	**0.1503***	0.0182	0.0213	

Level of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

species of the *Mytilus* genus, high similarity was observed among species with only slight differences in some indicators (for further details, see Astorga et al. 2015).

In the nine microsatellite loci analyzed, the average allelic richness was 4.896, which was lower than that observed by Diz and Presa (2008, 2009) in six microsatellite loci (allelic richness > 7.15) or that observed by Ouagajjou and Presa (2015) in seven loci (allelic richness > 10.986) both for *Mytilus galloprovincialis*. The observed heterozygosity data (Ho = 0.376) were similar to those obtained for *Mytilus chilensis* by Larraín et al. (2015) from microsatellites and higher than those obtained by Araneda et al. (2016) from SNPs. These differences in only some parameters of genetic diversity are probably driven by the use of different types of molecular markers or different microsatellite loci, as is the case when compared with Larraín et al. (2015).

The genetic diversity patterns observed within the analyzed locations show significant differences between some locations, which may be explained by the different characteristics of each locality. For example, YAL, which presents the highest value of inbreeding Fis, is the site where the development of this aquaculture resource first started. Because of the aquaculture production at this site, there has been high sampling and extractive pressure over time, first by artisanal fishermen and later through the extraction of seeds for aquaculture using long-line systems (Gonzalez-Poblete et al. 2018). Long-line systems are composed of ropes strung over the natural beds to which the seeds adhere; the captured seeds are then transferred to aquaculture systems for fattening. It has since been discovered that these artificial seed capture systems compete with natural beds for recruits and settlers (Molinet et al. 2017). This leads to a reduction in the bed size because they are only used as a seed source, and fewer recruits return to maintain the bed over time. Furthermore, the site that presents the lowest value for inbreeding (HUI) is located in the Comau Fjord, which was decreed a marine protected area (MPA) in 2001 (www.leychile.cl) and is closed to fishing and seafood extraction. There is therefore no extractive pressure on the natural *Mytilus* beds at this site, and no cultivation systems have been installed for seed capture. The difference in the inbreeding values between the site that has historically suffered the greatest extractive pressure versus a protected area may indicate that protected areas are effective in preserving the genetic diversity of natural beds. It has indeed been observed that MPA contain a greater biodiversity and biomass of organisms than areas open to fishing (Edgar et al. 2014). Thus, the creation of more protected areas aimed at maintaining the sustainability of particular resources could have a positive effect on maintaining the genetic diversity of populations. Similar patterns were observed with both types of markers for the locations mentioned. For HUI, the highest genetic diversity was observed by COI gene and the lowest rate of inbreeding with microsatellites; inversely, the locality of YAL showed the lowest genetic diversity with COI gene and the highest inbreeding with microsatellites.

Congruent patterns of genetic differentiation were observed with the two marker types, but the values appear to be larger

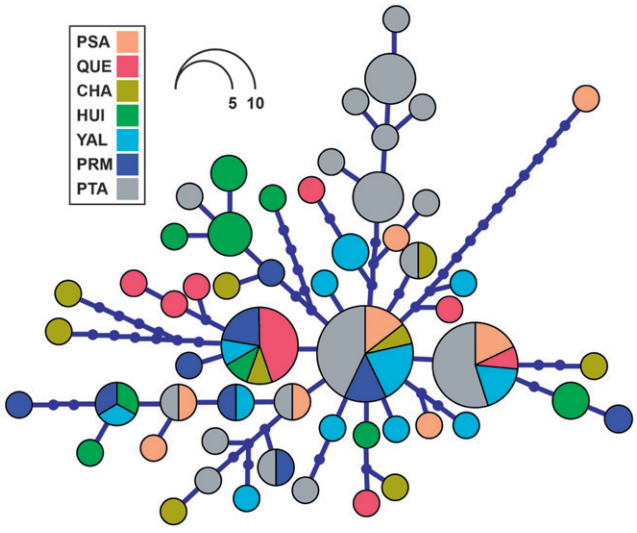

Figure 2. Network analysis using the COI mitochondrial gene sequencing data for the seven study sites.

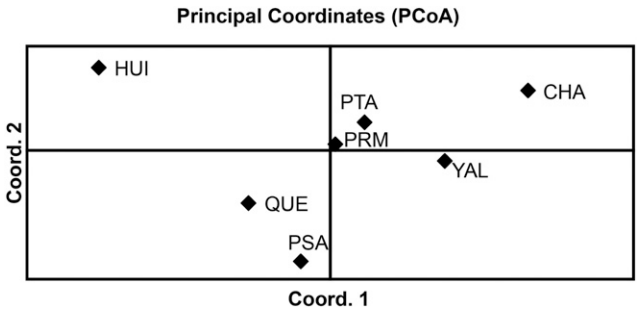

Figure 3. Principal components analysis of the results obtained from the nine microsatellite loci in the study sites.

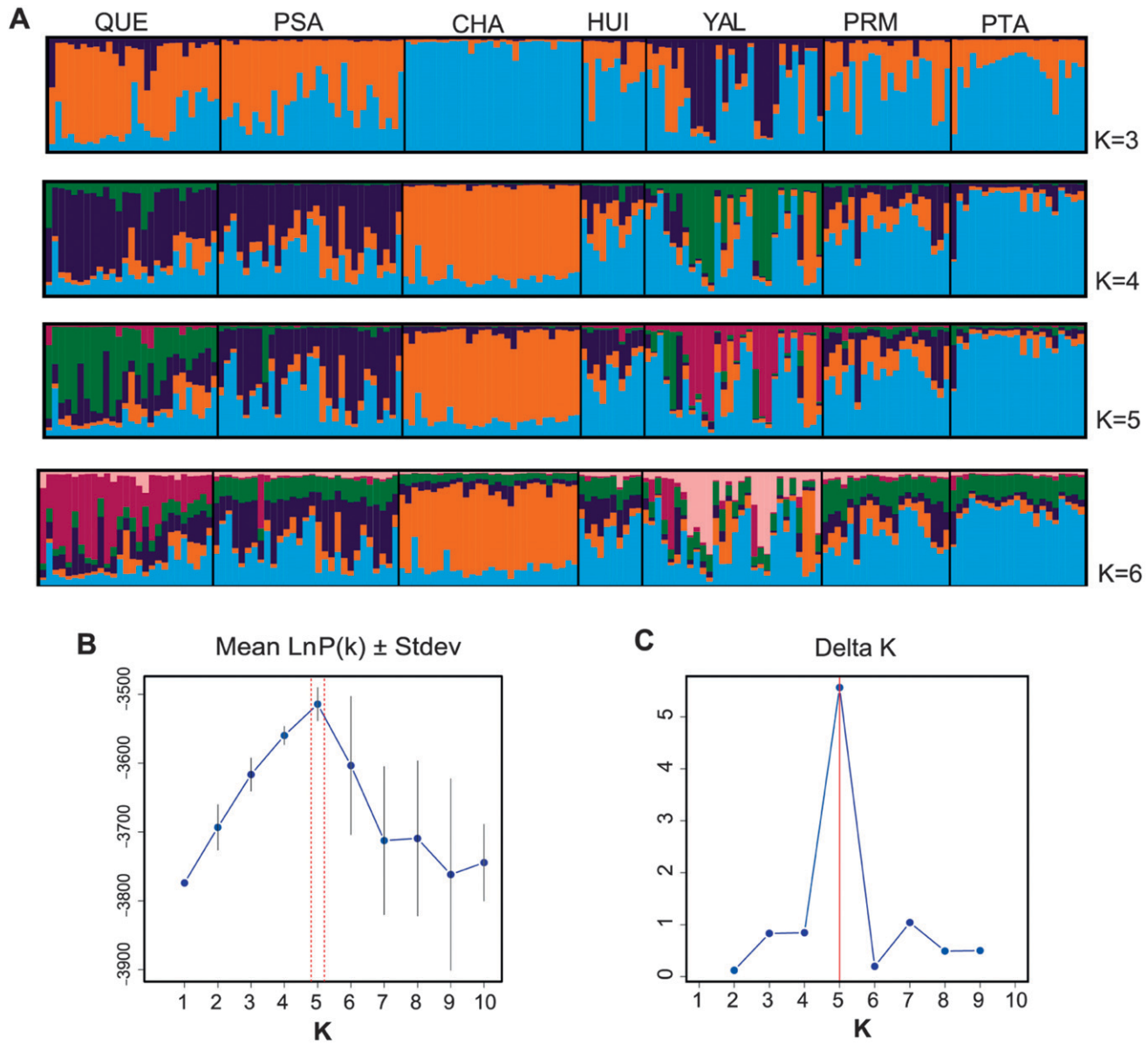

Figure 4. Structure analysis. (A) The most probable number of clusters from $K = 2$ to $K = 6$. Each graph represents the estimated percentage ancestry of each individual in a hypothetical color-coded population (Y axis) grouped by collection site names (X axis) as provided in Table 1. (B, C) Evanno et al. (2005) plots for detecting the number of K groups that best fit the data, mean likelihood $L(K)$, and variance per K value from structure.

than have previously been observed for this species. The values observed in this work show greater genetic differentiation than those recorded for the same species using other types of markers such as SNP (Fst = 0.005) (Araneda et al. 2016) and allozymes (Fst = 0.011–0.055) (Toro et al. 2006), but less than that observed using RAPD (Gst = 0.244) (the estimate from the data of Toro et al. 2004). The estimates of genetic structure in this work were also higher than those observed by Diz and Presa (2008, 2009) for *Mytilus galloprovincialis* on the shores of Galicia (Fst = 0.012), and on the Atlantic and Mediterranean coasts of the Iberian Peninsula (Fst = 0.0306), estimated using six microsatellites. The estimates are also higher than those reported by Ouagajjou and Presa (2015) for *M. galloprovincialis* on the Atlantic coast of Morocco (Fst = 0.012) and between Atlantic Morocco and Alboran Morocco (Fst = 0.038). The relatively higher differentiation observed between populations of mussels along the Chilean coast may be due to the use of different types of molecular markers (higher resolution microsatellites) and because the sampled populations represent a larger scale than in the aforementioned works (2,000 km among extreme localities), with the exception of Diz and Presa (2008), who analyzed samples spaced around 4,000 km apart and observed two highly differentiated groups.

Analysis of genealogical relationships from the CO1 gene provides further insight into potential gene flow patterns. The haplotype network shows the presence of different haplotypes in individuals from all locations along the coastal range studied, which have developed from three high-frequency central haplotypes. This pattern provides evidence that the mussel has shared haplotypes, suggesting historical connectivity between

natural beds, in contrast with the situation observed in macroalgae (Tellier et al. 2009) or barnacles (Zakas et al. 2009).

Along the southeastern Pacific coast of Chile, high differentiation has been noted in other species, for which clines or breaks in distribution have been observed; for example, genetic breaks associated with geographical breaks have been detected along the Chilean coast in species of macroalgae such as *Lessonia nigrescens* (Tellier et al. 2009). A restriction of gene flow has been observed in barnacles related with biogeographical and oceanographic transition zones (Zakas et al. 2009). In contrast to these species, network analysis shows the absence of any clines or genetic breaks across the distribution of *Mytilus chilensis*, which is corroborated by the overall differentiation detected by the Fst estimator. This is principally explained by sharp differences between certain locations (specific pairwise comparisons), for example, PSA representing the far north of the range; PTA in the far south; CHA, an intermediate site; and to a lesser degree HUI, the only marine reserve.

Given the genetic data described earlier for other marine species on the Chilean coast, it is proposed that there are three major factors that may be driving the diversity patterns observed among mussel populations on the Chilean coast: dispersal potential, oceanographic conditions, and high reproductive or recruitment variance. First, *Mytilus chilensis* has a long larval stage lasting 30–40 days, suggesting a higher dispersal potential than is observed for other species examined from the Chilean coast, including the macroalga *Lessonia nigrescens* (Tellier et al. 2009) and barnacles (Zakas et al. 2009), both of which have short dispersal distances. Because of this factor, greater gene flow is expected for this species, as is observed in the number of migrants estimated, and in the population differentiation and structure results.

Second, oceanography may play a large role in the genetic patterns observed. The Chilean coast is affected by the superficial influence of a mass of subantarctic water associated with the West Wind Drift, which enters the eastern Pacific from the west at latitude 43–45° S, where the YAL and PRM sites are located. This current then divides into two parts: a northern, oceanic flow called the Chile–Peru current (Bernal et al. 1983) or the Humboldt current system; and a southerly flow called the Cape Horn current, which follows the southeastern Pacific coast, and for the purposes of this work only affects the PTA site. This current may explain the greater differentiation observed at the last location because its flow direction generates a degree of isolation from the other sites; this would coincide with the observation of isolation by distance found for this site. Punta Arenas has been previously described as having a different population of *Mytilus chilensis* by Toro et al. (2004, 2006, using RAPD-PCR and allozymes, respectively), in which these differences as being caused by ocean currents are explained. Furthermore, southward from 41° S, the coast changes drastically, forming an island system known as the Chilean archipelago in which the coastline is broken up into numerous gulfs, channels, and fjords (Camus 2001). The system here is strongly influenced by rivers, forming estuarine environments with a marked pycnocline (Pickard 1971, Silva et al. 1995), whereas tidal flows and strong winds cause local retention processes in the channels and fjords (Valle-Levinson et al. 2001, Cáceres et al. 2003, Cáceres & Valle-Levinson 2004, Valle-Levinson & Blanco 2004). These oceanographic processes could reduce the real dispersal of the larvae. Furthermore, each channel or fjord presents specific ecological and physical conditions, leading to local processes that may accentuate differentiation. The locations of HUI, located in a fjord; YAL, which is a bay; PRM, located in a fjord; and PTA, located on the Magellan Strait, all lie in this zone, strongly influenced by rivers, tidal flows, and strong winds. All these sites present unique, differentiated geomorphological and oceanographic characteristics, which could favor larval retention and genetic differentiation between locations. The genetic differences between these sites were most marked in PTA in the far south, and HUI, located in the narrow Comau Fjord.

Finally, the phenomenon of high reproductive and recruitment variance has been observed in other marine species with broadcast spawning and long-distance larval dispersal, and can be detected using high variability markers, such as microsatellites, which provide greater resolution (Hedgecock 1994, Moberg & Burton 2000, Robainas-Barcia et al. 2008, Calderón et al. 2012, Kesäniemi et al. 2014). Because connectivity exists between the sites examined in this study, the finding of genotypic differences between certain locations is unlikely to result from isolation between locations, but instead may be driven by the very high reproductive variance between one year and another, leading to differences between cohorts. This proposal could be investigated in future work.

In conclusion, the genetic analysis of *Mytilus chilensis* from along the Chilean coast shows that genetic diversity can be higher in protected areas where they are less exploited; it also shows a complex pattern of spatial population differentiation. Observations of relatively high gene flow suggests the possibility of larval exchange between natural mussel beds distributed along the study zone of the Pacific coast; the local genetic differentiation detected in some sites may result from oceanographically or geomorphologically driven larval retention, or differential recruitment. Thus, because these natural mussel beds sustain a very large aquaculture industry, it is suggested that measures to protect the beds or regulate extraction should be introduced to ensure their maintenance over time, because they already display high genetic differentiation according to some estimators. The maintenance of the existing HUI MPA and the creation of new MPA may help to ensure the maintenance of these beds over time. Multiple use coastal MPA (MU-MPA), where fishing and resource extraction are permitted, principally by local artisanal fishing communities (Costello & Ballantine 2015), are common in Chile. It is very important to maintain such areas along the coast to ensure that biodiversity is sustained, including species which are extracted as resources (Gelcich et al. 2009, Costello & Ballantine 2015, Gelcich & Donlan 2015).

The sustainability of these natural beds used both in fishing and aquaculture, can be ensured for the future through the tracking and monitoring of their genetic diversity and population structure to evaluate their continued capacity to support the aquaculture of this resource. These results will allow the future needs to be defined for MPA containing natural beds of this species with sufficient levels of genetic diversity.

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