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Short Communication

Allozymic variation and differentiation in the chilean blue mussel, *Mytilus chilensis*, along its natural distribution

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Abstract

Genetic differentiation in the Chilean blue mussel *Mytilus chilensis* (Hupé 1854) was investigated based on the variation in the allozyme frequencies of *Pgm, Gpi, Icd, Me, Gsr, Lap* and *Pep* in eight samples collected along 1800 km from Arauco (VIII Region) to Punta Arenas (XII Region). Despite the large geographic separations, values of Neis unbiased genetic distance, D (0.004-0.048) and standardised genetic variation among populations, Fst (0.011-0.055) were small. The levels of gene flow (Nm = 8) found in this study prevent the effect of differentiation among populations by genetic drift. This findings indicate that its long-lived planktotrophic larvae provides this species with considerable dispersal ability throughout its range which is favoured by the ocean currents along the chilean coast. In terms of management of the *M. chilensis* fishery, the results provide no evidence for discrete stocks, with the possible exception of the Punta Arenas population. Considering the intensive aquaculture activities with this species the present study provide preliminary data which can be used as a baseline for further characterization and /or monitoring these mussel populations.

Key words: allozyme, *Mytilus chilensis*, population genetics, gene flow, Chile.

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The Chilean blue mussel (*Mytilus chilensis*, Hupe 1854) is an economically important resource in southern Chile, commonly known as chorito or quilmahue. It is widely distributed on hard substrata in the lower intertidal zone to 25 m depths, along the chilean coast line (Brattström and Johanssen, 1983). The range of the species extends over 40° of latitude from Arica to Cape Horn (Lancellotti and Vázquez, 2000) and it is impacted by a major oceanographic feature, the West Wind Drift (43°S) that defines the boundary between the main current directions along the chilean Coast (Strub and Mesías, 1998). Winter *et al.* (1984), determined that there was an annual reproductive cycle, with spawning occurring during spring and summer. Toro and Sastre (1995) and Toro *et al.* (2004) have shown that *M. chilensis* possesses a planktotrophic larval stage with a pelagic existence of 45 d. The species thus has the potential for long-distance dispersal over a scale of hundreds of kilometers along the chilean coast. Its culture began in 1943 in the Chiloé Island, southern Chile (Osorio *et al.*, 1979) and the aquaculture production raised from 3,864 t in 1993 to 41,406 t in 2001. Despite this explosive development of *M. chilensis* aquaculture, population genetics studies in the chilean mussel (*Mytilus chilensis*) are scarce in the literature. Only a few studies with limited sampling are reported in the scientific literature mainly focused to dilucidate the distribution of the *Mytilus edulis* species complex (Koehn, 1991; McDonald *et al.*, 1991).

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An important question, from both evolutionary and fishery amangement perspectives, is the extent of gene flow and associated genetic differentiation among *Mytilus chilensis* populations at a macrogeographic (over 100 km) scale. Marine species with longer larval phases are generally though to disperse further and have more gene flow, larger geographic ranges, lower levels of genetic differentiation among populations, and high levels of genetic variation within populations (Scheltema and Williams, 1983; Waples, 1987; Palumbi, 1995; Williams and Benzie, 1993). However, expected patterns of gene flow and population connectivity in *Mytilus chilensis* could be altered by oceanographic features peculiar to its range, such as the presence of the west wind drift (WWD) which bisects the flow of currents along the chilean coast (Strub and Mesías, 1998). Also, human mediated dispersal may have heavely affected the levels of genetic variation in several southern Chile stocks, because most of the mussel cultures used juveniles (seed) transferred from Yaldad Bay (Winter *et al.*, 1984) that may caused in some locations the restocking of local natural populations with juveniles from the Yaldad population.

In this paper we examine the genetic structure of *Mytilus chilensis* collected from eight widely separated (up to 1800 km) sites along the chilean coast, using allozyme electrophoresis to establish the extend of gene flow and levels of genetic differentiation.

Samples of mussels were collected by dredging or by diving at eight localities extending over 1800 km along the chilean coast (covering the whole range of the species natural distribution) from Arauco (VIII Región) to Pta. Arenas (XII Región) (Figure 1). The mussels (N = 120 from each population) were delivered alive to the laboratory where they were immediately dissected and isolated tissues were stored in liquid nitrogen for subsequent electrophoretic analysis.

Tissue samples from abductor muscle and hepatopancreas were homogenized in 50-100 µL of 0.05 M Tris-HCl buffer, pH 8.0. Standard techniques of horizontal starch (12%) gel electrophoresis were assayed in the following loci: PGM (2.7.5.1), GPI (5.3.1.9), LAP (3.4.11.1), EST (3.1.1.1), ODH (1.5.1.11), XDH (1.2.1.37), Alfa GPD (1.1.1.8), MDH (1.1.1.37), ME (1.1.1.40), AAT (2.6.1.1), PEP (3.4.11.13), IDH (1.1.1.42), ICD (1.1.1.2), MPI (5.3.1.8) y GSR (1.1.4.2). Starch gels were stained according to (Shaw and Prassad, 1970; Harris and Hopkinson, 1976; Selander *et al.*, 1983; Beaumont and Toro, 1996). Alleles were designated alphabetically according to their migration relative to the most common electromorph. A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. Data were analysed using the computer programs POPGENE V. 1.31 (Yeh and Boyle, 1997) and GENETIX V 4.05.2. These programs were used to test for Hardy-Weinberg equilibrium, to provide F-statistics with their significance and Neis (Nei, 1978) unbiased genetic distances (D) and identities (I). A UPGMA cluster analysis was performed using I values. The Mantel test using 50,000 randomisations was carried out for association between geographic distance and the genetic distance, using PopTools (Hood, 2004).

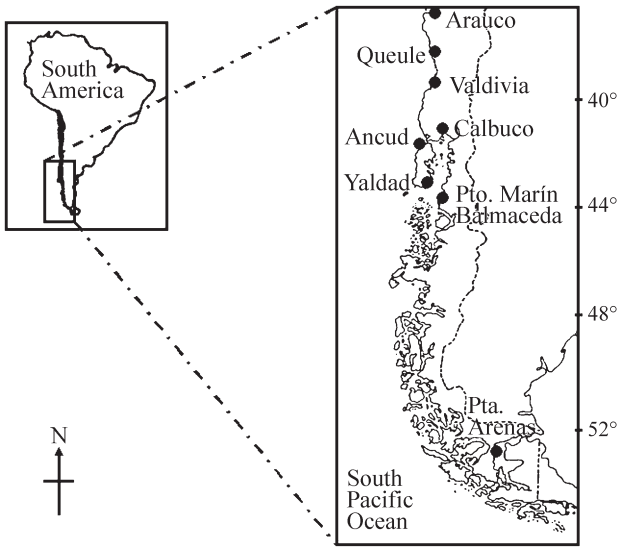

Figure 1 - Locations of the eight natural populations of the chilean blue mussel (*Mytilus chilensis*) sampled along its whole natural distribution in southern Chile (•).

Only seven variable loci (40.8% range = 33.3-46.7%) differing in gene frequencies were found across the geographic samples, Leucine aminopeptidase, glucose phosphate isomerase, Malic Enzime, Peptidase, Isocitrate Deshidrogenase, Glutatione reductase and phospho glucomutase. The allele frequencies for the seven polymorphic

Table 1 - Allele frequencies for the seven polymorphic loci screened in eight populations of the chilean blue mussel (*Mytilus chilensis*).

Locus allele	Arauco	Queule	Valdivia	Calbuco	Ancud	Yaldad	Pto. M.B.	Pta. Arenas
PGM								
n	109	108	116	110	99	128	99	100
A	0.0275	0.0694	0.0603	0.0136	0.0101	0.0078	0.0051	0.2950
B	0.7936	0.7454	0.7802	0.7864	0.8283	0.7969	0.8384	0.4650
C	0.1786	0.1852	0.1595	0.2000	0.1616	0.1953	0.1566	0.2350
D	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0050
GPI								
n	112	80	102	110	110	111	99	107
A	0.1071	0.0312	0.0294	0.0955	0.0909	0.0721	0.1263	0.1262
B	0.7277	0.8625	0.8775	0.8000	0.6636	0.8423	0.7727	0.5794
C	0.1607	0.1062	0.0931	0.1045	0.2455	0.0856	0.1010	0.2944
D	0.0045	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ICD								
n	112	108	115	110	110	141	99	107
A	0.0268	0.0417	0.0087	0.0500	0.0227	0.0177	0.0253	0.0327
B	0.9375	0.9444	0.9565	0.8773	0.9545	0.9645	0.9646	0.9252
C	0.0357	0.0139	0.0348	0.0727	0.0227	0.0177	0.0101	0.0421

Table 1 (cont.)

Locus allele	Arauco	Queule	Valdivia	Calbuco	Ancud	Yaldad	Pto. M.B.	Pta. Arenas
ME								
n	112	108	116	110	110	111	98	106
A	0.0223	0.1852	0.1078	0.0227	0.0318	0.0721	0.0816	0.0377
B	0.1652	0.2778	0.3233	0.2455	0.1864	0.2973	0.2959	0.2311
C	0.4554	0.2454	0.2974	0.4182	0.3727	0.3874	0.2959	0.3962
D	0.2857	0.1806	0.2112	0.2818	0.2364	0.2207	0.2500	0.2406
E	0.0714	0.1111	0.0603	0.0318	0.1727	0.0225	0.0765	0.0943
GSR								
n	110	108	116	110	110	111	97	107
A	0.0227	0.0000	0.0086	0.0364	0.0182	0.0450	0.0155	0.0514
B	0.9409	0.9306	0.9181	0.9545	0.9812	0.9505	0.8969	0.8972
C	0.0364	0.0694	0.0733	0.0091	0.0000	0.0450	0.0876	0.0514
LAP								
n	111	106	116	112	107	115	93	100
A	0.2252	0.2736	0.2069	0.0938	0.1963	0.1565	0.2312	0.2600
B	0.5090	0.5330	0.4784	0.4777	0.5234	0.5217	0.6720	0.4800
C	0.2432	0.1887	0.2802	0.3438	0.2523	0.2783	0.0968	0.2400
D	0.0225	0.0047	0.0345	0.0848	0.0280	0.0435	0.0000	0.0200
PEP								
n	111	108	107	112	80	136	96	101
A	0.1486	0.4815	0.5047	0.3705	0.2313	0.4449	0.4583	0.3119
B	0.3874	0.4398	0.3458	0.4062	0.4750	0.4338	0.4583	0.4208
C	0.3604	0.0648	0.1262	0.1786	0.2938	0.1176	0.0833	0.1881
D	0.1036	0.0139	0.0234	0.0446	0.0000	0.0037	0.0000	0.0792

Key: [PGM: phosphoglucomutase; GPI: glucose phosphate isomerase; IDH: isocitrate dehydrogenase; ME: malic enzyme; GSR: glutathione reductase; LAP: leucine aminopeptidase; PEP: peptidase]; n = number of specimins scored.

loci in each population analysed are shown in Table 1. The ME locus was the most variable in all eight populations of *Mytilus chilensis*, followed by LAP and PEP (Table 1). The same common allele at the ICD and GSR loci were the most frequent in all populations, with two other alleles present at lower frequencies. Three alleles were present for PGM and GPI loci, with one allele predominant in all populations, a fourth rare allele was found for PGM in Pta. Arenas and for GPI in Arauco (Table 1).

Mean number of alleles per locus ranged from 3.28 to 3.71 among the eight populations of *M. chilensis*. Mean heterozygosities for the populations ranged from 25.0 to 32.0 (Table 2) with a generalized heterozygote deficiency in all loci in Hardy-Weinberg disequilibrium (Table 2). Genetic distances, D, among eight populations of *M. chilensis* were on the whole small, ranging from 0.003 to 0.048 (Figure 2). A Mantel test using 50,000 randomisations showed evidence for a significant correlation ($p < 0.05$) between genetic and geographic (coastal) distance (with and without the the inclusion of Punta Arenas population).

The standarized variance of gene frequencies that measures the fractional reduction in heterozygosity relative to random mating was small ($Fst = 0.0303$, $p < 0.05$). In fact, only 3.0% of the variance in gene frequency was atributable to among-population variation whereas most differences found within populations. The estimates of the individual reduction in heterozygosity relative to its population indicate an excess of homozygotes ($Fis = 0.29$, $p < 0.05$), observed at many loci across all populations (Table 2). The migration rate deduced from the Fst value suggested a theorical exchange of $Nm = 8$ individuals per generation between pair of samples.

A cluster analysis based on the unweighted pair group method and Neis co-efficient of unbiased genetic identity (Nei, 1978) showed that all populations were genetically quite similar, with the Pta. Arenas population most distinct (Figure 2).

A hight genetic (allozymic) variation has been reported in marine organisms (Ayala *et al.*, 1973; Levington, 1975; Koehn *et al.*, 1976). The 40.8% polymorphism reported here is within the range reported for other marine invertebrates (Nevo *et al.*, 1984). The mean heterozygosity level in *M. chilensis* (29.7%) is within the range of variation reported for other bivalves (Buroker *et al.*, 1979a, 1979b; McDonald *et al.*, 1991; Toro and Vergara, 1995).

Heterozygote deficiency has been widely reported in marine invertebrates (Zouros and Foltz, 1984; Mallet *et al.*, 1985; Gaffney *et al.*, 1990; Beaumont, 1991; Toro and

Table 2 - Genetic variability measures for eight populations of *Mytilus chilensis*.

	Arauco				Queule			
Locus	Ho	He	Hd		Ho	He	Hd	
PGM	0.33	0.33	0.00	ns	0.36	0.40	-0.10	*
GPI	0.38	0.43	-0.11	a	0.25	0.24	0.04	ns
ICD	0.03	0.11	-0.72	*	0.03	0.10	-0.70	*
ME	0.18	0.31	-0.41	*	0.70	0.78	-0.10	*
GSR	0.10	0.11	0.00	ns	0.10	0.12	-0.16	*
LAP	0.49	0.63	-0.22	*	0.50	0.60	-0.17	*
PEP	0.54	0.68	-0.20	*	0.29	0.57	-0.49	*
Means	0.29	0.37	-0.30		0.32	0.4	-0.20	
	Valdivia				Calbuco			
Locus	Ho	He	Hd		Ho	He	Hd	
PGM	0.28	0.36	-0.22	*	0.28	0.34	-0.17	a
GPI	0.16	0.22	-0.27	*	0.34	0.34	0.00	ns
ICD	0.00	0.08	-1.00	*	0.02	0.22	-0.90	*
ME	0.62	0.74	-0.16	*	0.34	0.68	-0.50	*
GSR	0.14	0.15	-0.06	ns	0.05	0.08	-0.37	*
LAP	0.53	0.64	-0.17	*	0.57	0.63	-0.09	*
PEP	0.42	0.60	-0.30	*	0.50	0.66	-0.24	*
Means	0.31	0.4	-0.22		0.30	0.42	-0.28	
	Ancud				Yaldad			
Locus	Ho	He	Hd		Ho	He	Hd	
PGM	0.34	0.28	0.21	a	0.32	0.32	0.00	ns
GPI	0.44	0.49	-0.10	*	0.29	0.27	0.07	a
ICD	0.01	0.08	-0.87	*	0.05	0.06	-0.16	*
ME	0.40	0.73	-0.45	*	0.35	0.70	-0.50	*
GSR	0.01	0.03	-0.66	*	0.08	0.09	-0.11	ns
LAP	0.56	0.62	-0.09	*	0.42	0.62	-0.32	*
PEP	0.46	0.63	-0.26	*	0.33	0.60	-0.45	*
Means	0.32	0.41	-0.21		0.26	0.38	-0.31	
	Pto. M. Balmaceda				PTA. Arenas			
Locus	Ho	He	Hd		Ho	He	Hd	
PGM	0.24	0.27	-0.11	ns	0.18	0.64	-0.71	*
GPI	0.36	0.37	-0.02	ns	0.47	0.56	-0.16	*
ICD	0.01	0.06	-0.83	*	0.01	0.14	-0.92	*
ME	0.58	0.74	-0.21	*	0.43	0.72	-0.40	*
GSR	0.02	0.18	-0.75	*	0.07	0.18	-0.61	*
LAP	0.20	0.48	-0.58	*	0.41	0.64	-0.35	*
PEP	0.35	0.57	-0.38	*	0.52	0.68	-0.23	*
Means	0.25	0.38	-0.34		0.30	0.51	-0.41	

Key: Ho = observed heterozygosity (direct count); He +expected heterozygosity; Hd, degree of departure of genotypic frequencies from Hardy-Weinberg expectations where Hd = (Ho- He)/He (Selander, 1970).
[PGM: phosphoglucomutase; GPI: glucose phosphate isomerase; IDH: isocitrate dehydrogenase; ME: malic enzyme; GSR: glutathione reductase; LAP: leucine aminopeptidase; PEP: peptidase].
*: Significant deviation from expected at $p < 0.05$. ns: Non significant. a : No test possible, less mussels than expected for one genotypic class.

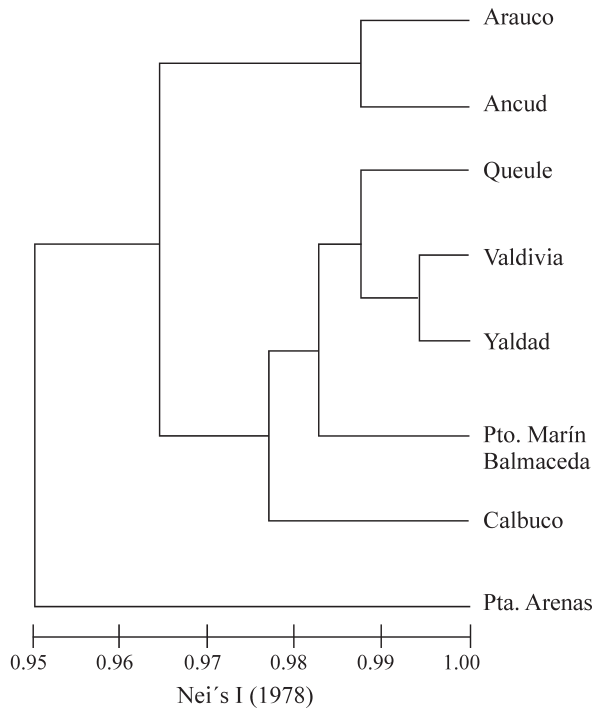

Figure 2 - A phenetic dendrogram showing the relationship of eight populations of the chilean blue mussel (*Mytilus chilensis*), based on seven polymorphic loci.

Vergara, 1995). Significant heterozygote deficiency was also found in this study, and corroborate by the positive Fis (0.29, P) which indicates and excess of homozygous animals within each population. There are several possible explanations (relatively high levels of inbreeding, null alleles, aneuploidy, molecular imprinting, selection, Wahlund effect) which have been discussed extensively elsewhere (Fujio *et al.*, 1983; Zouros and Foltz, 1984; Mallet *et al.*, 1985; Gaffney *et al.*, 1990; Beaumont, 1991; Toro and Vergara, 1995). No one of these seems either sufficient or plausible alone for *M. chilensis*, and the overall deficit probably results from several small effects, all tending to reduce heterozygosity, which add to a significant overall deficit. Nevertheless, the causal mechanism of the heterozygote deficiency reported here remain to be studied.

Very low genetic diffentiation was found among eight populations of *M. chilensis* sampled over distances greater than 1800 km along the Chilean coastline (D = 0.003-0.048, Fst = 0.01-0.05). This indicate high levels of gene flow and suggests that these populations act as a single interbreeding population. However, one population sampled in the Magellanican Channel, Pta. Arenas, was genetically distinct from the others, although the scale of differentiation was not large (D = 0.032-0.048). This differentiation, even low could be the result of the West Wind Drift (WWD) and the Cape Horn Current (Strub and Mesías, 1998) which precludes the larval transport towards the northern mussel populations. A Mantel test excluding the Punta Arenas population gave a significan correlation

value ($r = 0.113$, $p < 0.05$), indicating more evidence of an isolation by distance scenario.

Theory predicts that a global value of $Nm > 1$ prevents random differentiation by genetic drift (Slakin, 1987). Thus the migration rate reported here ($Nm = 8$) suggest that some deterministic factors are involved in the among population differentiation of *M. chilensis*. Our results seems to indicate that the long lived (45 d) larvae of *M. chilensis* provide the species with considerable dispersal ability throughout its range, and that this dispersal is not affected but enhanced by oceanographic features such as the WWD and several coastal currents along the Chilean coastline (Strub and Mesías, 1998; Camus, 2001). Indeed, the very low genetic differentiation between such populations as Valdivia and Yaldad, Queule and Yaldad, Ancud and Arauco (Figure 2) suggests that larval transport along the coast does take place, perhaps in stepping-stone fashion, with each generation of larvae dispersing short distances along the coast, especially under the 45°S tending to homogenize the populations with the exception of the one from Pta. Arenas.

Interpopulational genetic homogenity has been described for other marine invertebrates along the Chilean coastline (Gallardo and Carrasco, 1996; Toro and Aguila 1996; Gallardo *et al.*, 1998). Even when the results from the present study are consistent with the life-history features of the chilean blue mussel with a very prolonged larval life span (40-45 d) suggesting a potential for genetic homogenization over large geographical distances, we must keep in mind that this is a farming species, so there is a lot of gene flow through "seed" transplantation and restocking of juveniles (human activity), especially from Yaldad Bay, which is the main natural source of juveniles for culturing purposes (Winter *et al.*, 1984) to other localities in southern Chile.

In terms of management of the *M. chilensis* fishery, our results provided no evidence for discrete stocks with the possible exception of the Pta. Arenas population. Management practices for this species thus can be independent of macrogeography, and they should be focused on a smaller spacial scale, dealing with differentiation of populations caused by environmental factors. Finally, our survey for natural populations of *M. chilensis* along the coastline of Chile gave as a result that the natural distribution of this species described by Lancellotti and Vásquez (2000) is actually highly reduced at its northern border from the 18°S to 35°S. Further sampling will be carried out within the same localities to evaluate and/or monitoring the genetic variation of these populations, using PCR based DNA markers (RAPDs).

Acknowledgments

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