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Note

Genetic differentiation of populations of the oyster *Ostrea chilensis* in southern Chile

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Différentiation génétique des populations de l'huître Ostrea chilensis *du Sud du Chili*

Population genetics studies in the Chilean oyster (*Ostrea chilensis*) are scarce in the literature. Guiñez and Gallegillos (1984, 1985, 1986) and Guiñez *et al.* (1986) report some studies using the Carbonic anhydrase locus in *O. chilensis*, to evaluate relationships between size, heterozygosity, and clinal variation in morphological distance between genotypes at the carbonic anhydrase locus. Recently, Toro and Vergara (1995) report that, in the Chilean oyster, heterozygote deficiencies in a cohort obtained by mass spawning in the laboratory are not randomly distributed in time among genotypes. *O. chilensis* is a brooding oyster with limited larval dispersal capabilities because their extremely short pelagic stage (Toro and Chaparro, 1990; Toro *et al.*, 1995), thus genetic differentiation among populations is assumed to be a consequence of restricted gene flow. The range of species distribution for the Chilean oyster is restricted to the Chiloé Island and southern islands such as Las Guaitecas (Winter *et al.*, 1984). Most of the populations within the northern range of the species distribution have suffered strong declines due to overfishing (Toro and Chaparro, 1990) that caused in some locations the restocking with oysters from other local populations, usually from Pullinque population. These human mediated dispersal may have affected the levels of genetic variation in several northern stocks.

The purpose of this study was to determine the degree of genetic subdivision in the Chilean oyster by examining the geographic distribution of allozymes.

MATERIALS AND METHODS

Samples of oysters (*Ostrea chilensis* Philippi, 1845) were collected by dredging or by diving at four locations within the Chiloé island and one location (Melinka) in the Guaiteca Islands (*fig.* 1) covering the whole range of the species natural distribution. The oysters (N=150 from each population) were delivered alive to the laboratory where they were immediately dissected and isolated tissues were stored at −30°C for subsequent electrophoretic analysis. Since highly polymorphic loci give more information on the genetic structure of populations, four polymorphic loci (Toro and Vergara, 1995) were chosen. Horizontal starch (12%) gel electrophoresis was used to score the loci leucine aminopeptidase (LAP, Enzyme Commission number 3.4.1.1), glucose phosphate isomerase (GPI, EC 5.3.1.9), carbonic anhydrase (CA, EC 4.2.1.1) and phosphoglucomutase (PGM, EC 2.5.7.1) following Shaw and Prasad (1970), Selander *et al.* (1971) and Ahmad *et al.* (1977).

Statistical computer analysis of electrophoretic data were conducted using BIOSYS-1 (Swofford and Selander, 1981). The pairwise comparisons between populations were carried out using Nei's (1972) genetic identity (*I*) and were illustrated by UPGMA cluster analysis.

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Table 1. – *Ostrea chilensis.* Allelic frequencies, locus heterozygosities (h), sample size (N) for 4 loci analysed in five Chilean oyster populations.

Enzyme	Locus		Population				
			Pullinque	Quempillén	Rilán	Yaldad	Melinka
Leucine aminopeptidase	LAP						
		N	147	150	150	143	145
		90	0.316	0.265	0.382	0.045	0.244
		100	0.684	0.735	0.618	0.943	0.756
		110	0.000	0.000	0.000	0.011	0.000
		h	0.347	0.449	0.529	0.114	0.400
Carbonic anhydrase	CA						
		N	149	149	149	141	143
		100	0.653	0.400	0.667	0.802	0.167
		150	0.102	0.260	0.088	0.198	0.389
		200	0.245	0.340	0.245	0.000	0.444
		h	0.449	0.580	0.275	0.302	0.556
Glucose phosphate isomerase	GPI						
		N	150	150	149	145	146
		90	0.214	0.140	0.200	0.655	0.389
		100	0.786	0.860	0.800	0.345	0.611
		h	0.306	0.280	0.240	0.310	0.511
Phosphoglucomutase	PGM						
		N	150	150	150	142	146
		95	0.286	0.245	0.382	0.720	0.136
		100	0.714	0.755	0.618	0.280	0.864
		h	0.204	0.345	0.412	0.317	0.273

RESULTS AND DISCUSSION

The allele frequencies for the four polymorphic loci in each population analysed are shown in *table* 1. The mean value of alleles per locus over all populations was 2.3. Measures of genetic variation at the four polymorphic loci studied in the five populations are given in *table* 2. We have found Hardy-Weinberg equilibrium at most of the loci examined in the samples. However, significant deficiencies of heterozygotes were found in the PGM and CA loci in the Pullinque and Rilán populations with D values of -0.505 and -0.443 respectively.

Some of the pairwise comparisons between populations show low values of Nei's genetic identity (I) indicating that the genetic differences between them are high. Most of the values are under 0.869 with the lowest value being 0.677 between the Yaldad population and the Melinka population. The highest value being 0.994 was found between Pullinque and Rilán. The genetic differences between populations, based on UPGMA cluster analysis of values of I, are illustrated in *figure* 2.

Levels of genetic variation, measured as observed heterozygosity ($H_0 = 0.360 \pm 0.056$, averaged over all five populations) are high compared to other molluscs (Beaumont, 1986; Leclair and Phelps, 1994), but in line with other species of oysters (Buroker, 1983). Fujio *et al.* (1983) reported mean heterozygosities ranging from 0.059 in *Ostrea circumpicta* to 0.216 in *Tiostrea lutaria*. The high genetic variability found in populations have been explained by the very unstable environment in which these populations inhabit (Ayala *et al.*, 1973; Johnson, 1974). The relatively large number of alleles per locus and the high average heterozygosity among populations in *O. chilensis* could suggests a great potential for genetic adaptation to environmental change (Leclair and Phelps, 1994).

Several studies of bivalve molluscs report an overall deficiency of heterozygotes (*e.g.* Zouros *et al.*, 1988; Gaffney *et al.*, 1990). However the mechanism or mechanisms producing this phenomenon have not been well defined. According to recent evidence of laboratory studies by Beaumont (1991) working with *Mytilus edulis* and Toro and Vergara (1995) working with *O. chilensis*, suggests that post-settlement selection against heterozygotes may be an important cause of heterozygote deficiencies. An alternative hypothesis, considering the human-mediated movements of oysters between at least two of the localities (Pullinque and Rilán) in the present study is the Wahlund principle. Deficiencies of heterozygotes at the PGM and CA are only significant in these two locations and the average D values shown in *table* 2 are also the greatest in these two locations. This could suggest a two-way movement producing admixtures in both populations.

The genetic identities displayed in *figure* 2, shows significant genetic differentiation between some of the populations sampled in this study. According

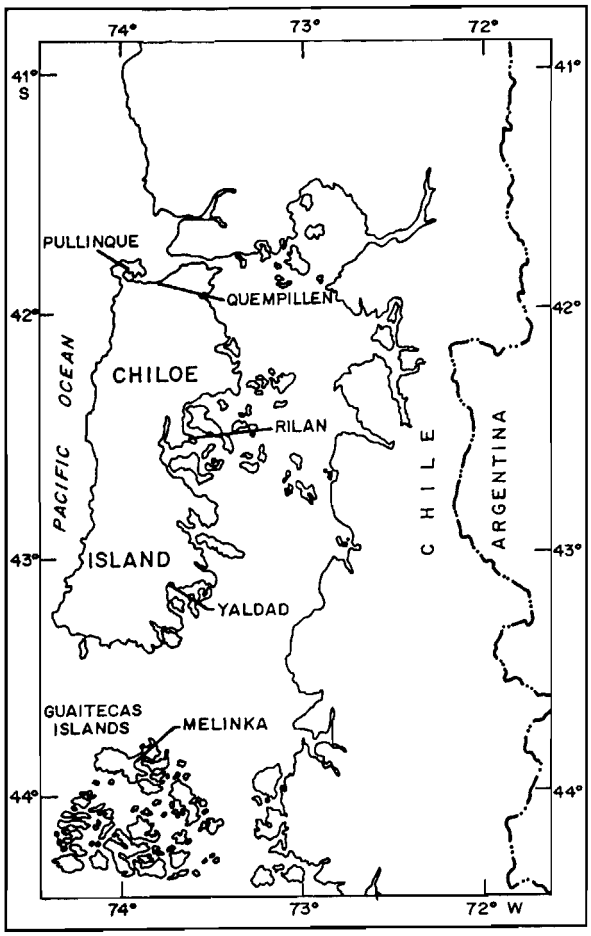

Figure 1. – Localities where *Ostrea chilensis* populations were sampled in the present study: Bay of Pullinque, Estuary of Quempillén, Channel of Rilán, Bay of Yaldad and Island of Melinka.

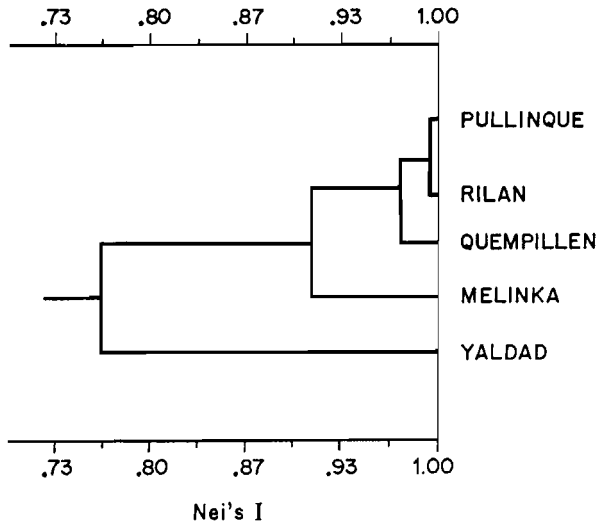

Figure 2. – UPGMA dendrogram of Nei's genetic identity (*I*) between 5 populations of *O. chilensis*.

Table 2. – *Ostrea chilensis*. Genetic variability at four loci in all populations. Mean (±S.E.) observed heterozygosity (H_0) and expected heterozygosity (H_e).

Population	H_0	H_e
Bay of Pullinque	0.327±0.051	0.424±0.035
Estuary of Quempilléen	0.414±0.065	0.419±0.088
Channel of Rilán	0.364±0.066	0.442±0.040
Bay of Yaldad	0.261±0.049	0.324±0.077
Island of Melinka	0.435±0.063	0.431±0.083

to Beaumont *et al.* (1993), the average value of Nei's *I* between populations of *Pecten maximus* (sampled between Scotland and Brittany, France) was 0.989±0.001 and differences between species range from 0.3 to 0.6 (Ayala, 1983). Amongst the oyster populations sampled in the present study, the mean *I* was 0.846±0.122, and even when it is derived from a small sample of the genome (4 loci), it is however, a value that cannot be expected to occur purely as a result of sampling error. These findings are consistent with the life-history features of *O. chilensis*. The very restricted larval life span (5 minutes to 20 hours, Toro and Chaparro, 1990) suggests a potential for genetic differentiation even over short geographical distances. From *figure* 2, it can be seen that there is a tendency for the populations from the northern Chiloé Island (Pullinque, Rilán and Quempillén), to cluster together separately from the other two to the south (Yaldad and Melinka). This pattern could be due mainly to unreported [Sanhueza (1994) personnal communication] transplantation and restocking of juveniles from the Pullinque population to the northern local populations.

CONCLUSION

We conclude that Chilean oysters have a relatively small amount of genetic variation within populations and a relatively large amount of genetic diversity among populations ($F_{is}=0.109$ and $F_{st}=0.138$). Significant differences in allelic composition exist among all five *O. chilensis* populations sampled. Our data demonstrate that *O. chilensis* along the southern coast of Chile are not a single, genetically homogeneous population Northern Chiloé island populations (Pullinque, Quempilléen and Rilán) represent sympatric populations which seems to be reproductively isolated from the southern populations (Yaldad and Melinka). Further sampling within the same areas would be necessary to evaluate the genetic change within areas to the diversity among locations.

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REFERENCES

Ahmad M., D. O. F. Skibinsky, J. A. Bearmore 1977. An estimate of the amount of genetic variation in the common mussel *Mytilus edulis*. *Biochem. Genet.* **15**, 833-846.

Ayala F. J., D. Hedgecock, G. S. Zumwalt, J. M. Valentine 1973. Genetic variation in *Tridacna maxima*, an ecological analog of some unsuccessful evolutionary lineages. *Evolution* **27**, 177-191.

Ayala F. J. 1983. Enzymes as taxonomic characters. In: Protein polymorphism: Adaptative and taxonomic significance. G. S. Oxford, D. Rollinson eds. Academic Press, London, 3-26.

Beaumont A. R. 1986. Genetics aspects of hatchery rearing of the scallop, *Pecten maximus* (L.). *Aquaculture* **57**, 99-110.

Beaumont A. R. 1991. Genetics studies of laboratory reared mussels, *Mytilus edulis*: Heterozygosity deficiencies, heterozygosity and growth. *Biol. J. Linn. Soc.* **44**, 273-285.

Beaumont A. R., C. Morvan, S. Huelvan, A. Lucas, D. Ansell 1993. Genetics of indigenous and transplanted populations of *Pecten maximus*: no evidence for the existence of separate stocks. *J. Exp. Mar. Biol. Ecol.* **169**, 77-88.

Buroker N. E. 1983. Population genetics of the American oyster *Crassostrea virginica* along the Atlantic coast and the Gulf of Mexico. *Mar. Biol.* **75**, 99-112.

Fujio Y., Y. Ryoichi, P. Smith 1983. Genetic variation in marine molluscs. *Bull. Jpn. Soc. Sci. Fish.* **49**, 1809-1817.

Gaffney P. M., T. M. Scatt, R. K. Koehn, W. J. Diehl 1990. Interrelationships of heterozygosity, growth rate and heterozygote deficiencies in the coot clam, *Mulinia lateralis*. *Genetics* **124**, 687-699.

Guiñez R., R. Gallegillos 1984. Variation at carbonic anydrase (CA) locus in relation to geographic distribution and morphology of *Ostrea (=Tiostrea) chilensis*. *Arch. Biol. Med. Exp.* **18**, 69.

Guiñez R., R. Gallegillos 1985. Clinal variation in morphological distance between genotypes at the carbonic anhydrase locus in the Chilean oyster, *Tiostrea chilensis* (Philippi, 1845) Chanley and Dinamani, 1980. *Brazil. J. Genet.* **3**, 609-616.

Guiñez R., R. Gallegillos 1986. Variaciones de la heterocigosidad en el locus enzimático anhidrasa carbónica entre clases de tamaño en poblaciones de la ostra chilena (*Tiostrea chilensis*). *Boletín de la Sociedad de Biología de Concepción* **57**, 63-68.

Guiñez R., A. Monsalve, R. Gallegillos 1986. Correlaciones genético morfológicas en la ostra Chilena, *Tiostrea chilensis* (Philippi, 1845) Chanley and Dinamani 1980, del banco de Pullinque (Ancud, Chiloé), *Biol. Pesq.* (Chile) **15**, 17-25.

Johnson G. B. 1974. Enzyme polymorphisms and metabolism. *Science* **184**, 28-37.

Leclair L. L., S. R. Phelps 1994. Genetic characteristics and relationships of five razor clam (*Siliqua patula* Dixon) populations along the Pacific coast of North America. *J. Shellfish Res.* **13**, 207-216.

Nei M. 1972. Genetic distance between populations. *Am. Nat.* **106**, 283-292.

Selander R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, J. B. Gentry 1971. Biochemical polymorphism and systematics in the genus *Perumyscus*. I. Variation in the oldfield mouse (*Peromyscus polionotus*). University of Texas Publication n° 7103. *Stud. Genet.* **6**, 49-90.

Shaw C. R., R. Prasad 1970. Starch gel electrophoresis of enzymes. A compilation of recipes. *Biochem. Genet.* **4**, 292-520.

Swofford D. L., R. B. Selander 1981. BIOSYS-1: a Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Heredity* **72**, 281-283.

Toro J. E., O. R. Chaparro 1990. Conocimiento biológico de *Ostrea chilensis* Philippi, 1845. Impacto y perspectivas en el desarrollo de la ostricultura en Chile. In: Cultivo de Moluscos en America Latina. A. Hernandez ed. CIID-Canadá, Bogotá, Colombia, 231-264.

Toro J. E., A. M. Vergara 1995. Evidence for selection against heterozygotes: post-settlement excess of allozyme homozygosity in a cohort of the Chilean oyster; *Ostrea chilensis* Philippi, 1845. *Biol. Bull.* **188**, 117-119.

Toro J. E., M. A. Sanhueza, J. E. Winter, P. Aguila, A. M. Vergara 1995. Selection response and heritability estimates for growth in the Chilean oyster *Ostrea chilensis* (Philippi, 1845). *J. Shellfish Res.* **14**, 97-92.

Winter J. E., J. E. Toro, J. M. Navarro, G. S. Valenzuela, O. R. Chaparro 1984. Recent developments, status and prospects of molluscan aquaculture on the Pacific coast of South American. *Aquaculture* **39**, 95-134.

Zouros E., M. Romero, A. Mallet 1988. Heterozygosity and growth in marine bivalves: further data and possible explanations. *Evolution* **42**, 1332-1341.