



Genetic structure of the massive coral *Porites panamensis* (Anthozoa: Scleractinia) from the Mexican Pacific

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Abstract. Genetic structure was studied in the brooding coral *Porites panamensis* along of the Mexican Pacific (MP). We collected in three locations inside of the Gulf of California, two zones at the entrance of the Gulf, and one location at south of MP. Exclusive genotypes in two of six allozyme loci were observed in northern (LGG-1^{DE} and LGG-1^{EE}) and southern (EST-1^{AA} and EST-1^{AB}) populations. All populations presented significant deficits of heterozygotes. These deficits could be for different recruitment and temporal events of larvae expulsion along the MP, high local recruitment and inbreeding by limited dispersion of larvae, and different mortality events by natural disturbances. Cluster analysis of genetic distance showed three groups by geographic proximity: the populations from inside of the GC, two populations from the entrance of the gulf, and the southern population of MP. AMOVA indicated a significant differentiation among the three groups (11.93%, $p = 0.016$). Mean significant F_{ST} value ($F_{ST} = 0.104$, $p < 0.01$) revealed a genetic structure. The oceanic patterns coupled with restricted dispersion of this brooding coral species could be the principal factor that generating the genetic structure observed.

Key words: Population structure, Scleractinian coral, brooding coral, population genetics

Introduction

Porites panamensis is the most abundant coral in the central and north of the Gulf of California and is observed in most coral communities from the Mexican Pacific (Glynn and Ault, 2000; Reyes-Bonilla, 2003). This coral has been reported from 31°N in Mexico to Isla Gorgona (3°N), Colombia (Glynn and Ault, 2000; López-Pérez *et al.*, 2003). *P. panamensis* is a gonochoric species with internal fertilization and colonies begin to reproduce at 5 cm² (Glynn *et al.*, 1994). Asexual reproduction by fragmentation has not been observed as important roll in their recruitment (Glynn *et al.*, 1994; Reyes-Bonilla and Calderón-Aguilera, 1994; Paz-García *et al.*, 2009b). Their larvae settle a few meters from the parental colonies (Glynn *et al.*, 1994) and perhaps this reflects a limited capacity of dispersion (Glynn and Ault, 2000). Substrate cover of this coral species in the Gulf of California varies between 1 to 7% of the substrate; in some areas it exhibits a dense covering and in other this totally absent (Reyes-Bonilla and Calderón-Aguilera, 1994; Halfar *et al.*, 2005). ENSO 1982-83 event produced massive mortalities in coral

communities of Central America and this species disappeared almost completely in this region (Guzmán *et al.*, 1987; Weil, 1992; Glynn *et al.*, 1994). In Mexico, the damage in the coral communities of this species was lower, and the reproduction and local recruitment of this species only diminished during the ENSO events (Reyes-Bonilla and Calderón-Aguilera, 1994; Medina-Rosas *et al.*, 2005; López-Pérez *et al.*, 2007). Our aim was to determine the genetic structure of the massive coral *P. panamensis* along of the Mexican Pacific (MP).

Material and Methods

Field Work. Collections were conducted from 2004 to 2006 in different areas of the Mexican Pacific (Fig. 1): Bahía de Los Angeles (BLA), Isla San Marcos (ISM), Bahía Concepción (BCO) and South of Bahía de La Paz (BLP), Punta Arenas (PAV), Isla Redonda (IRD) and La Entrega (LET). The specimens were collected in shallow coral communities (1-5 m). The coral fragments were frozen in liquid nitrogen, transported to the laboratory, and subsequently stored at -80°C.

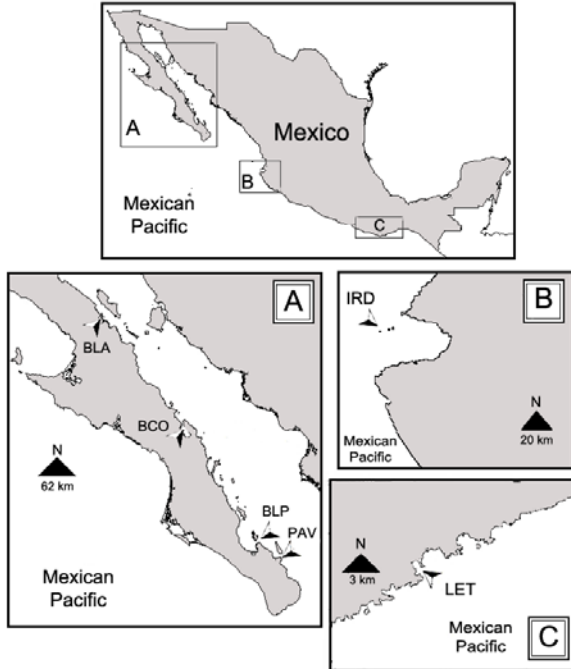


Figure 1: Map of Mexican Pacific showing the collection populations. **BLA** Bahía de Los Angeles, **BCO** Bahía Concepción, **BLP** South of Bahía de La Paz, **PAV** Punta Arenas, **IRD** Isla Redonda, **LET** La Entrega.

Allozyme Electrophoresis. 3g of superficial tissue-skeleton coral were placed in two vials with 0.7 ml of Stoddart's buffer (Stoddart, 1983; Weil, 1992). The vials were placed in a cold bath of a sonicator and two milliliters of blastate was centrifugated at 2600 *g* for 10 min at 4°C. The resulting supernatant was placed in vials and concentration of total proteins for each sample was determined by Bradford's method (Bradford, 1976) and 25 µg was used for the analysis of each enzyme system (Paz-García *et al.*, 2009b). Allozyme analysis was carried out using the method of Polyacrilamide Gel Electrophoresis (PAGE) by discontinuous gel system in native conditions (Laemmli, 1970; Manchenko, 1994). Four enzyme systems were used: leucine-glycyl-glycyl peptidase (LGG, E.C.3.4.11.1), malic enzyme (ME, E.C.1.1.1.40), glutamate dehydrogenase (GDH, E.C.1.4.1.3) and esterase (EST, E.C. 3.1.1.1).

Statistical analysis. Genetic variability was calculated for each population using BIOSYS-1 software package (Swofford and Selander, 1981). Unbiased genetic distances (Nei, 1978) were used for cluster analysis, as implemented in TFGA (Miller, 1997). We performed an analysis of molecular variance (AMOVA) to compare genetic similarity among three groups: I) populations from the GC, II) entrance of GC, and III) the population from the south of MP. P-values were calculated from a random

permutation test with 16 000 replicates (Excoffier *et al.*, 1992). The level of genetic heterogeneity was estimated by F_{ST} . We calculated pairwise F_{ST} estimates between each pair of populations. F_{ST} were tested for difference from zero permuting (10 000 replicates) alleles between samples with exact G-test (Goudet *et al.*, 1996), as implemented in FSTAT v. 2.8 (Goudet, 1995). We applied a sequential Bonferroni correction to reduce the chance of type I errors (Rice, 1989).

Results

Five loci were detected using four enzyme systems. We observed exclusive genotypes from the most northern (BLA: LGG-1^{DE} and LGG-1^{EE}) and southern (LET: EST-1^{AA} and EST-1^{AB}) populations (Anexus I). The allelic diversity at each location ranged from 2.4 to 2.8 (Fig. 2a). The observed heterozygosities were lower than the expected under Hardy-Weinberg equilibrium in all populations, ranging from 0.106 to 0.260 (Fig. 2b).

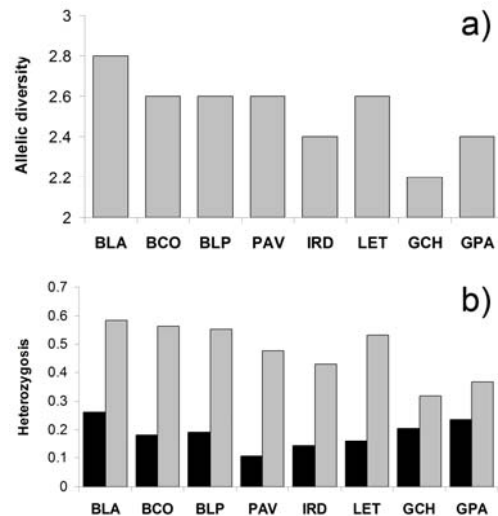


Figure 2. a) Allelic diversity. b) Heterozygosity. Observed and Expected Heterozygosity, black and gray, respectively. Population abbreviation as in Fig. 1. **GCH** Gulf of Chiriqui and **GPA** Gulf of Panama (data from Weil, 1992).

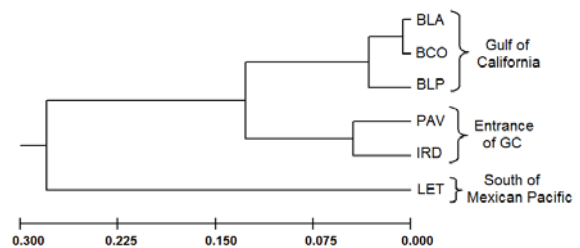


Figure 3. UPGMA dendrogram based on Nei's (1978) unbiased genetic distance. Population abbreviation as in Fig. 1.

Cluster analysis based on Nei's (1978) unbiased genetic distance, showed three groups by geographical proximity: (I) the populations from inside of the GC, (II) two populations from the entrance of the Gulf, and (III) the southern population of MP as other cluster (Fig. 3). AMOVA indicated a significant differentiation among the three groups (11.93%, $p = 0.016$), among populations within groups (3.38%, $p = 0.036$), and within populations (84.68%, $p < 0.001$). Mean significant F_{ST} value ($F_{ST}=0.104$, $p < 0.01$) was observed for the massive populations of *P. panamensis*. Pairwise F_{ST} estimates revealed significant differences among populations along the MP, except the populations from inside of the GC (Table 1).

Table 1. F_{ST} values (below diagonal) and p values (above diagonal) for the massive coral *Porites panamensis* from the MP. Population abbreviations as in Figure 1. F_{ST} were tested for difference from zero permuting (10 000 replicates) alleles between samples with exact G-test (Goudet *et al.*, 1996). NS = not significant, * $p < 0.001$ after Bonferroni correction.

Population	1	2	3	4	5	6
1 BLA	—	0.014 ^{NS}	0.010 ^{NS}	0.000	0.000	0.000
2 BCO	0.013	—	0.000	0.000	0.000	0.000
3 BLP	0.016	0.024	—	0.000	0.000	0.000
4 PAV	0.048	0.071	0.062	—	0.000	0.000
5 BB	0.070	0.082	0.103	0.031	—	0.000
6 OAX	0.096	0.095	0.118	0.124	0.118	—

Discussion

Allelic diversity in two populations of *P. panamensis* from the Panamanian Pacific showed values ranged from 2.2 to 2.4 alleles per locus (Fig. 2a; Weil, 1992). For the present work, most populations of *P. panamensis* from the MP presented slight higher values (2.4-2.8). The observed heterozygosities, ranged from 0.205 to 0.236 in two populations of *P. panamensis* from Panama (Fig. 2b; Weil, 1992). All populations of *P. panamensis* from the MP were slight higher than this (Fig. 2). During the 1982-83 ENSO event, populations of *P. panamensis* from the Panamanian Pacific suffered severe mortalities that caused their disappearance almost completely (Guzmán *et al.*, 1987; Glynn *et al.*, 1994). In Mexico, the recruitment of this species diminished during the ENSO events (Reyes-Bonilla and Calderón-Aguilera, 1994), but contrary to the reefs of Central America, this species suffered lower mortalities, it has continued reproducing and their larvae have recruited locally (Medina-Rosas *et al.*, 2005; Mora-Pérez, 2005; López-Pérez *et al.*, 2007). Genetic variation observed among the populations of *P. panamensis* from the Mexican and the Panamanian Pacific, may be due to the difference in the mortality presented in both regions, since it has been mentioned that the heterozygosities diminishes fast when the size population is reduced (Gillespie, 1998).

A deficit of heterozygosity is characteristic of several coral species and several explanations for such heterozygosity deficits have been proposed (Ayre *et al.*, 1997; Ayre y Hugnes, 2004). Heterozygosity deficits observed in populations of *P. panamensis* along of the MP could be due to different recruitment events of cohorts and mixes of adult colonies from diverse coral communities (Medina-Rosas *et al.*, 2005; López-Pérez *et al.*, 2007), different temporal events along the MP when larvae expulsion takes place (Vizcaíno-Ochoa, 2003; Mora-Pérez, 2005; Rodríguez-Troncoso, 2006; Paz-García *et al.*, 2009b), high local recruitment and inbreeding by limited dispersion of larvae (Glynn y Ault, 2000); and different mortality events by natural disturbances (Reyes-Bonilla *et al.*, 2002).

The dendrogram based on Nei's (1978) unbiased genetic distance showed three groups by geographic proximity (Fig. 3). Our data supports the similarity among the populations inside of the GC and the differentiation between the entrance of the GC (PAV y IRD), and the southern population of the MP (LET). Same pattern of population subdivision in the MP was observed in *Pocillopora damicornis* (Chávez-Romo *et al.*, 2008). These results suggest that the inside populations of the GC may be a group more homogeneous genetically, while the populations of the entrance of the GC (PAV y IRD) and southern of MP (LET) present a genetic differentiation that may be due at the high frequency of natural phenomenon (e.g. hurricanes, upwelling zones and mortality by ENSO events).

Genetic structure among populations of *P. panamensis* in the MP was supported by mean AMOVA (11.93%, $p = 0.016$) and significant F_{ST} value ($F_{ST}=0.104$, $p < 0.01$). Previous studies in the GC and MP have found population subdivision in different marine invertebrates and fishes groups (De la Rosa-Vélez *et al.*, 2000; Riginos y Nachman, 2001; Valles-Jimenez *et al.*, 2005). Genetic structure was reported in other coral species along of the California coast (*Balanophyllia elegans*, mean $F_{ST} = 0.195$; Hellberg, 1996), and along the MP in the species *Pocillopora damicornis* ($F_{ST} 0.153$, Chávez-Romo *et al.*, 2008) and *Pavona gigantea* ($F_{ST} 0.10-0.20$; Saavedra-Sotelo, 2007). Several factors may help generate population genetic subdivision in the MP, including biogeography, geographic distance, habitat discontinuities, current direction, and differences in the environmental conditions (e.g. temperature, tide, eutrophic conditions by upwelling zones), could result in different evolutionary histories among populations (De la Rosa-Vélez *et al.*, 2000; Riginos and Nachman, 2001; Halfar *et al.*, 2005; Valles-Jimenez *et al.*, 2005). We suggest other factors that could be generating the pattern observed: differences in

reproductive seasons among coral communities along the MP (Paz-García *et al.*, 2009b; Chávez-Romo *et al.*, 2008), selection of resistance genotypes due at the differential bleaching and mortality events (Reyes-Bonilla *et al.*, 2002), the presence of different symbiont clades in one same host species and the possibility of different combination between host and symbiont along the MP, could be represent different opportunities to support low light conditions and high level of environment stress (LaJeunesse *et al.*, 2007a, b; Paz-García *et al.*, 2009a, b), and the existence of long sand barriers and mangroves communities that may limit the larval dispersion between coral communities (Glynn y Ault, 2000). Also, the oceanic patterns coupled with restricted dispersion of brooding coral *P. panamensis* could be the principal factor that is generating the genetic structure observed on the populations in the MP. However, detailed studies in ecology, reproduction and genetics are necessary to understand better the relationships between the coral populations in the MP.

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Annex I. Allele frequency of six populations of *P. panamensis* from the Mexican Pacific. Population abbreviation as in Fig. 1. *N* number of samples analyzed at each population.

Población	BLA	BCO	BLP	PAV	IRD	LET
LOCI						
<i>ME-1</i>						
N	20	20	20	34	20	25
A	0.675	0.625	0.625	0.868	0.775	0.360
B	0.325	0.375	0.375	0.132	0.225	0.640
<i>GDH-1</i>						
N	20	13	20	34	20	25
A	0.450	0.462	0.400	0.706	0.650	0.720
B	0.550	0.538	0.600	0.294	0.350	0.280
<i>GDH-2</i>						
N	20	19	20	34	20	25
A	0.250	0.210	0.300	0.353	0.200	0.240
B	0.450	0.474	0.500	0.412	0.600	0.480
C	0.300	0.316	0.200	0.235	0.200	0.280
<i>EST-1</i>						
N	20	20	20	34	20	25
A	—	—	—	—	—	0.660
B	0.475	0.375	0.425	0.750	0.900	0.340
C	0.525	0.625	0.575	0.250	0.100	—
<i>LGG-1</i>						
N	20	20	20	34	19	25
A	0.150	0.400	0.075	0.059	0.237	0.260
B	0.300	0.350	0.125	0.338	0.605	0.480
C	0.125	0.200	0.500	0.382	0.158	0.080
D	0.300	0.050	0.300	0.221	—	0.180
E	0.125	—	—	—	—	—