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-1DE and LGG-1EE) and southern (EST-1AA and EST-1AB) 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 cm$^2$ (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 (PAN), 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.
Allozyme Electrophoresis. 3 g 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-glycil-glycil 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 TFPGA (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 FST. We calculated pairwise FST estimates between each pair of populations. FST 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-1DE and LGG-1DI) and southern (LET: EST-1AA and EST-1AB) populations (Appendix I). The allelic diversity at each location ranged from 2.4 to 2.8 (Fig. 2a). The observed heterozygosities were lower that the expected under Hardy–Weinberg equilibrium in all populations, ranging from 0.106 to 0.260 (Fig. 2b).
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 \( P. panamensis \) 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.

<table>
<thead>
<tr>
<th>Population</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong> BLA</td>
<td>0.016</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>2</strong> BCO</td>
<td>0.013</td>
<td>0.014**</td>
<td>0.010**</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>3</strong> BLP</td>
<td>0.048</td>
<td>0.071</td>
<td>0.062</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>4</strong> PAV</td>
<td>0.070</td>
<td>0.062</td>
<td>0.103</td>
<td>0.031</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>5</strong> BB</td>
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<td>0.095</td>
<td>0.118</td>
<td>0.124</td>
<td>0.118</td>
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</tr>
</tbody>
</table>

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 that 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 heterozygous 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; Lópeze-Pérez et al., 2007), different temporal events along the MP when larvae expulsion takes place (Vizcaíno-Ochoa, 2003; Mora-Perez, 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-Romó 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 cost (\( Balanophyllia elegans \), mean \( F_{ST} = 0.195 \); Hellberg, 1996), and along the MP in the species \( Pocillopora damicornis \) (\( F_{ST} = 0.153, \) Chávez-Romó 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 to the differential branching 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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References
Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590
Paz-García DA, LaJeunesse TC, Chávez-Romo HE, Correa-Sandoval F, Reyes-Bonilla H (2009a) Differences in the distribution of Symbiodinium spp. among morphotypes and genotypes of Porites panamensis from the Gulf of California, Mexico. Proc 11th Int Coral Reef Sym
Paz-García DA, Reyes-Bonilla H, Hernández-Cortés MP (2009b) Genetic variation in two morphotypes of Porites panamensis from the Gulf of California, Mexico. Proc 11th Int Coral Reef Sym
Rodríguez-Troncoso AP (2006) Ciclo reproductivo de tres especies de corales formadoras de arrecife en Bahía La Entrega, Oaxaca,
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.

<table>
<thead>
<tr>
<th>Población</th>
<th>BLA</th>
<th>BCO</th>
<th>BLP</th>
<th>PAV</th>
<th>IRD</th>
<th>LET</th>
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<tr>
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<td>0.625</td>
<td>0.868</td>
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<td>0.375</td>
<td>0.132</td>
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<tr>
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<td>A</td>
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<tr>
<td>A</td>
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