

Differences in the distribution of *Symbiodinium* spp. among morphotypes and genotypes of *Porites panamensis* from the Gulf of California, Mexico

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Abstract. Cnidarian-algal symbioses are important in sustaining coral reef ecosystems. Little is known about how these associations respond ecologically and evolutionarily to environmental change. We examined the diversity of zooxanthellae populations in two morphotypes of *Porites panamensis* in the southern Gulf of California. Additionally, we analyzed the host genetic information by allozyme electrophoresis in order to demonstrate if the species of symbiont corresponds with the genotype and/or morphotype of the host individual. The specimens (N = 20) were collected from shallow coral communities (1-2 m). *Symbiodinium* C66a associated with columnar colonies while C66 occurred commonly in colonies with mounded or massive morphologies. Both colony forms associated with C1. Higher allelic diversity and different symbiont clades were found in columnar colonies, relative to massive colonies in the same habitat. Certain host genotypes associated specifically with a particular *Symbiodinium* type. These preliminary findings indicate that host-symbiont co-evolution is important in shaping partner combinations and that the mode of symbiont acquisition by maternal or vertical transmission facilitates this process.

Key words: host-symbiont specificity, *Symbiodinium*, host-genetic data, species complex, scleractinian coral

Introduction

Partner specificity is important to the biology of all reef corals maintaining obligate associations with endosymbiotic dinoflagellates in the genus *Symbiodinium* (zooxanthellae) (Baker 2003; Stat et al. 2006). Ecological and physiological studies indicate that tolerance to low and/or high light and temperature differs among *Symbiodinium* (Iglesias-Prieto et al. 2004; LaJeunesse et al. 2008). Hosting a particular symbiont may therefore determine the longevity of the colony when exposed to episodes of stress. Of critical interest is determining how changing environmental conditions affect the stability and evolution of these partnerships over time (LaJeunesse 2004; Stat et al. 2006). Initial analyses of corals in the Gulf of California (GC), have showed that they maintain highly specific and stable associations, despite wide seasonal fluctuations in light and temperature (Iglesias-Prieto et al. 2004; LaJeunesse 2004; LaJeunesse et al. 2008).

The coral *Porites panamensis* is widely distributed in the Eastern Pacific. In the Gulf, this species has two colonial morphologies with slight differences in corallite morphology (Ketchum and Reyes-Bonilla

2001), ability to recover from physical damage (Paz-García and Reyes-Bonilla 2006), and vertical distribution (López-Pérez et al. 2003; Paz-García and Reyes-Bonilla 2006), and appear to be genetically distinct (Paz-García et al. 2008b). These data suggest that several factors could explain the variation observed in both morphotypes (Paz-García et al. 2008b). *Porites panamensis* harbors different symbiont types (LaJeunesse et al. 2008), but it is unknown whether the distribution of these symbionts relates to host-morphotype. Our aim was to examine the diversity of *Symbiodinium* in these two morphotypes of the coral *P. panamensis* in the southern Gulf of California. Additionally, We analyzed the host genotypes by allozyme electrophoresis in order to demonstrate if the species of symbiont corresponds with the genotype and/or morphotype of the host individual. This study is among the first to provide evidence that host genotype dictates the distribution of different symbiont types.

Material and Methods

Field Work. The collections were conducted in May 2006 in two locations in the southern Gulf of

California, Mexico (Fig. 1). The columnar morphotype of *P. panamensis* is more prevalent at Punta Gaviotas, the massive one at Punta Galeras (Paz-García and Reyes-Bonilla 2006). The specimens ($n = 10$ for each morphotype) were collected from shallow coral communities (1-2 m).



Figure 1: Map showing the locations, in the south of the Gulf of California.

Symbiont Identification. Coral fragments were stripped of tissue using an airbrush and the symbiont cells isolated as described by LaJeunesse et al. (2003). Algal pellets were transferred to 1.5 mL microcentrifuge tubes and preserved in 20% dimethylsulfoxide (DMSO), 0.25 mol L⁻¹ ethylenediaminetetraacetic acid (EDTA), in sodium chloride-saturated water solution (Seutin et al. 1991). The genetic identity of the *Symbiodinium* populations in each sample was later analyzed via PCR-denaturing gradient gel electrophoresis (DGGE) of the ITS regions 1 and 2 as described by LaJeunesse et al. (2008).

Host genetic analysis. We conducted a coral tissue extraction as described by Paz-García et al. (2008a). We determined the concentration of total proteins from each sample by Bradford's method (Bradford 1978) and 25 µg were used for the analysis of each enzyme system. Allozyme analysis was carried out using polyacrylamide gels. Four enzyme systems were used: LGG (E.C.3.4.11.1), ME (E.C.1.1.1.40), GDH (E.C.1.4.1.3) and EST (E.C. 3.1.1.1).

To examine the differences between host morphotype and symbiont ITS type, we scored and compared the frequencies of allozyme alleles between colonies that were either columnar or massive and/or associated with C66, C66a, or C1. Host allelic and genotype frequencies were tested by the Markov chain method (Raymond and Rousset 1995). Host

unbiased genetic distance (Nei 1978) was used to carry out cluster analysis among clade groups.

Results

Six colonies of columnar *P. panamensis* associated with *Symbiodinium* C66a while the other four colonies harbored C1. The distribution of these combinations appeared random. All 10 shallow massive colony morphotypes collected from Punta Galeras associated with C66 (Fig. 2). A single symbiont type was detected in each colony analyzed.

During the animal genotyping, two to four alleles were found for each allozyme locus analyzed. Higher allelic diversity was found in columnar colonies relative to massive colonies (Fig. 3). One genotype of locus LGG-1 and three genotypes of locus ME-1 were observed in colonies associating with a particular symbiont type (Table 1). Significant differences in host allelic and genotype frequencies were determined by Markov chain analyses of columnar C1 and massive C66 colonies (Table 2), but allelic and genotype diversity in colonies with C66 and C66a were not statistically different or were columnar colonies with C66a or C1 (Table 2). The relationship between host-genotype and symbiont type was supported by cluster analysis based on host Nei's unbiased genetic distance that showed massive colonies with C66 and columnar colonies with C66a clustered together before joining columnar colonies hosting C1 (Fig. 4).

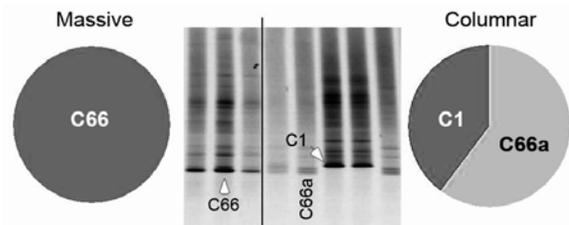


Figure 2: ITS-DGGE fingerprinting analysis of *Symbiodinium* spp. identity and type frequencies in each host morphotype.

Table 1. Host genotypes compared with the *Symbiodinium* type harbored by *P. panamensis* individuals. Animal genotypes observed with only one symbiont type are in bold.

	Massive C66	Columnar C66a	Columnar C1
ME-1	AA, AB	AA, AB	BB
GDH-1	AA, BB	AA, BB	BB
GDH-2	AA, BB	AA, BB, CC	AA, BB, CC
EST-1	BB, BC, CC	BB, BC, CC	BC, CC
LGG-1	CC, DD	AA, AB, CC, CD, DD	BB, CC

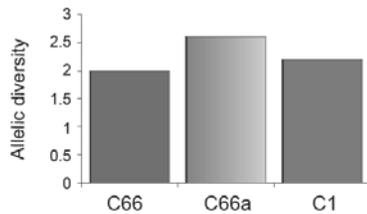


Figure 3: Host allelic diversity based on the symbiont type hosted. Massive morphotype (C66), Columnar morphotype (C66a and C1).

Table 2. Allelic (below diagonal) and genotypic (above diagonal) host differentiation frequency test.

	Massive C66	Columnar C66a	Columnar C1
Massive C66	—	NS	*
Columnar C66a	NS	—	NS
Columnar C1	**	NS	—

* $\chi^2=21.378$ 10 df, $p=0.018$. ** $\chi^2=38.810$ 10 df, $p<0.001$.

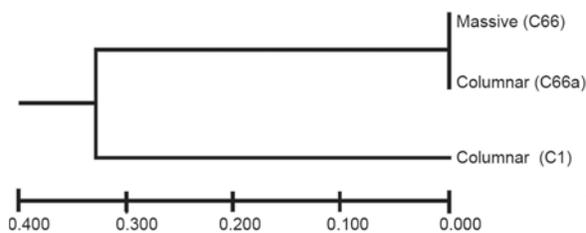


Figure 4: Cluster analysis based on host Nei's unbiased genetic distance.

Discussion

Inter-colony variability in symbiont type among colonies of *Porites panamensis* appears to be influenced by environmental and biological factors. Five different *Symbiodinium* types are known to associate with *P. panamensis* in the Gulf of California (LaJeunesse et al. 2008). Depth strongly influences the frequency of occurrence of particular symbionts in populations of host individuals. *Symbiodinium* C1 is prevalent among colonies living below 2 meters. At depths below 5 meters colonies are occasionally found associating with C75. Closely related C66, C66a, and C66b occur in *P. panamensis* living in shallow environments (0.5 and 1.5 m). These ecological patterns suggest that significant differences in physiology exist among Clade C *Symbiodinium* (Sampayo et al. 2008).

Columnar and massive morphotypes of *P. panamensis* also exhibit differences in vertical distribution throughout the Gulf (López-Pérez et al. 2003; Paz-García and Reyes-Bonilla 2006). Columnar colonies are more limited in their distributions to

shallow habitats, typically 0.5 to 4.0 meters from the surface. While these data are limited, the presence of a particular symbiont does not explain this pattern of zonation.

Specific host-symbiont combinations involving *Symbiodinium* adapted to different light regimes may result in ecological zonation along an irradiance gradient (Iglesias-Prieto and Trench 1994; Iglesias-Prieto et al. 2004). This coral belongs to one of the few genera that produce eggs containing symbiont cells (Richmond and Hunter 1990; Glynn et al. 1994). Therefore, the presence of a high or low light-adapted symbiont may directly affect the differential success of larvae settling in deep or shallow environments. Further understanding of the physiological performances of these different host-symbiont combinations (holobionts) under different environmental conditions would help explain these ecological patterns.

There appears to be a close correspondence with symbiont identity and host genotype (Fig. 4). While the columnar or massive morphology does not appear to influence symbiont distribution, the presence of C66 or C66a in hosts with similar genotypes suggests that host genetic background is important to specificity. While external physical factors affect the symbioses in some hosts (Rowan et al. 1997), host-symbiont specificity plays a crucial role in dictating the distribution of certain partner combinations.

Thoroughly describing the ecological and geographic distributions of specific host-symbiont combinations is important for ultimately understanding co-evolutionary processes that occur between host and symbiont lineages. Associations with C1, C66 (a and b variants), and C75 are unique for Indo-Pacific *Porites* (LaJeunesse 2004). The evolution of unusual host-symbiont combinations is probably influenced by populations living for many generations in isolation while subjected to wide ranges in temperature and water clarity, as is characteristic of the northern Eastern Pacific. The presence of different symbionts among colonies of *P. panamensis* may provide the natural variation that may eventually be important in determining how these coral populations respond to selection pressures created by global warming.

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