

# CORAL SYMBIONT DIVERSITY ALONG A SEAWATER TEMPERATURE GRADIENT

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## Abstract

The genetic diversity of endosymbiotic algae (zooxanthellae) occurring within the tissues of some Pacific reef-building coral species may vary according to environmental conditions: Most colonies of most species contain clade C, while those occupying habitats subjected to elevated seawater temperatures or large diurnal temperature fluctuations often contain clade D. On Ofu and Olosega Islands, seawater temperatures were logged every 30 minutes for a 1-year period at three sites along a seawater temperature gradient (all 1 -1.5 m depth): A forereef site with small daily temperature fluctuations and relatively low daily temperature maxima, a large back reef pool with intermediate daily temperature fluctuations and maxima, and a small back reef pool with large daily temperature fluctuations and high maxima. The mean daily temperatures at the three sites are very similar to one another. *Zooxanthella* samples were collected from each site from colonies of *Acropora gemmifera*, *Galaxea fascicularis*, *Pocillopora eydouxi* and *Porites lobata*. All species except *Por. lobata* had the highest proportion of clade D in colonies from the site with the greatest daily temperature fluctuations and highest maxima (Pool 300). In addition, all species except *Por. lobata* had more genotypes in samples from the back reef pools than in samples from the forereef. These results suggest that zooxanthella genotype diversity may increase the capacity of some reef-building coral species to tolerate the thermal conditions of shallow back reef pools. However, the less labile *Porites* species dominate the Ofu back reef coral community, suggesting this genus relies more on host coral mechanisms to cope with back reef conditions.

## Introduction

Skeletal growth in reef-building scleractinian corals occurs by the formation and precipitation of aragonite (Barnes 1970), a process greatly enhanced by symbiotic dinoflagellates (*Symbiodinium* spp.) known as zooxanthellae that provide up to 95 percent of the corals' carbon requirements for growth, reproduction, and maintenance (Muscatine 1990). Physiological differences in cultured isolates suggested genetic diversity within *Symbiodinium* (Kinzie and Chee 1979; Schoenberg and Trench 1980), providing the impetus to apply molecular systematics. The discovery of high sequence variability in nuclear small subunit ribosomal DNA (18S-rDNA) led to the classification of *Symbiodinium* into a growing number of clades (Rowan and Powers 1991a, 1991b; LaJeunesse 2001; Baker 2003). The inferred phylogeny was confirmed using other molecular markers (Santos et al. 2002; Pochon et al. 2006), and eight clades are recognized, designated A – H, five of which (A – D, F) are known to form associations with scleractinian corals (Coffroth and Santos 2005; Pochon et al. 2006; Stat et al. 2006).

Clade-level zooxanthella genotypes found in a reef-building coral species may vary spatially between colonies according to seawater temperature maxima (Berkelmans and van Oppen 2006), irradiance level (Robison and Warner 2006), or degree of sedimentation (Garren et al. 2006). Spatial variability also occurs within a single colony, and multiple clades may occur simultaneously within a colony (reviewed by Stat et al. 2006). In the Pacific, clade C is by far the most common genotype (LaJeunesse et al. 2004b), but clade D is found in colonies of some coral species occupying habitats typified by elevated seawater temperatures, large temperature fluctuations, and high irradiance levels (Fabricius et al. 2004; Rowan 2004). Clade D appears to

increase the tolerances of host colonies to elevated seawater temperatures (Berkelmans and van Oppen 2006) and high irradiance levels (Robison and Warner 2006), while reducing skeletal growth rates of the host coral (Little et al. 2004).

Clade-level variability in zooxanthella genotypes can also occur temporally, especially in highly-fluctuating habitats and before-and-after bleaching events. Reef-building coral colonies occupying back reef habitats where seawater temperature and irradiance level rapidly fluctuate may have highly dynamic zooxanthella communities, with multiple clades and sub-clades in a state of flux (Magalon et al. 2006; Baker and Romanski 2007). Zooxanthellae density also may vary seasonally, with higher densities during cooler seawater temperatures in the winter (Stimson 1997; Fagoonee et al. 1999). Temporal variability is also demonstrated by the increased frequency of clade D in colonies recovering from bleaching than in colonies that have not recently bleached (Baker 2001; Glynn et al. 2001; Baker et al. 2004; van Oppen et al. 2005).

Though clade-level spatial and temporal variability in zooxanthella genotypes occurs in some species of reef-building corals, it is important to note that many coral-algal symbioses remain stable both spatially and temporally as long as the external environment remains similar (Iglesias-Prieto et al. 2004; LaJeunesse et al. 2004a; LaJeunesse et al. 2004b; Thornhill et al. 2005). Furthermore, zooxanthellae variability is uncommon both spatially and temporally in certain reef-building coral genera, especially *Porites*, regardless of environmental disturbances such as warming events resulting in mass bleaching (Baker et al. 2004; Fabricius et al. 2004). Yet *Porites* is one of the most common reef-building coral genera in shallow reef habitats where seawater temperatures and irradiance levels commonly reach high levels (Veron 2000). One such reef area is the back reef on the south side of Ofu Island, American Samoa (Craig et al. 2001).

## Methods

### *Study sites & Seawater temperatures*

This study was carried out at the three sites collectively used for the coral reciprocal transplant experiments described in Chapters 3 – 5 above; a shallow forereef site, a large back reef pool, and a small back reef pool (Figure 6.1). Shaded seawater temperatures were recorded simultaneously every 30 minutes at the three sites for a 1-year period from 1-Apr 2004 to 31-Mar 2005. Attempts were made to record temperatures at 1 – 1.5 m low tide depth at all three sites with Onset Water Temp Pro<sup>®</sup> temperature loggers, but extreme water motion at the forereef site repeatedly tore loose the logger at that site. However, the forereef site was originally selected in part because the National Marine Fisheries Service's Coral Reef Ecosystem Division (CRED) had been logging seawater temperatures at the site since 2004 with a Seabird 39<sup>®</sup> logger placed at 5 m low tide depth. Thus, forereef temperature data was provided by CRED's logger. At the two back reef sites, seawater temperatures were recorded with the Water Temp Pro loggers placed at 1 – 1.5 m depth. The loggers were tested indoors against one another and a calibrated thermometer, deployed for six months, then retrieved and tested again. Loggers always read < 0.1°C of one another and the calibrated thermometer. In addition, seawater temperatures at the two back reef sites have been monitored in this fashion by the National Park of American Samoa since 2000, thus back reef temperature data for a 6-year period (1 Apr-00 – 31 Mar-06) are also provided for reference.

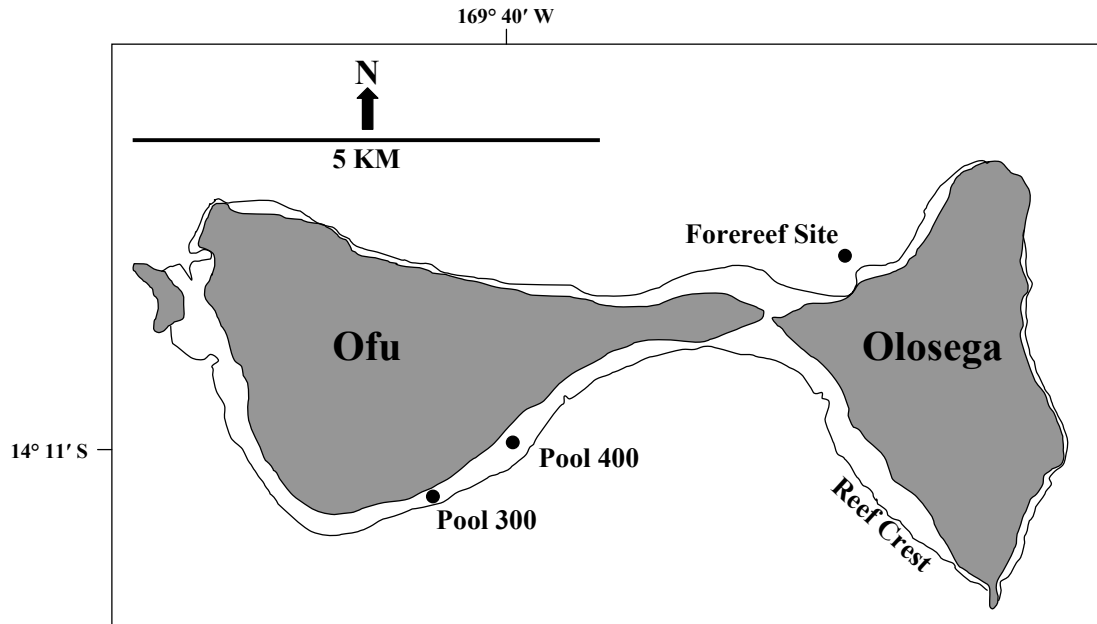


Figure 6.1. Map of study area in American Samoa, showing the three sites where seawater temperatures were recorded and zooxanthella samples were collected.

### *Zooxanthellae sampling*

*Acropora*, *Pocillopora*, and *Porites* species make up 70 percent of live coral cover in the back reef (Craig et al. 2001). Zooxanthella samples were taken from colonies of *A. gemmifera*, *Galaxea fascicularis*, *Poc. eydouxi*, and *Por. lobata* using a 13 mm punch from the top of each source colony in September 2004, near the end of the southern winter. Coral colonies were sampled in Pool 300, Pool 400 and on the shallow forereef (Figure 6.1; all colonies 1 – 3 m low tide depth) to test for spatial variability in zooxanthella genotypes within each coral species. At least 6 colonies of each species were sampled at each site, except *Por. lobata*, because few colonies of this species could be positively distinguished from other massive *Por.* species. *Poc. eydouxi* and *Por. lobata* were also sampled in March 2006 (same colonies as Sep-04 samples) during the southern summer to test for temporal variability in zooxanthella genotypes.

Samples were preserved in 95% ethanol, and total DNA was extracted using established methods (Baker et al. 1997). Using primers with a Guanine-Cytosine clamp (GC clamp), the internal transcribed spacer-2 (ITS-2) region of nuclear ribosomal DNA was amplified using the polymerase chain reaction (PCR). The PCR product was then run on an acrylamide Denaturing-Gradient Gel Electrophoresis (DGGE) gel with a 35% - 75% chemical gradient (formamide and urea) from low to high following established methods (LaJeunesse 2001). The diagnostic bands were excised and reamplified using PCR with primers not containing the GC clamp. The PCR products from the cut bands were then sequenced, and the edited sequences run through a Basic Local Alignment Search Tool (BLAST) search in GenBank for a *Symbiodinium* type match.

## Results

### *Seawater temperatures*

Pool 300 had much higher daily maxima and daily fluctuations than the forereef year-round except during stormy, well-mixed conditions (e.g., most of June and July, Figure 6.2), while Pool 400 was intermediate. Though mean annual temperatures were nearly identical at the three sites (Table 6.1), the temperature gradient suggested by the raw data (Figure 6.2) is born out by an analysis of the summer (Nov-Mar) temperature data: The local summer mean in the back reef pools was 29.4 °C for the six summers between Nov-00 and Mar-06 (Table 6.1). The frequency and duration of temperatures exceeding the local summer mean were greatest in Pool 300, intermediate in Pool 400, and lowest on the forereef (Figure 6.3, left and middle panels), a pattern also followed by maximum daily temperature fluctuations (Figure 6.3, right panel).

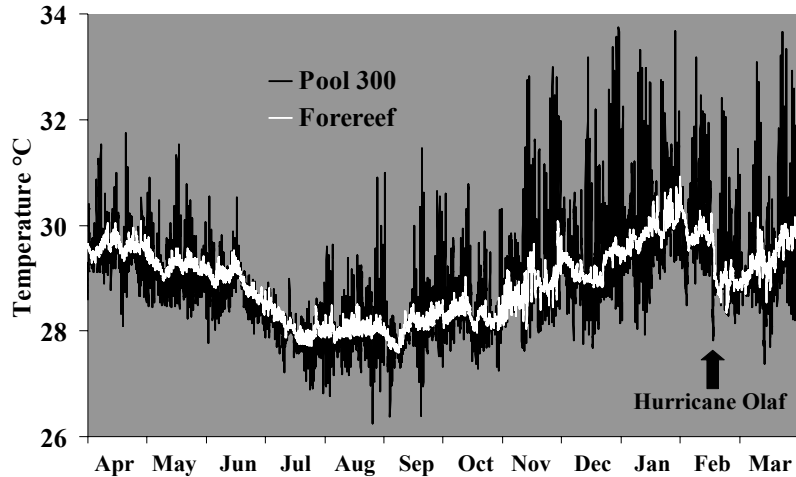


Figure 6.2. Seawater temperature raw data (every 30 minutes) at the Pool 300 and forereef sites during a 1-year period (1 Apr-04 to 31 Mar-05). Temperatures in Pool 400 had intermediate daily maxima and fluctuations (Figure 6.3, right panel).

Table 6.1. Temperature summaries for all three sites (1 Apr-04 – 31 Mar-05) and the back reef pools (1 Apr-00 – 31 Mar-06; MDR = maximum daily range).

Site	Dates	n	Mean	Max	Min	Range	MDR
			Year-round				
Pool 300	1 Apr-04 – 31 Mar-05	17,520	28.97	33.76	26.26	7.50	5.61
Pool 400	"	17,520	29.00	33.03	27.38	5.65	4.34
Forereef	"	17,520	28.92	30.93	27.58	3.35	1.49
Pool 300	1 Apr-00 – 31 Mar-06	105,120	28.92	35.47	25.12	10.35	6.51
Pool 400	"	105,120	28.84	33.03	26.23	6.80	4.42
Summers Only (Nov-Mar)							
Pool 300	1 Nov-04 – 31 Mar-05	7,248	29.55	33.76	27.38	6.38	5.61
Pool 400	"	7,248	29.52	33.03	28.05	4.98	4.34
Forereef	"	7,248	29.37	30.93	28.04	2.89	1.49
Pool 300	1 Nov-00 – 31 Mar-06	43,488	29.50	35.47	25.94	9.53	6.51
Pool 400	"	43,488	29.35	33.03	27.04	5.99	4.42

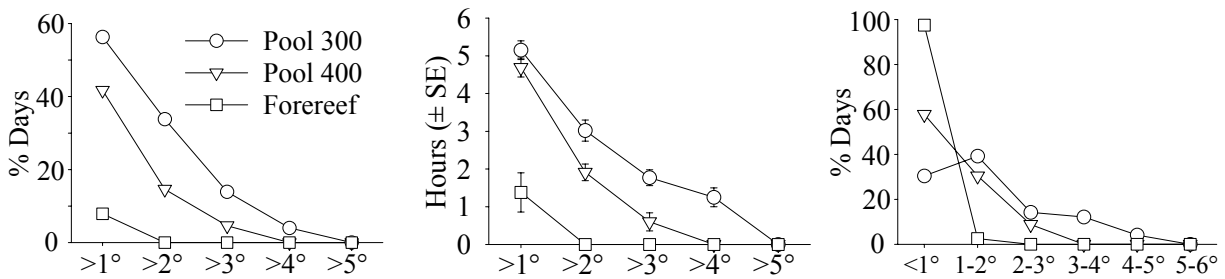


Figure 6.3. Frequency (left panel) and duration (middle panel) of summer (1 Nov-04 to 31 Mar-05) seawater temperatures exceeding 1, 2, 3, 4, and 5 °C above the local summer mean (29.4 °C) at the three sites. Also shown are maximum daily temperature fluctuations (right panel).

### *Zooxanthella* genotypes

*Zooxanthella* genotypes showed clade-level spatial variability for *A. gemmifera*, *G. fascicularis*, and *Poc. eydouxi* during the winter. The maximum proportion of clade D was found in Pool 300 colonies for all three species, though the same proportion (100%) was found in *A. gemmifera* colonies from the two back reef pools, and there was no data for *G. fascicularis* from Pool 400. Only *Poc. eydouxi* colonies in Pool 300 contained a mix of clades C and D. Summer sampling was only done for *Poc. eydouxi* and *Por. lobata*: *Zooxanthella* genotypes of *Poc. eydouxi* varied temporally, having a higher number of genotypes and a larger proportion of clade D in the summer than in the winter. In contrast, *Por. lobata* showed no spatial or temporal variability between the three sites, even at the sub-clade level (Figure 6.4, Appendix 6.1). During the winter, *A. gemmifera*, *G. fascicularis*, and *Poc. eydouxi* colonies in the back reef pools contained 1 – 3 *Zooxanthella* genotypes, and these same *Poc. eydouxi* colonies contained 1 – 5 *Zooxanthella* genotypes in the summer (Appendix 6.1).

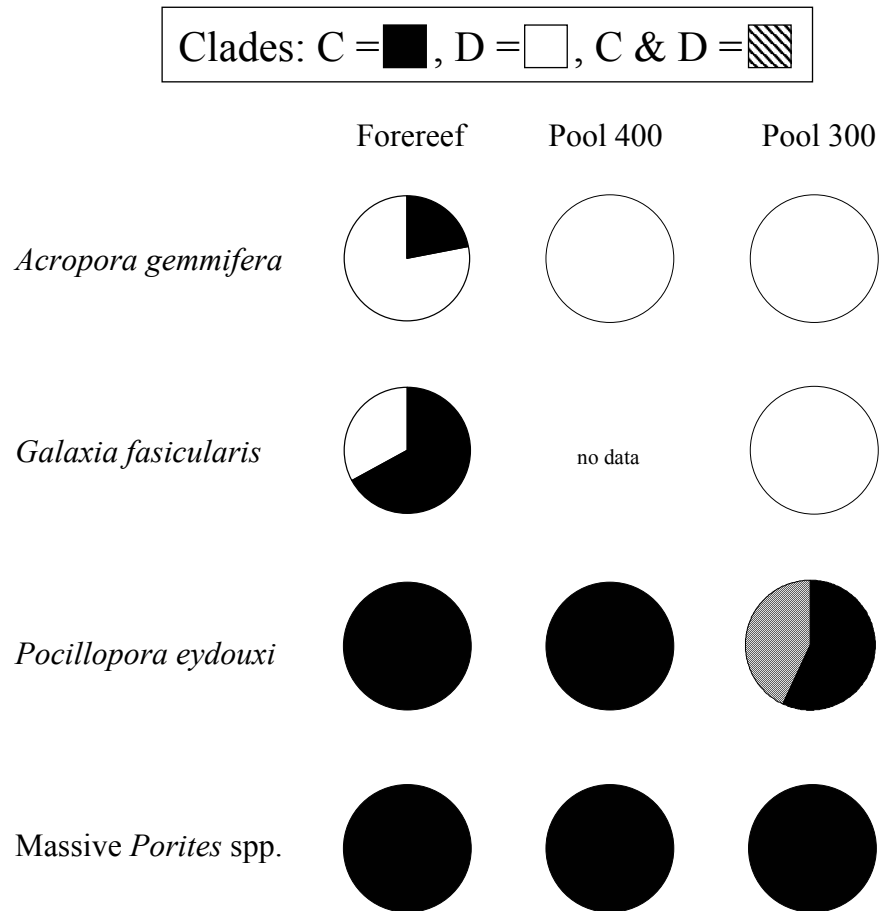


Figure 6.4. Proportions of colonies with clade C only (black), clade D only (white), and both zooxanthella clades (hatched) in *Acropora gemmifera*, *Galaxea fascicularis*, *Pocillopora eydouxi*, and *Porites lobata* at the three sites during the winter (Sep-04).

## Discussion

Although the three sites had nearly identical mean seawater temperatures over the 1-year period, the differences in daily temperature maxima and fluctuations produced a seawater temperature gradient from least-fluctuating (forereef) to most-fluctuating (Pool 300; Figure 3). Many shallow reef habitats are subjected to elevated daily maxima and large daily fluctuations in

seawater temperature, such as reef flats (Glynn 1973; Brown 1997), areas that receive upwelling or advective flow of subsurface water (Coles 1997), back reef pools (Orr 1933; Craig et al. 2001), and marine lakes (Fabricius et al. 2004). Such habitats also typically experience major seasonal fluctuations in seawater temperature and other environmental variables, yet all support diverse communities of reef-building corals (Brown 1997; Coles 1997; Craig et al. 2001; Fabricius et al. 2004).

Overall, the site with highest maxima and great fluctuations in daily seawater temperatures had the highest proportion of coral colonies containing clade D zooxanthella genotypes (Figure 4). Similarly, a study of zooxanthella genotypes in *Acropora hyacinthus* colonies from the three sites carried out during summer 2006 also showed that colonies in Pool 300 had a higher proportion of colonies containing clade D (100%) than colonies in Pool 400 (80%) or on the forereef (70%; Tom Oliver, in preparation). Also, zooxanthella genotypes of *Poc. eydouxi* colonies in Pool 300 showed an increase in clade D during the summer (Appendix 6.1). These results are consistent with other studies of spatial and temporal variability in zooxanthella genotypes, which have also found the highest proportion of clade D in colonies inhabiting the warmest and/or most fluctuating environment (Baker et al. 2004; Fabricius et al. 2004). These environments typically also have very high irradiance levels, and tolerance of high irradiance may also vary by zooxanthellae genotype (Robison and Warner 2006). Thus, utilization of clade D zooxanthellae appears to be a mechanism contributing to the tolerance of some reef-building corals to environmental conditions in the Ofu back reef pools, such as elevated seawater temperatures, large temperature fluctuations, and high irradiance levels.

The symbiont acquisition portion of a reef-building coral species' life history may affect spatial and temporal variability in its zooxanthella genotypes (LaJeunesse et al. 2004b): Most

Pacific taxa broadcast spawn eggs and sperm that do not contain zooxanthellae, so symbionts are acquired by juvenile corals from the environments (horizontal transmitters), whereas some taxa transmit zooxanthellae from parent to offspring (vertical transmitters). For example, *Acropora* and *Galaxea* are horizontal transmitters, while *Pocillopora* and *Porites* are vertical transmitters (Richmond and Hunter 1990; Glynn et al. 1991; Kinzie 1993). In this study, *A. gemmifera* and *G. fascicularis*, both horizontal transmitters, had only clade D in their Pool 300 colonies, while *Poc. eydouxi* and *Por. lobata*, both vertical transmitters, had either clade C or D (*Poc. eydouxi*) or only clade C (*Por. lobata*). In addition, *Poc. eydouxi* colonies often had several zooxanthella genotypes, especially in the summer, resulting in a mix of clades C and D (Appendix 6.1). These results suggest that horizontal transmitters more easily exchange zooxanthella genotypes (like *A. gemmifera* and *G. fascicularis*), whereas vertical transmitters either add additional genotypes as needed (like the Pool 300 *Poc. eydouxi* colonies) or do not change genotypes at all (like *Por. lobata*).

Though *Acropora* and *Pocillopora* species are important components of the Ofu back reef coral community (21% and 17% of live coral cover in the pools in 2000, respectively, while the ubiquitous *G. fascicularis* and other *Galaxea* species made up < 1 % of live coral cover in 2000), *Porites* species dominate the pools (32% of live coral cover in the pools in 2000)(Craig et al. 2001). However, *Por. lobata* showed no spatial or temporal variability in zooxanthella genotypes, even at the sub-clade level (all samples contained only genotype C15; Appendix 6.1). In addition, other *Porites* samples (10 samples of *Por. cylindrica* from each of the two pools, one or two samples of *Por. lichen*, *Por. lutea*, and *Por. solida* from the pools, all taken in Sep-04) also all contained only genotype C15.

Other studies have also shown that even in reef habitats with elevated seawater temperatures (Fabricius et al. 2004) or in corals recovering from bleaching (Baker et al. 2004), *Porites* colonies do not contain clade D zooxanthellae (although a minority of *Porites* colonies contained clade D in the study by Fabricius et al. 2004). If clade D zooxanthella genotypes increase tolerances of reef-building corals to elevated seawater temperatures and high irradiance levels, why is a warm, shallow reef habitat like the Ofu back reef dominated by *Porites*, which does not appear to utilize clade D? The high tolerances of Ofu back reef *Porites* species to elevated seawater temperatures and high irradiance levels (Smith and Birkeland 2007), and the lack of variability in zooxanthella genotypes in *Porites*, suggest that host coral mechanisms rather than zooxanthellae may be the source of high tolerances. This concept is supported by a parallel study of *Por. lobata* in the back reef and forereef sites, which demonstrated contrasting physiological capacities of the host corals (production of heat shock proteins and anti-oxidants) between the two sites (Barshis et al. Submitted). An alternative explanation for the tolerances of *Porites* species to elevated seawater temperatures is that genotype C15 is more similar to clade D zooxanthellae than other clade C genotypes in terms of affording the host coral high tolerances (Fabricius et al. 2004), though no data are currently available to support or rule out this hypothesis.

Appendix 6.1. Zooxanthella genotypes (LaJeunesse 2001; LaJeunesse et al. 2004b) detected in individual samples of *Acropora gemmifera*, *Galaxea fascicularis*, *Pocillopora eydouxi* and *Porites lobata* from Pool 300, Pool 400 and the forereef during the winter (Sep-04) and summer (Mar-06), showing proportion of clade D genotypes (**bold**) for each coral species.

	Winter			Summer		
	Forereef	Pool 400	Pool 300	Forereef	Pool 400	Pool 300
<i>Acropora gemmifera</i>	C	<b>D2</b>	<b>D2</b>	no data		
	<b>D2</b>	<b>D1, D2</b>	<b>D2</b>			
	<b>D2</b>	<b>D1, D3</b>	<b>D2</b>			
	<b>D2</b>	<b>D1, D2</b>	<b>D2</b>			
	<b>D2</b>	<b>D1, D2</b>	<b>D2</b>			
	C15	<b>D2</b>	<b>D2</b>			
	<b>D2</b>	<b>D2</b>	<b>D2</b>			
	<b>D2</b>	<b>D2</b>	<b>D2</b>			
	<b>D2</b>	<b>D2</b>	<b>D2</b>			
<b>Proportion w/Clade D</b>	<b>78%</b>	<b>100%</b>	<b>100%</b>			
<i>Galaxea fascicularis</i>	C		<b>D1, D1a</b>	no data		
	C		<b>D1, D1a</b>			
	C	no data	<b>D1, D1a, D2</b>			
	C		<b>D1, D1a</b>			
	<b>D</b>		<b>D1, D1a</b>			
	<b>D</b>		<b>D1, D1a</b>			
<b>Proportion w/Clade D</b>	<b>33%</b>	<b>100%</b>	<b>100%</b>			
<i>Pocillopora eydouxi</i>	C1c	C1c	C1,C1c	C1c	C1c	C1,C1c,C42, <b>D,D1a</b>
	C1c	C1c	C1,C1c	C1c	C1c	C1,C1c
	C1c	C1c	<b>D1a</b>	C1c	C1c	C1,C1c,C42, <b>D,D1a</b>
	C1c	C1c	C1,C1c, <b>D1a</b>	C1c	C1c	C1,C1c,C42, <b>D,D1a</b>
	C1c	C1c	C1,C1c	C1c	C1c	C1,C1c,C42, <b>D,D1a</b>
	C1c	C1c	<b>D1a</b>	C1c	C1c	C1,C1c
	C1c	C1c,C42	C1,C1c	C1c	C1c,C42	C1,C1c
	<b>Proportion w/Clade D</b>	<b>0%</b>	<b>0%</b>	<b>43%</b>	<b>0%</b>	<b>0%</b>
<i>Porites lobata</i>	C15	C15	C15	C15	C15	C15
	C15	C15	C15	C15	C15	C15
	C15	C15	C15	C15	C15	C15
<b>Proportion w/Clade D</b>	<b>0%</b>	<b>0%</b>	<b>0%</b>	<b>0%</b>	<b>0%</b>	<b>0%</b>

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