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Symbiodinium sp. associations in the gorgonian *Pseudopterogorgia elisabethae* in the Bahamas: high levels of genetic variability and population structure in symbiotic dinoflagellates

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Abstract Little is known concerning the fine-scale diversity, population structure, and biogeography for *Symbiodinium* spp. populations inhabiting particular invertebrate species, including the gorgonian corals, which are prevalent members of reef communities in the Gulf of Mexico, the Caribbean, and the western Atlantic. This study examined the *Symbiodinium* sp. clade B symbionts hosted by the Caribbean gorgonian *Pseudopterogorgia elisabethae* (Bayer). A total of 575 colonies of *P. elisabethae* were sampled in 1995 and 1998–2000 from 12 populations lying along an ~450 km transect in the Bahamas and their *Symbiodinium* sp. clade B symbionts genotyped at two polymorphic dinucleotide microsatellite loci. Twenty-three unique, two-locus genotypes were identified in association with these *P. elisabethae* colonies. Most colonies hosted only a single *Symbiodinium* sp. clade B genotype; however, in some instances ($n = 25$), two genotypes were harbored simultaneously. For 10 of the 12 populations, 66–100% of the *P. elisabethae* colonies hosted the same symbiont genotype. Added to this, in 9 of the 12 populations, a *Symbiodinium* sp. clade B genotype was either unique to a population or found infrequently in other populations. This distribution of *Symbiodinium* sp. clade B genotypes resulted in statistically significant ($P < 0.05$ or < 0.001) differentiation in 62 of 66 pairwise comparisons of *P. elisabethae* populations. Tests of linkage disequilibrium suggested that a combination of clonal propagation of the haploid phase and recombination is responsible for maintaining these distinct *Symbiodinium* sp. clade B populations.
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Introduction

Mutualistic associations between invertebrates and dinoflagellates are common features of tropical marine communities. These algae, collectively known as zooxanthellae and predominately belonging to the genus *Symbiodinium* Freudenthal (Taylor 1974), play a vital role in their host's nutrition and physiology (reviewed in Davies 1993). It was once thought that all invertebrate–*Symbiodinium* associations involved a single species of dinoflagellate, *Symbiodinium microadriaticum* (Taylor 1974). However, infectivity, ultrastructural, behavioral, and molecular studies on cultured, as well as freshly isolated and in hospite, algae have demonstrated that zooxanthellae comprise a heterogeneous group of many species and strains (reviewed by Rowan 1998). To date, 10 species of *Symbiodinium* and 14 other species of symbiotic dinoflagellates have been described, either formally or informally (Trench and Blank 1987; Banaszak et al. 1993; Trench 1993, 2000).

Early studies quantifying *Symbiodinium* spp. diversity and phylogenetic relationships relied on cultured materials (Schoenberg and Trench 1980a, 1980b, 1980c; Blank and Huss 1989). Unfortunately, *Symbiodinium* spp. cultures can be difficult to establish (Schoenberg and Trench 1980a) and may not be representative of the host or population from which they were isolated (Santos et al. 2001; LaJeunesse 2002). The application of polymerase chain reaction (PCR)-based molecular

techniques has circumvented the need for cultures, leading to an explosion of data regarding *Symbiodinium* spp. diversity and phylogenetics, particularly from natural populations. It is now known that the genus is highly diverse, on a level comparable to that observed among orders of free-living dinoflagellates (Rowan and Powers 1992). Members of *Symbiodinium* are typically identified as belonging to one of several large groups, or clades [i.e. *Symbiodinium* spp. clades A, B, C (Rowan and Powers 1991), D (Carlos et al. 1999), E (*S. californium*; LaJeunesse and Trench 2000; LaJeunesse 2001), F (LaJeunesse 2001), and the symbionts of foraminiferans (Pawlowski et al. 2001; Pochon et al. 2001)] based on analyses of nuclear ribosomal DNA (rDNA) genes. Phylogenies inferred from nuclear 5.8S- (LaJeunesse 2001) and large subunit (28S)-rDNA (Pawlowski et al. 2001; Pochon et al. 2001) group *Symbiodinium* sp. clade A into one assemblage, while *Symbiodinium* spp. clades B/C/D/E/F and the formaniniferan symbionts form a second, closely related group. These relationships are also supported by phylogenies based on organellar (i.e. chloroplast) large subunit (cp23S)-rDNA (Santos et al. 2002).

Although the diversity and phylogenetics of the entire complex of taxa is now well established, there are few data on the population structure and biogeography of *Symbiodinium* spp. over the geographic range of a host species. For hosts that associate with more than a single clade of *Symbiodinium* spp., population structure and biogeography of the dinoflagellate symbionts appear to depend on geography (LaJeunesse and Trench 2000; Rodriguez-Lanetty et al. 2001; Burnett 2002). In contrast, population structure and biogeography may or may not be apparent in hosts that harbor a single clade of *Symbiodinium* spp. For example, populations of *Symbiodinium* sp. clade C associated with the scleractinian corals *Acropora longicyathus* and *Seriatopora hystrix* exhibit significant genetic differentiation between geographical regions of the Indo-West Pacific (Loh et al. 2001). On the other hand, *Symbiodinium* sp. clade A of giant clams are nearly indistinguishable throughout the Indo-Pacific (Baillie et al. 2000). In all of these cases, host species were from the Pacific or Indian Oceans. For Atlantic hosts, data concerning *Symbiodinium* spp. population structure and biogeography over a geographic range are almost nonexistent. The gorgonian *Plexaura kuna* has been found to host *Symbiodinium* sp. clade B at various geographic sites and depths in the Caribbean (Goulet and Coffroth 2003). A study of the *Symbiodinium* spp. populations from two widely separated Caribbean reefs found that many "types" [defined as possessing a distinctive internal transcribed spacer 2 (ITS-2) sequence] occurred at both sites and were shared by individuals of the same host species (LaJeunesse 2002). To date, a comprehensive survey of the fine-scale diversity, population structure, and biogeography of *Symbiodinium* spp. from a single host species distributed in the Gulf of Mexico, Caribbean, or western Atlantic has not been conducted.

The present study addresses this deficiency by using microsatellites to characterize the *Symbiodinium* sp. clade B populations hosted by the gorgonian *Pseudopterogorgia elisabethae* in the Bahamas in the Caribbean. Microsatellites are segments of DNA in which a specific motif of one to six bases is repeated in an array. Length variability in a microsatellite array is typically generated by polymerase slippage during DNA replication and variants (i.e. alleles) detected by PCR and gel electrophoresis. Over the last decade, these single locus, multiallelic, codominant segments of DNA have proved to be versatile and accessible markers for population genetic studies (reviewed by Chambers and MacAvoy 2000). Previously, microsatellites have been used to assess the relatedness between *Symbiodinium* sp. clade B cultures and populations from which they were established (Santos et al. 2001) and demonstrate haploidy in the vegetative (i.e. coccoid) life stage of *Symbiodinium* spp. (Santos and Coffroth 2003).

The economically important Caribbean gorgonian *P. elisabethae* is the sole source of pseudopterosins, natural products with anti-inflammatory properties (Fenical 1987), which are used as a topical agent in cosmetics. This gorgonian is geographically widespread on coral reefs of the Caribbean (Bayer 1961; Kinzie 1970; Lasker and Coffroth 1983); however, little is known about the biology, ecology, and genetics of *P. elisabethae* or its algal symbionts. Sexual reproduction in *P. elisabethae* results in brooded asymbiotic planulae (Kinzie 1974) that take up *Symbiodinium* sp. clade B (Goulet 1999; Santos et al. 2001, 2002, 2003) from the immediate environment following settlement and metamorphosis (horizontal transmission of symbionts). Thus, *Symbiodinium* sp. clade B symbionts could be heterogeneous either within the same colony or among different colonies. Based on cp23S-rDNA genotyping, it has been demonstrated that *P. elisabethae* associates specifically with a single lineage of *Symbiodinium* sp. clade B (*Symbiodinium* sp. B184, cf. Santos et al. 2003). To characterize the fine-scale diversity, population structure, and biogeography of these *Symbiodinium* sp. clade B symbionts, 575 colonies of *P. elisabethae* were sampled from 12 sites along an ~450 km transect in the Bahamas, and their symbionts were genotyped at two polymorphic dinucleotide microsatellite loci.

Materials and methods

Biological materials and nucleic acid isolation

Pseudopterogorgia elisabethae (Bayer) colonies were sampled either in 1995 (Sweetings Cay) or between 1998 and 2000 (all other sites) using SCUBA. Collections were made at 12 sites along an ~450 km transect of the Bahamas: Sweetings Cay (SC; 21.6 m), Gorda Rock (GR; 16.7 m), Abaco Shallow (AS; 8.7 m), and Abaco Deep (AD; 16.7 m) at Abaco Island; South Hampton Reef (SH; 16 m) and East End Point (EE; 12.7 m) at Eleuthera Island; Little San Salvador (LS; 10.3 m), Cat Island (CI; 26 m), Hog Cay (HC; 12.3 m), Rum Cay (RC; 21.6 m), and Pillar Reef (PR; 11 m); and Riding Rock (RR; 17 m) at San Salvador (Fig. 1). For the sites at San Salvador, the two *P. elisabethae* populations were within ~1 km of each other, allowing for a within-reef comparison. Sampling was conducted in two ways. Where *P. elisabethae* was abundant,

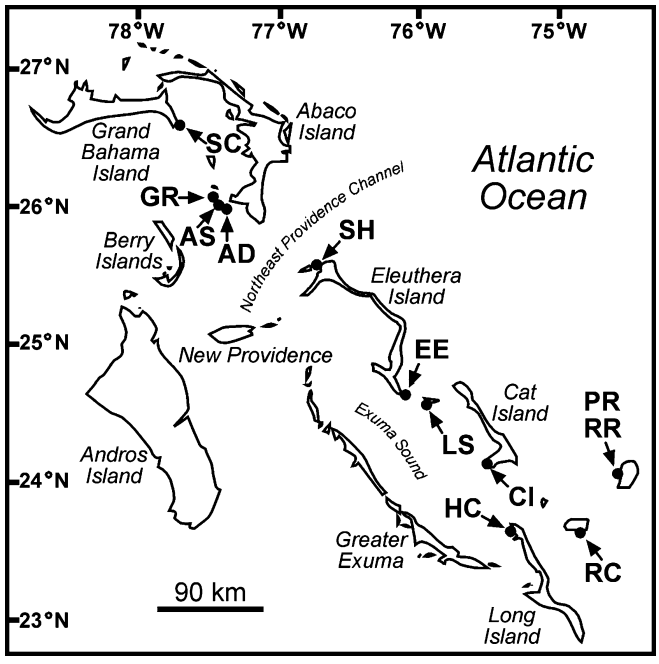

Fig. 1 *Symbiodinium* sp. clade B. Map of 12 *Pseudopterogorgia elisabethae* populations in the Bahamas [denoted by two-letter abbreviations as follows: *SC* Sweetings Cay (26°34′N, 77°48′W); *GR* Gorda Rock (26°07′N, 77°33′W); *AS* Abaco Shallow (26°02′N, 77°28′W); *AD* Abaco Deep (25°59′N, 77°25′W); *SH* South Hampton Reef (25°25′N, 76°48′W); *EE* East End Point of Eleuthera (24°37′N, 76°10′W); *LS* Little San Salvador (24°35′N, 75°58′W); *CI* Cat Island (24°09′N, 75°32′W); *HC* Hog Cay (23°37′N, 75°21′W); *RC* Rum Cay (23°37′N, 74°52′W); *PR* Pillar Reef; *RR* Riding Rock (24°04′N, 74°32.5′W)]

collections were performed along 20-m transects, collecting a branch of each individual found within 1 m of the transect line. Where *P. elisabethae* was less abundant (e.g. GR, EE, CI, and RC), all colonies encountered were sampled until the desired sample size was achieved. Tissue samples were preserved in either 95% ethanol or salt-saturated DMSO preservation buffer (Seutin et al. 1991) immediately following collection. Between 43 and 50 *P. elisabethae* colonies were selected haphazardly from each collection (total sample size = 575 colonies) for the study. Total nucleic acids were extracted from approximately 3 cm of *P. elisabethae* tissue by first grinding the tissue in STE buffer [0.05 M Tris-HCl (pH 8.0), 0.1 M EDTA, 0.1 M NaCl, 0.2% SDS] followed by a modified extraction protocol using the Prep-A-Gene DNA extraction kit (Bio-Rad Laboratories, Hercules, Calif.).

Isolation and screening of *Symbiodinium* sp. clade B microsatellite loci

Two *Symbiodinium* sp. clade B microsatellite loci, CA4.86 and CA6.38, were used in this study (Table 1). Details regarding the isolation of these microsatellite loci and screening of the PCR primers are presented in Santos and Coffroth (2003).

Microsatellite amplifications and allele detection from *Symbiodinium* sp. clade B populations

PCR reactions for each microsatellite locus were performed in 10 μl volumes containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, 200 μM dNTPs, 1 U *Taq* polymerase, and 10 ng of template DNA. PCR primer and $MgCl_2$ concentrations varied for each locus. For loci CA4.86 and CA6.38, $MgCl_2$ concentrations were 2.5 mM and 1.5 mM, respectively. Primer concentrations for locus CA4.86 were 0.2 pmol CA4.86R, 0.18 pmol CA4.86L, and 0.02 pmol 5′-IRD800 M13 forward primer. For locus CA6.38, primer concentrations were 3 pmol CA6.38R, 2 pmol CA6.38L, and 0.2 pmol 5′-IRD800 M13 forward primer. Nineteen nucleotides (5′-CACGACGTTGTAAAACGAC-3′) were added to the 5′ end of primers CA4.86L and CA6.38L to allow incorporation of the 5′-IRD800 fluorescent-labeled M13 forward primer (LI-COR Biotechnology Division, Lincoln, Neb.) into PCR products. Thermocycling conditions were: initial denaturing step of 94°C for 2 min, 35 cycles of 94°C for 30 s, annealing temperature of 50°C or 56°C (see Table 1) for 30 s, 72°C for 30 s, and a final extension of 72°C for 3 min. Following amplification, reactions were diluted with 5 μl loading buffer [95% (v/v) formamide, 10 mM EDTA, 0.01% bromophenol blue (final pH 9.0)], denatured for 3 min at 95°C, and snap-cooled on ice immediately prior to polyacrylamide gel electrophoresis.

Electrophoresis was done in 25 cm long, 0.25 mm thick 6.5% Long Ranger (FMC Bioproducts, Rockland, Me.)/1× Tris-borate (TBE) polyacrylamide gels under denaturing (7 M urea) conditions and visualized using a LI-COR NEN Global IR2 DNA sequencer system (LI-COR Biotechnology Division). Conditions were 1,500 V, 40 W, 40 mA, 50°C, and the default scan speed. DNA ladders, spanning either 97–147 bp or 172–272 bp at 25 bp increments, were run in every fifth lane across the gel for size reference. A single gel was reloaded up to three times over a 5 h period. Sizing of alleles was done with the fragment analysis program Gene ImagIR v3.55 (Scanalytics, Fairfax, Va.), using the DNA ladders as size references. Alleles were scored according to "true" base pair (bp) number, excluding the nineteen 5′-nucleotides of the fluorescent-labeled M13 forward primer.

Data analysis

A variety of descriptive and statistical analyses were conducted on the data set. First, allelic frequencies were derived from the total number of alleles observed for a locus from all samples, while allelic diversities were estimated as the expected heterozygosity index (*H*; Nei 1978). Allelic frequencies and diversities were calculated separately for each population, as well as across all populations, using the program "Tools For Population Genetic Analysis" (TFPGA) v1.3 (Miller 1997). Estimates of population structure were made using both infinite-alleles (IAM; Kimura and Crow 1964) and stepwise-mutation (SMM; Kimura and Ohta 1978) evolutionary models. F_{ST}, which assumes an IAM, was estimated using θ (Weir and Cockerham 1984). Single-locus estimates of θ were weighted as described by Weir and Cockerham (1984) to create a combined measure. R_{ST}, an analog of θ that assumes a SMM (Slatkin 1995), was estimated using ρ_{ST} (Rousset 1996). The standardization approach of Goodman (1997) was used to make a two-locus measure of R_{ST}. Values of F_{ST} and R_{ST} were obtained with FSTAT v2.9.3.2 (Goudet 2001).

Table 1 *Symbiodinium* sp. clade B. Characterization of microsatellite loci and primer sets employed in this study

Locus	Motif type	Array sequence	Primer sequences	Anneal (°C)
CA4.86	CA, interrupted complex	$(CACC)CA(CACC)_2(CA)_9$	CA4.86R: 5′-GCCTTCAATGCAATCACCTT-3′ CA4.86L: 5′-GGAATTGGCCATCCCTCTAT-3′	50
CA6.38	CA, interrupted pure	$(CA)_9CG(CA)_4$	CA 6.38R: 5′-CAAAGAATATTCGGGGGTCA-3′ CA6.38L: 5′-AGTTGATACGCCGGATGTGT-3′	56

Second, the alleles detected at the loci were used to construct a two-locus genotype [i.e. haplotype, as recent work has demonstrated that the vegetative form of *Symbiodinium* spp. is haploid (Santos and Coffroth 2003)] for the *Symbiodinium* sp. clade B population of each *P. elisabethae* colony. To test for geographical differentiation, a categorical analysis consisting of a χ^2 test of absolute genotype frequencies at each site (Hudson et al. 1992) was conducted. Due to bias with low expected values, the *P* value was obtained by simulating the null distribution of no geographic subdivision (10,000 permutations) using the algorithm of Roff and Bentzen (1989) implemented in CHIPERM v1.2 (written by D. Posada; available at http://bioag.byu.edu/zoology/crandall_lab/programs.htm). Pairwise tests for *Symbiodinium* sp. clade B population differentiation were also conducted by randomizing genotypes between pairs of populations using FSTAT v2.9.3.2. To graphically depict the relationship between *Symbiodinium* sp. clade B populations, an unweighted pair group method using arithmetic averages (UPGMA) dendrogram was constructed using Nei's minimum genetic distance (Nei 1972) in TFPGA v1.3. In addition, a Mantel test (Mantel 1967) was performed to test for a relationship between geographic distance and genetic distance. The Mantel test was conducted in TFPGA v1.3 using Nei's minimum genetic distance and 1,000 permutations.

Third, to determine if recombination was occurring among the *Symbiodinium* sp. clade B genotypes, the null hypothesis of random association among alleles at loci was tested with a permutation procedure. The program LIAN v3.1 (Haubold and Hudson 2000) was used to calculate the number of alleles shared (*D*) between all pairwise comparisons of genotypes and to measure the variance of this distance measurement (V_D). To test if the observed data set differed from random expectations, the observed V_D was compared to the distribution of V_D values in 10,000 simulated data sets. Simulated data sets were generated by random reshuffling of alleles among *Symbiodinium* sp. clade B genotypes. Significant linkage disequilibrium (LD), or non-random association of alleles, was detected if the observed V_D was >95% of the values generated in the reshuffled data sets. A "standardized" index of association (I_A^S; Haubold and Hudson 2000) was used to measure the strength of LD. Testing for LD was conducted separately on two data sets. The first comprised the *Symbiodinium* sp. clade B genotypes of all *P. elisabethae* colonies in the study, while the second included only a single representative of each unique genotype.

For the descriptive and statistical analyses, we assumed that alleles identical in state (i.e. size) were identical by descent. Although microsatellite alleles may sometimes violate this assumption (e.g. Viard et al. 1998), the occurrence of size homoplasy (alleles identical in state, but not identical by descent) is not considered a significant problem for analyses of population genetic structure (Estoup et al. 2002).

Results

Symbiodinium sp. clade B microsatellites from *Pseudopterogorgia elisabethae*

Screening of the *Symbiodinium* sp. clade B populations of *P. elisabethae* at CA4.86 and CA6.38 revealed that both loci were polymorphic. Eight and ten distinct alleles were identified from CA4.86 and CA6.38, respectively (Table 2). Allele sizes for CA4.86 ranged between 185 and 207 bp, while alleles for CA6.38 ranged between 96 and 122 bp (Table 2). For most *P. elisabethae* colonies ($n = 533$, 92.7% of the colonies), only a single *Symbiodinium* sp. clade B microsatellite allele was detected per locus. A single allele per microsatellite locus was also recovered from the majority of *Symbiodinium* sp. clade B populations of the Caribbean gorgonian *Plexaura kuna* and a series of *Symbiodinium* sp. clade B cultures (Santos and Coffroth 2003). This is consistent with *Symbiodinium* spp., like other dinoflagellates, possessing a haploid vegetative life stage (Blank 1987; Pfiester and Anderson 1987; Santos and Coffroth 2003). In some cases ($n = 25$, 4.3% of the colonies), two alleles

Table 2 *Symbiodinium* sp. clade B. Symbiont microsatellite allele frequencies at loci CA4.86 and CA6.38 for *Pseudopterogorgia elisabethae* at 12 sites in the Bahamas (*n* total number of alleles recovered from all colonies at each site; *site abbreviations*, see Fig. 1)

Allele size	Site											
	SC	GR	AS	AD	SH	EE	LS	CI	HC	RC	PR	RR
Locus CA4.86												
185 bp	0.022	–	–	–	–	–	–	–	–	–	–	–
189 bp	0.089	0.043	–	–	–	–	–	–	–	–	–	–
191 bp	0.823	–	0.151	0.44	–	–	0.435	–	–	–	0.04	–
193 bp	0.044	0.893	0.717	0.56	0.979	0.085	0.098	0.083	0.187	0.891	0.96	1.0
195 bp	–	0.021	0.132	–	0.021	0.894	0.435	0.917	0.813	0.109	–	–
197 bp	–	–	–	–	–	–	0.016	–	–	–	–	–
199 bp	–	0.043	–	–	–	0.021	0.016	–	–	–	–	–
207 bp	0.022	–	–	–	–	–	–	–	–	–	–	–
n	45	47	53	50	47	47	62	48	48	46	50	43
Locus CA6.38												
96 bp	0.022	–	–	–	–	0.023	–	1.0	0.065	0.143	–	–
98 bp	0.844	–	–	–	–	–	–	–	0.935	–	–	–
100 bp	–	0.041	–	–	0.04	0.045	0.02	–	–	0.02	–	–
104 bp	0.022	–	–	–	–	–	–	–	–	–	–	–
112 bp	–	–	–	–	–	–	–	–	–	–	1.0	1.0
114 bp	–	–	–	–	–	–	0.02	–	–	–	–	–
116 bp	–	0.245	0.22	0.36	0.02	0.182	0.082	–	–	–	–	–
118 bp	0.112	0.714	0.78	0.64	0.94	0.727	0.878	–	–	0.041	–	–
120 bp	–	–	–	–	–	0.023	–	–	–	0.041	–	–
122 bp	–	–	–	–	–	–	–	–	–	0.755	–	–
n	45	49	50	50	50	44	49	45	46	49	50	43

were recovered from one or both loci. The recovery of two alleles from a locus occurred in one to two *P. elisabethae* colonies at 8 of the 12 sites. For the *P. elisabethae* population at Little San Salvador, 12 of 50 colonies had *Symbiodinium* sp. clade B populations with two alleles at one or both loci. These 25 colonies are thought to host two genotypes of *Symbiodinium* sp. clade B simultaneously (Santos and Coffroth 2003). In addition, the *Symbiodinium* sp. clade B populations of some *P. elisabethae* colonies ($n = 17$, 3.2% of the colonies) did not amplify at one or both loci despite multiple PCR attempts. For the purposes of this study, we assumed that the absence of PCR amplification at a locus represented a null allele for that locus. Details regarding samples with two alleles or null alleles are presented in Electronic Appendix 1.

Genetic diversity of *Symbiodinium* sp. clade B populations from *P. elisabethae*

In each of the *Symbiodinium* sp. clade B populations, one of three alleles at locus CA4.86 was most prevalent, and in 10 of the 12 populations, one of these three alleles was present in >70% of the *P. elisabethae* colonies (Table 2). For locus CA6.38, one of five alleles was most prevalent in a population, being present in >60% of the colonies (Table 2). At Abaco Deep and Little San Salvador, two alleles were numerically dominant at locus CA4.86 and were recovered at approximately equal frequencies (Table 2). There was a substantial range in genetic variation among the *Symbiodinium* sp. clade B populations. Allelic diversity (H) ranged from 0 to 0.611 for locus CA4.86 and from 0 to 0.461 for locus CA6.38 (Electronic Appendix 2). The average H over all populations for CA4.86 and CA6.38 was 0.611 and 0.760, respectively. The H for both loci and over all populations was 0.685, suggesting a high level of genetic diversity among the *Symbiodinium* sp. clade B populations of *P. elisabethae*.

Estimates of population structure were indicative of strong subdivision in the *Symbiodinium* sp. clade B populations of *P. elisabethae*. F_{ST} estimates were 0.579 and 0.701 for CA4.86 and CA6.38, respectively, with an F_{ST} for both loci of 0.647. Estimates of R_{ST} were similar to those for F_{ST}; 0.479 for CA4.86 and 0.794 for CA6.38, with an R_{ST} for both loci of 0.639. Prior to constructing *Symbiodinium* sp. clade B genotypes, the 42 *P. elisabethae* colonies for which two alleles and/or null alleles were detected at a locus were excluded since a genotype could not be determined unambiguously. From the remaining 533 colonies, 23 unique *Symbiodinium* sp. clade B genotypes were found to associate with *P. elisabethae* in the Bahamas (Table 3).

Population structure and geographical differentiation of *Symbiodinium* sp. clade B populations from *P. elisabethae*

Striking differences in the distribution of *Symbiodinium* sp. clade B genotypes were evident across the Bahamas

Table 3 *Symbiodinium* sp. clade B. Symbiont genotype frequencies for *Pseudopterogorgia elisabethae* at 12 sites in the Bahamas. *Genotype* is defined as a unique combination of microsatellite allele sizes at loci CA4.86 and CA6.38 (n number of colonies from each site for which a symbiont genotype could be unambiguously identified; *site abbreviations*, see Fig. 1

Genotype		Site											
Locus CA4.86	Locus CA6.38	SC	GR	AS	AD	SH	EE	LS	CI	HC	RC	PR	RR
191 bp	98 bp	0.815	–	–	–	–	–	–	–	–	–	–	–
193 bp	118 bp	0.023	0.668	0.702	0.456	0.956	0.068	0.083	–	–	–	–	–
191 bp	96 bp	0.023	–	–	–	–	–	–	–	–	–	–	–
189 bp	118 bp	0.047	0.044	–	–	–	–	–	–	–	–	–	–
193 bp	98 bp	0.023	–	–	–	–	–	–	–	0.091	–	–	–
191 bp	118 bp	0.023	–	0.128	0.196	–	–	0.416	–	–	–	–	–
185 bp	98 bp	0.023	–	–	–	–	–	–	–	–	–	–	–
207 bp	104 bp	0.023	–	–	–	–	–	–	–	–	–	–	–
193 bp	116 bp	–	0.222	0.064	0.109	0.022	–	–	–	–	–	–	–
199 bp	100 bp	–	0.044	–	–	–	0.023	0.028	–	–	–	–	–
195 bp	118 bp	–	0.022	–	–	0.022	0.658	0.361	–	–	0.045	–	–
195 bp	116 bp	–	–	0.106	–	–	0.182	0.056	–	–	–	–	–
191 bp	116 bp	–	–	–	0.239	–	–	0.028	–	–	–	–	–
195 bp	114 bp	–	–	–	–	–	–	0.028	–	–	–	–	–
195 bp	100 bp	–	–	–	–	–	0.023	–	–	–	–	–	–
193 bp	96 bp	–	–	–	–	–	0.023	–	0.089	0.068	0.045	–	–
195 bp	120 bp	–	–	–	–	–	0.023	–	–	–	–	–	–
195 bp	96 bp	–	–	–	–	–	–	–	0.911	–	0.069	–	–
195 bp	98 bp	–	–	–	–	–	–	–	–	0.841	–	–	–
193 bp	122 bp	–	–	–	–	–	–	–	–	–	0.796	–	–
193 bp	120 bp	–	–	–	–	–	–	–	–	–	0.045	–	–
191 bp	112 bp	–	–	–	–	–	–	–	–	–	–	0.040	–
193 bp	112 bp	–	–	–	–	–	–	–	–	–	–	0.960	1.000
n		43	45	47	46	46	44	36	45	44	44	50	43

Table 4 *Symbiodinium* sp. clade B. Pairwise tests of symbiont population differentiation for *Pseudopterogorgia elisabethae* at 12 sites in the Bahamas (*site abbreviations*, see Fig. 1; *NS* not significant; $*P < 0.05$; $***P < 0.001$)

Site	GR	AS	AD	SH	EE	LS	CI	HC	RC	PR	RR
SC	***	***	***	***	***	***	***	***	***	***	***
GR		NS	***	NS	***	***	***	***	***	***	***
AS			*	NS	***	***	***	***	***	***	***
AD				***	***	***	***	***	***	***	***
SH					***	***	***	***	***	***	***
EE						***	***	***	***	***	***
LS							***	***	***	***	***
CI								***	***	***	***
HC									***	***	***
RC										***	***
PR											NS

(Table 3). In 10 of the 12 *P. elisabethae* populations, a single *Symbiodinium* sp. clade B genotype was recovered from ~66% to 100% of the colonies at a site. In six of these populations (SC, CI, HC, RC, PR, and RR), the numerically dominant *Symbiodinium* sp. clade B genotype was either unique to that population or found infrequently in other populations. In three populations (GR, AS, and SH), the same numerically dominant *Symbiodinium* sp. clade B genotype was shared by 66.8–95.6% of the *P. elisabethae* colonies. This symbiont genotype was predominately confined to these sites. Many (45.6%) of the *P. elisabethae* colonies at Abaco Deep harbored the numerically dominant *Symbiodinium* sp. clade B genotype found at Gorda Rock, Abaco Shallow, and South Hampton Reef, but also associated with a genotype found almost exclusively in this population (23.9% of the colonies). The remaining *P. elisabethae* populations (EE and LS) also shared a common *Symbiodinium* sp. clade B genotype in 36.1% and 65.9%, respectively, of the colonies. However, colonies at Little San Salvador also hosted a *Symbiodinium* sp. clade B genotype (41.6% of the colonies) that was not found at East End Point and only found infrequently in other populations. For all populations other than Riding Rock, in addition to the numerically dominant *Symbiodinium* sp. clade B genotype, between one and seven additional genotypes were observed in a population, but typically at low frequencies (Table 3). The distribution of *Symbiodinium* sp. clade B genotypes followed no patterns related to depth; distinct genotypes were found at the same depth (e.g. SC and RC), while the same *Symbiodinium* sp. clade B genotype could be found at different depths (e.g. PR and RR; Table 3).

The categorical analysis of *Symbiodinium* sp. clade B genotype frequencies by site revealed highly significant differentiation ($\chi^2 = 3{,}072.74$; $P < 10^{-4}$) between populations. Pairwise tests of *Symbiodinium* sp. clade B population differentiation gave similar results. Of the 66 pairwise comparisons, 62 were significant at P values of from < 0.05 to < 0.001 (Table 4). For the within-reef comparison, the *Symbiodinium* sp. clade B populations at Riding Rock and Pillar Reef were not significantly different. The other three pairwise comparisons that did not differ significantly involved the *Symbiodinium* sp. clade B populations at Gorda Rock, Abaco Shallow, and South Hampton Reef, all of which shared a common genotype.

Comparisons of the *Symbiodinium* sp. clade B populations from *P. elisabethae* based on genetic distance detected some correspondence to geographic location in the Bahamas (Electronic Appendix 3). Although the correlation was low ($r = 0.268$), the Mantel test detected a significant positive correlation between genetic and geographic distance ($P = 0.017$). Furthermore, the UPGMA dendrogram identified several clusters that also corresponded to the sites' geography (Fig. 2). In general, similar *Symbiodinium* sp. clade B populations were recovered from sites that were very close geographically. Thus, Riding Rock and Pillar Reef, which are located within 1 km on the same reef, formed a cluster. In the northwestern Bahamas, Gorda Rock, Abaco Shallow, Abaco Deep, and South Hampton Reef also formed a cluster. The first three of these populations are geographically close, but South Hampton Reef is ~90 km from the nearest other site. In the middle Bahamas, *Symbiodinium* sp. clade B populations from the geographically close sites of East End Point and Little San Salvador also grouped

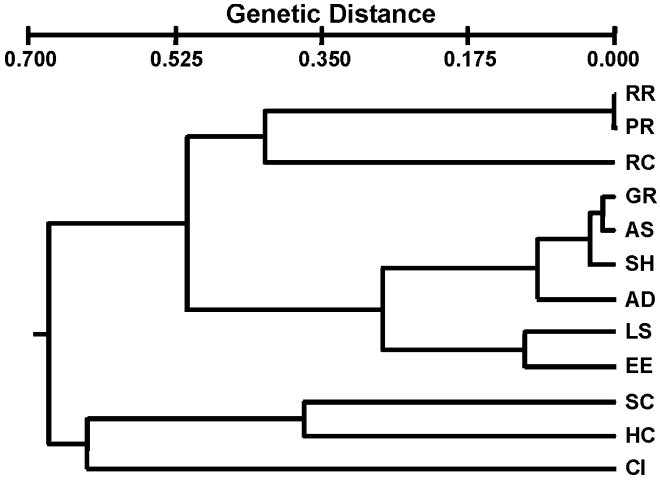

Fig. 2 *Symbiodinium* sp. clade B. Dendrogram by unweighted pair group method using arithmetic averages, depicting relationships between symbiont populations of *Pseudopterogorgia elisabethae* at 12 sites in the Bahamas

together. On the other hand, Cat Island and Hog Cay, which like East End Point and Little San Salvador, are within Exuma Sound (Fig. 1), showed significant differentiation (Fig. 2; Table 4). The *Symbiodinium* sp. clade B populations of Sweetings Cay, at the northwestern end of the Bahamas, clustered more closely with populations from Hog Cay than Hog Cay did with Cat Island *Symbiodinium* sp. clade B populations. The relationships are driven in large part by the high similarity of populations separated by tens of kilometers. At greater distances, there was no clear relationship between genetic and geographic distance.

Multilocus linkage disequilibrium of *Symbiodinium* sp. clade B populations from *P. elisabethae*

Analysis of the complete *Symbiodinium* sp. clade B population data set identified a highly significant deviation ($I_A^S = 0.139$; $P < 10^{-4}$) from random association among alleles. However, 17 (73.9%) of the 23 *Symbiodinium* sp. clade B genotypes were represented in the data set more than once. Among clonal organisms, I_A^S is often >0 when one or a few genotypes are widespread and/or abundant (Maynard-Smith et al. 1993). In order to test for true LD, Maynard-Smith et al. (1993) suggest analysis of a data set comprising only one representative of each unique genotype. The curtailed data set of 23 unique *Symbiodinium* sp. clade B genotypes revealed no significant deviation ($I_A^S = -0.114$; $P = 0.999$) from random associations.

Discussion

This study is the first to explore the fine-scale diversity, population structure, and biogeography of *Symbiodinium* sp. clade B populations from a single, geographically widespread Caribbean host species using microsatellites. Using only two microsatellites, we have identified 23 unique *Symbiodinium* sp. clade B genotypes in *Pseudopterogorgia elisabethae*, all belonging to a single lineage, *Symbiodinium* sp. B184 (cf. Santos et al. 2003, unpublished data). In addition, we have documented significant population differentiation among the *Symbiodinium* sp. clade B from different Bahamian reefs. These microsatellite loci also appear to be stable over time. For example, *Symbiodinium* sp. clade B culture SSPe, which was established from a *P. elisabethae* colony collected at Riding Rock in January 1999 (Santos et al. 2001), had the same alleles as those in the symbiont populations of Riding Rock *P. elisabethae* colonies in the present study. The SSPe culture has been transferred into fresh medium every 3–4 weeks since its isolation so this represents allelic stability over ~36 transfers in 3 years. Taken together, these properties make microsatellites powerful tools for exploring questions of *Symbiodinium* spp. biology.

Mechanisms driving population differentiation of *Symbiodinium* sp. clade B across the Bahamas

Our documentation of striking population differentiation in *Symbiodinium* sp. clade B across the Bahamas is surprising given the recovery of *Symbiodinium* spp. isolates with identical rDNA sequences, such as cp23S-rDNA (Santos et al. 2002) and the less conserved ITS-rDNA (Baillie et al. 2000; LaJeunesse 2001, 2002; Santos et al. 2001), from Pacific and Atlantic hosts. This implies widespread dispersal of these symbiotic algae. Furthermore, the recovery of *Symbiodinium* spp. from environmental water samples (Loeblich and Sherley 1979; Chang 1983; Carlos et al. 1999) lends support for the existence of planktonic or benthic populations that are capable of dispersing. We suggest the following hypotheses to explain the discrepancy between the data presented here and these previous studies. Firstly, we are capable of identifying significant levels of differentiation among these populations of *Symbiodinium* sp. clade B since microsatellites reveal substantially more within-lineage variation than other molecular markers (e.g. Blackston et al. 2001). Secondly, the symbiotic algae of *P. elisabethae* may have greater levels of genetic variation due to limited intrinsic dispersal abilities. *Symbiodinium* spp. isolates display characteristic motility patterns (Fitt et al. 1981; Fitt and Trench 1983; Crafts and Tuliszewski 1995), and differences in this trait may have important implications in the dispersal of some *Symbiodinium* spp., such as those associated with *P. elisabethae*. A third hypothesis is that the *Symbiodinium* sp. clade B populations at each site have been/are subjected to selection greater than the effects of migration between habitats. Thus, it is possible that all the symbiont genotypes identified in this study are found throughout the Bahamas. However, particular *Symbiodinium* sp. clade B genotypes may be selected differentially in each *P. elisabethae* population since they have a selective advantage, ultimately leading to the prevalence of a single genotype within a population. Of the possible forms that a selective advantage between symbiont genotypes may take, the most likely involves differences in physiological and/or biochemical characteristics. Variation in photobiology (Chang et al. 1983; Iglesias-Prieto and Trench 1994, 1997; Perez et al. 2001), in water-soluble peridinin–chl *a* protein (sPCP) synthesis and form (Govind et al 1990; Iglesias-Prieto and Trench 1997), and in isozyme profiles (Colley 1984; Baillie et al. 1998), as well as temperature tolerance (Kinzie et al. 2001; Perez et al. 2001), have been documented between different *Symbiodinium* spp. isolates. However, these data are limited, making extrapolation to other isolates or in hospite populations difficult. Establishing cultures of the *Symbiodinium* sp. clade B genotypes associated with *P. elisabethae*, followed by detailed characterization, would provide a way to test this hypothesis and to add to the information on *Symbiodinium* spp. physiology and biochemistry. These hypotheses are also not mutually exclusive, and, if we accept that the first

hypothesis is correct, then some mechanism such as drift and/or selection is still required to generate the differences among populations that we have identified using microsatellites.

Oceanic currents and *Symbiodinium* sp. clade B biogeography

In a few cases, *Symbiodinium* sp. clade B populations at different sites in the Bahamas shared the same numerically dominant genotype. We hypothesize that oceanic currents, driven by trade winds as well as tidal flows, produce the observed patterns by aiding in the transport of *Symbiodinium* sp. clade B genotypes between these populations. For example, of the two numerically dominant genotypes at Little San Salvador, one is unique to the population, while the other is identical to the numerically dominant *Symbiodinium* sp. clade B genotype at East End Point (Table 4). Although separated by deep water, the two populations are relatively close (22.5 km) and transport of a symbiont genotype from East End Point to Little San Salvador via oceanic currents is consistent with this distribution pattern. Similarly, a common symbiont genotype was found at Gorda Rock, Abaco Shallow, and South Hampton Reef, which span a distance of ~89 km. This implies transport of this *Symbiodinium* sp. clade B genotype across the Northeast Providence Channel, a deep (~3,600 m) channel that separates Abaco and Eleuthera Islands (Fig. 1). These observations also suggest that distance alone is not a good predictor of whether a symbiont genotype will be unique or shared between host populations. Thus, most *Symbiodinium* sp. clade B genotypes appear to be retained within local populations, similar to model simulations (Cowen et al. 2000) and empirical data (Taylor and Hellberg 2003) from larval dispersing organisms in the Caribbean; however, dispersal by oceanic currents may also influence the population structure of *Symbiodinium* spp.

Recombination in *Symbiodinium* sp. clade B populations

Tests for multilocus linkage disequilibrium have been used to estimate levels of recombination among protozoan and fungal populations (Maynard-Smith et al. 1993; Anderson et al. 2000; de Meeus et al. 2002; Fisher et al. 2002). Analyses of complete, as well as curtailed, data sets distinguish if an organism is panmictic (random association among alleles due to recombination), "epidemic" (LD resulting from temporal expansion of particular genotypes in an otherwise recombining population), or truly clonal (levels of recombination insufficient to break down lineages) (Maynard-Smith et al. 1993). We found strong LD for the complete data set of *Symbiodinium* sp. clade B genotypes from *P. elisabethae*; on the other hand, linkage equilibrium was restored when a data set comprised solely of unique genotypes was reanalyzed. These results suggest that the symbiont populations of *P. elisabethae* are "epidemic", being maintained by a combination of clonal propagation of the haploid phase and recombination. Our study is the first to document this form of population structure in *Symbiodinium* spp., which was hypothesized by LaJeunesse (2001). Furthermore, although recombination has been rarely observed for dinoflagellates (Pfiester and Anderson 1987) and inconsistent descriptions of recombination have been reported for *Symbiodinium* spp. (Freudenthal 1962; Taylor 1974), our study contributes to the growing evidence that these dinoflagellates undergo recombination during their life cycle (reviewed by Santos and Coffroth 2003). However, the frequency of recombination, where it occurs (e.g. within an invertebrate host or in the environment) and the circumstances that induce it remain unknown and may prove difficult to establish. Thus, molecular data, such as those presented here, may prove to be the only method of documenting recombination in these enigmatic dinoflagellates.

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References

Anderson TJC, Haubold B, Williams JT, Estrada-Franco JG, Richardson L, Mollinedo R, Bockarie M, Mokili J, Mharakura S, French N, Whitworth J, Velez ID, Brockman AH, Nosten F, Ferreira MU, Day KP (2000) Microsatellite markers reveal a spectrum of population structures in the malaria parasite *Plasmodium falciparum*. Mol Biol Evol 17:1467–1482

Baillie BK, Monje VA, Silvestre V, Sison M, Belda-Baillie CA (1998) Allozyme electrophoresis as a tool for distinguishing different zooxanthellae symbiotic with giant clams. Proc R Soc Lond B Biol Sci 256:1949–1956

Baillie BK, Belda-Baillie CA, Maruyama T (2000) Conspecificity and Indo-Pacific distribution of *Symbiodinium* genotypes (Dinophyceae) from giant clams. J Phycol 36:1153–1161

Banaszak AT, Iglesias-Prieto R, Trench RK (1993) *Scrippsiella velellae* sp. nov. (Peridiniales) and *Gloeodinium viscum* sp. nov. (Phytodiniales), dinoflagellate symbionts of two hydrozoans (Cnidaria). J Phycol 29:517–528

Bayer FM (1961) The shallow water Octocorallia of the East Indian region. Martinus Nijoff, The Hague

Blackston CR, Dubey JP, Dotson E, Su C, Thulliez P, Sibley D, Lehmann T (2001) High-resolution typing of *Toxoplasma gondii* using microsatellite loci. J Parasitol 87:1472–1475

Blank RJ (1987) Cell architecture of the dinoflagellate *Symbiodinium* sp. inhabiting the Hawaiian stony coral *Montipora verrucosa*. Mar Biol 94:143–155

Blank RJ, Huss VAR (1989) DNA divergency and speciation in *Symbiodinium* (Dinophyceae). Plant Syst Evol 163:153–163

Burnett WJ (2002) Longitudinal variation in algal symbionts (zooxanthellae) from the Indian Ocean zoanthid *Palythoa caesia*. Mar Ecol Prog Ser 234:105–109

Carlos AA, Baillie BK, Kawachi M, Maruyama T (1999) Phylogenetic position of *Symbiodinium* (Dinophyceae) isolates from tridacnids (Bivalvia), cardiids (Bivalvia), a sponge (Porifera), a soft coral (Anthozoa), and a free-living strain. J Phycol 35:1054–1062

Chambers GK, MacAvoy ES (2000) Microsatellites: consensus and controversy. Comp Biochem Physiol 126:455–476

Chang FH (1983) Winter phytoplankton and microzooplankton populations off the coast of Westland, New Zealand, 1979. NZ J Mar Freshw Res 17:279–304

Chang SS, Prezelin BB, Trench RK (1983) Mechanisms of photoadaptation in three strains of the symbiotic dinoflagellate *Symbiodinium microadriaticum*. Mar Biol 76:219–229

Colley NJ (1984) The cell biology of dinoflagellate symbiosis in a coelenterate. PhD dissertation, University of California, Santa Barbara

Cowen RK, Lwiza KMM, Sponaugle S, Paris CB, Olson DB (2000) Connectivity of marine populations: open or closed? Science 287:857–859

Crafts CB, Tuliszewski JR (1995) Motility rhythms in cultured zooxanthellae isolated from the scyphomedusa *Linuche unguiculata*. Bull Mar Sci 56:822–825

Davies PS (1993) Endosymbiosis in marine cnidarians. In: John DM, Hawkins SJ, Price JH (eds) Plant–Animal interactions in the marine benthos. Clarendon, Oxford, pp 511–540

de Meeus T, Renaud F, Mouveroux E, Reynes J, Galeazzi G, Mallie M, Bastide JM (2002) Genetic structure of *Candida glabrata* populations in AIDS and non-AIDS patients. J Clin Microbiol 40:2199–2206

Estoup A, Jarne P, Cornuet JM (2002) Homoplasy and mutation model at microsatellite loci and their consequences for population genetic analysis. Mol Ecol 11:1591–1604

Fenical W (1987) Marine soft corals of the genus *Pseudopterogorgia*: a resource for novel anti-flamatory diterpenoids. J Nat Prod 6:1001–1008

Fisher MC, Rannala B, Chaturvedi V, Taylor JW (2002) Disease surveillance in recombining pathogens: multilocus genotypes identify sources of human *Coccidioides* infections. Proc Natl Acad Sci USA 99:9067–9071

Fitt WK, Trench RK (1983) The relation of diel patterns of cell division to diel patterns of motility in the symbiotic dinoflagellate *Symbiodinium microadriaticum* Freudenthal in culture. New Phytol 94:421–432

Fitt WK, Chang SS, Trench RK (1981) Motility patterns of different strains of the symbiotic dinoflagellate *Symbiodinium* (=*Gymnodinium*) *microadriaticum* (Freudenthal) in culture. Bull Mar Sci 31:436–443

Freudenthal HD (1962) *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a zooxanthella: taxonomy, life cycle and morphology. J Protozool 9:45–52

Goodman SJ (1997) Rst Calc: a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. Mol Ecol 6:881–885

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). Available from http://www.unil.ch/izea/softwares/fstat.html

Goulet TL (1999) Temporal and spatial stability of zooxanthellae in octocorals. PhD dissertation, State University of New York at Buffalo, Buffalo

Goulet TL, Coffroth MA (2003) Genetic composition of zooxanthellae between and within colonies of the octocoral *Plexaura kuna*, based on small subunit rDNA and multilocus DNA fingerprinting. Mar Biol 142:233–239

Govind NS, Roman SJ, Iglesias-Prieto R, Trench RK, Triplett EL, Prezelin BB (1990) An analysis of the light-harvesting peridinin-chlorophyll a-proteins from dinoflagellates by immunoblotting techniques. Proc R Soc Lond B Biol Sci 240:187–195

Haubold B, Hudson RR (2000) LIAN version 3: detecting linkage disequilibrium in multilocus data. Bioinformatics 16:847–848

Hudson RR, Boos DD, Kaplan NL (1992) A statistical test for detecting geographic subdivision. Mol Biol Evol 9:138–151

Iglesias-Prieto R, Trench RK (1994) Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthethic unit to changes in photon flux density. Mar Ecol Prog Ser 113:163–175

Iglesias-Prieto R, Trench RK (1997) Acclimation and adaption to irradiance in symbiotic dinoflagellates. II. Response of chlorophyll-protein complexes to different photon-flux densities. Mar Biol 130:23–33

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. Genetics 49:725–738

Kimura M, Ohta T (1978) Stepwise mutation model and distribution of allelic frequencies in a finite population. Proc Natl Acad Sci USA 75:2868–2872

Kinzie III RA (1970) The ecology of the gorgonians (Cnidaria: Octocorallia) of Discovery Bay, Jamaica. PhD dissertation, Yale University, New Haven, Conn.

Kinzie III RA (1974) Experimental infection of aposymbiotic gorgonian polyps with zooxanthellae. J Exp Mar Biol Ecol 15:335–345

Kinzie III RA, Takayama M, Santos SR, Coffroth MA (2001) The adaptive bleaching hypothesis: experimental tests of critical assumptions. Biol Bull (Woods Hole) 200:51–58

LaJeunesse TC (2001) Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the internal transcribed spacer region: in search of a "species" level marker. J Phycol 37:866–880

LaJeunesse TC (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Mar Biol 141:387–400

LaJeunesse TC, Trench RK (2000) Biogeography of two species of *Symbiodinium* (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). Biol Bull (Woods Hole) 199:126–134

Lasker HR, Coffroth MA (1983) Octocoral distributions at Carrie Bow Cay, Belize. Mar Ecol Prog Ser 13:21–28

Loeblich III AR, Sherley JL (1979) Observations on the theca of the mobile phase of free-living and symbiotic isolates of *Zooxanthella microadriaticum* (Freudenthal) comb. nov. J Mar Biol Assoc UK 59:195–205

Loh WKW, Loi T, Carter D, Hoegh-Guldberg O (2001) Genetic variability of the symbiotic dinoflagellates from the wide ranging coral species *Seriatopora hystrix* and *Acropora longicyathus* in the Indo-West Pacific. Mar Ecol Prog Ser 222:97–107

Mantel NA (1967) The detection of disease clustering and a generalised regression approach. Cancer Res 27:209–220

Maynard-Smith J, Smith NH, O'Rourke M, Spratt BG (1993) How clonal are bacteria? Proc Natl Acad Sci USA 90:4384–4388

Miller MP (1997) Tools for population genetic analysis (TFPGA) 1.3: a Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author. Available from http://bioweb.usu.edu/mpmbio/index.htm

Nei M (1972) Genetic distance between populations. Am Nat 106:283–292

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of organisms. Genetics 89:583–590

Pawlowski J, Holzmann M, Fahrni JF, Pochon X, Lee JJ (2001) Molecular identification of algal endosymbionts in large miliolid foraminifera: dinoflagellates. J Eukaryot Microbiol 48:368–373

Perez SF, Cook CB, Brooks WR (2001) The role of symbiotic dinoflagellates in the temperature-induced bleaching response of the subtropical sea anemone *Aiptasia pallida*. J Exp Mar Biol Ecol 256:1–14

Pfiester LA, Anderson DM (1987) Dinoflagellate reproduction. In: Taylor FJR (ed) The biology of dinoflagellates. Blackwell, London, pp 611–648

Pochon X, Pawlowski J, Zaninetti L, Rowan R (2001) High genetic diversity and relative specificity among *Symbiodinium*-like endosymbiotic dinoflagellates in soritid foraminiferans. Mar Biol 139:1069–1078

Rodriguez-Lanetty M, Loh W, Carter D, Hoegh-Guldberg O (2001) Latitudinal variability in symbiont specificity within the widespread scleractinian coral *Plesiastrea versipora*. Mar Biol 138:1175–1181

Roff DA, Bentzen P (1989) The statistical analysis of mitochondrial DNA polymorphisms: chi-square and the problem of small samples. Mol Biol Evol 6:539–545

Rousset F (1996) Equilibrium values of measures of population subdivision for stepwise mutation processes. Genetics 142:1357–1362

Rowan R (1998) Diversity and ecology of zooxanthellae on coral reefs. J Phycol 34:407–417

Rowan R, Powers DA (1991) A molecular genetic identification of zooxanthellae and the evolution of animal–algal symbioses. Science 251:1348–1351

Rowan R, Powers DA (1992) Ribosomal RNA sequences and the diversity of symbiotic dinoflagellates (zooxanthellae). Proc Natl Acad Sci USA 89:3639–4363

Santos SR, Coffroth MA (2003) Molecular genetic evidence that dinoflagellates belonging to the genus *Symbiodinium* Freudenthal are haploid. Biol Bull (Woods Hole) 241:10–20

Santos SR, Taylor DJ, Coffroth MA (2001) Genetic comparisons of freshly isolated vs. cultured symbiotic dinoflagellates: implications for extrapolating to the intact symbiosis. J Phycol 37:900–912

Santos SR, Taylor DJ, Kinzie III RA, Hidaka M, Sakai K, Coffroth MA (2002) Molecular phylogeny of symbiotic dinoflagellates inferred from partial chloroplast large subunit (23S)-rDNA sequences. Mol Phylogenet Evol 23:97–111

Santos SR, Gutierrez-Rodriguez C, Coffroth MA (2003) Phylogenetic identification of symbiotic dinoflagellates via length heteroplasmy in domain V of chloroplast large subunit (cp23S)-rDNA sequences. Mar Biotechnol (in press)

Schoenberg DA, Trench RK (1980a) Genetic variation in *Symbiodinium* (=*Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. I. Isozyme and soluble protein patterns of axenic cultures of *Symbiodinium microadriaticum*. Proc R Soc Lond B Biol Sci 207:405–427

Schoenberg DA, Trench RK (1980b) Genetic variation in *Symbiodinium* (=*Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. II. Morphological variation in *S. microadriaticum*. Proc R Soc Lond B Biol Sci 207:429–444

Schoenberg DA, Trench RK (1980c) Genetic variation in *Symbiodinium* (=*Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. III. Specificity and infectivity of *S. microadriaticum*. Proc R Soc Lond B Biol Sci 207:445–460

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. Can J Zool 69:82–92

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462

Taylor DL (1974) Symbiotic marine algae: taxonomy and biological fitness. In: Vernberg WB (ed) Symbiosis in the sea. University of South Carolina Press, Coulmbia, pp 245–262

Taylor MS, Hellberg ME (2003) Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299:107–109

Trench RK (1993) Microalgal–invertebrate symbioses: a review. Endocyto Cell Res 9:135–175

Trench RK (2000) Validation of some currently used invalid names of dinoflagellates. J Phycol 36:972

Trench RK, Blank RJ (1987) *Symbiodinium microadriaticum* Freudenthal, *S. goreauii* sp. nov., *S. kawagutii* sp. nov. and *S. pilosum* sp. nov.: gymnodinioid dinoflagellate symbionts of marine invertebrates. J Phycol 23:469–481

Viard F, Franck P, Dubois M-P, Estoup A, Jarne P (1998) Variation of microsatellite size homoplasy across electromorphs, loci, and populations in three invertebrate species. J Mol Evol 47:42–51

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370