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Genetic composition of zooxanthellae between and within colonies of the octocoral *Plexaura kuna*, based on small subunit rDNA and multilocus DNA fingerprinting

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Abstract In intracellular symbiosis, the host is both the symbiotic partner and the habitat for the symbiont. Physical conditions may vary within a host individual, leading to niche partitioning within a host according to symbiont genotype. In this study, the algal symbionts (zooxanthellae) inhabiting the octocoral *Plexaura kuna* (Lasker, Kim, Coffroth) were characterized. Zooxanthellae are currently divided into clades based on sequence variation of the small and large subunit ribosomal gene and internal transcribed spacer regions. Zooxanthellae isolated from the top and bottom of a *P. kuna* colony, from colonies sampled at various depths and geographic locations (Bahamas, Florida Keys, Panama and St. Croix, USVI), from 1988 to 1998, all belonged to clade B. Since symbiont variability within a host is an individual-level question, in the second part of the study, multilocus DNA fingerprinting was used. Each genetically distinct *P. kuna* colony (i.e. non-clonemate) hosted a unique prominent zooxanthella genotype. This zooxanthella genotype was found at both the top and the bottom of the host colony, although physical conditions differed between these areas. The existence of one prominent zooxanthella genotype throughout a colony may indicate a fixed symbiont-acquisition period or the active maintenance of a host–symbiont genotypic association.

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Introduction

Many cnidarians (e.g. scleractinian corals, octocorals, and sea anemones) host intracellular dinoflagellate symbionts (“zooxanthellae”) belonging to the genus *Symbiodinium* (reviewed by Trench 1993). Recent research suggests that the symbiosis within a single host may be dynamic, changing in response to environmental conditions (Rowan et al. 1997; Baker 2001). Physiological responses of corals to depth or environmental conditions therefore may not be due to the plasticity of the host coral or the zooxanthellae within the coral, but to the plasticity of the association. Thus, an understanding of phenomena such as coral bleaching and the effects of global climate change on reef environments requires knowledge of the dynamics and diversity of zooxanthella populations.

The ability to answer questions regarding zooxanthella dynamics within a host species and within individuals of that host species has previously been hampered by the lack of taxonomic resolution among zooxanthellae. In the past decade, zooxanthellae have been classified based on sequence variation of the DNA coding for the small (ssRNA) (Rowan and Powers 1991a,b, 1992; McNally et al. 1994; Carlos et al. 1999; Darius et al. 2000) and the large (lsRNA) (Wilcox 1997, 1998; Baker 1999, 2001; Rodriguez-Lanetty et al. 2001) subunit ribosomal RNA gene. These sequences divide zooxanthellae into groups (clades). The clades include several described and undescribed species (Trench 1997; Rowan 1998). Currently, the zooxanthella cladal identity is known for 199 cnidarian hosts (scleractinians, octocorals, sea anemones, jellyfish, fire coral).

The field is further advancing with additional techniques. For example, Wilcox et al. (1999) and Baillie et al. (2000), using randomly amplified polymorphic DNA (RAPD), detected within-clade variation among zooxanthellae harbored by the jellyfish *Cassiopea xamachana* and by giant clams, respectively. Baillie et al. (1998) and Carlos et al. (2000) (using allozyme analysis

and temperature-gradient gel electrophoresis, respectively) found within-clade variation among zooxanthellae symbiotic with giant clams. Internal transcribed spacer (ITS) regions of nuclear DNA (Baillie et al. 2000; LaJeunesse 2001; van Oppen et al. 2001) and the 23S rDNA of chloroplasts (Santos et al. 2002) have also been used. None of these techniques, however, can detect zooxanthella individual level variability. Such an individual-level of resolution is important for ecological questions regarding a specific host/zooxanthella association. We therefore used multilocus DNA fingerprinting, a technique that allows us to focus on individual genotypes of both the host and the symbiont.

Zooxanthella genetic variability among individuals within a host cnidarian species may reflect the range of environmental conditions in host habitats such as different levels of light, nutrients, and water flow. For example, in the octocoral *Plexaura kuna*, deeper colonies encounter one order of magnitude less light than shallow colonies (Goulet 1999). Zooxanthella genetic variability with depth has been demonstrated for scleractinian coral species that host multiple zooxanthella clades (Rowan and Knowlton 1995; Rowan 1998; Baker 2001; Toller et al. 2001a). The majority of cnidarian hosts studied thus far (84%), however, host a single zooxanthella clade (Rowan and Powers 1991a,b, 1992; Baker and Rowan 1997; Baker et al. 1997; Billinghurst et al. 1997; Bythell et al. 1997; Wilcox 1997; Darius et al. 1998; Baker 1999; Goulet 1999; van Oppen et al. 2001; Diekmann et al. 2002). Whether the lack of zooxanthella cladal variability reflects a lack of zooxanthella genetic variability in most of these hosts species is currently unknown.

In addition to differing environmental conditions among cnidarian colonies of the same host species, environmental conditions may also vary within an individual host. For example, water movement between branches of branching corals (Chamberlain and Graus 1975; Sebens et al. 1998) and the gorgonian *Plexaura homomalla* (Kim and Lasker 1997) is greatly reduced compared to water flow around the colony. Reduction in water flow in different parts of a colony may, in turn, reduce nutrient and gas exchanges to both host and symbiont (Jokiel 1978; Dennison and Barnes 1988; Lesser and Shick 1989). Furthermore, different areas of a host colony may differ in their light levels. For example, light levels at the top of *P. kuna* are significantly higher than light levels at the base of the same colony (Goulet 1999). If microhabitat differences within a host colony affect zooxanthella genotypic distribution, then branches at the top of the colony might host different zooxanthellae from those in branches at the base of the colony.

Zooxanthella niche partitioning within a single host may also arise due to the timing of zooxanthella acquisition by the host. For example, new growth in a *P. kuna* colony occurs at the branch tips, so that the tissue at the base of a colony is the oldest while tissue at the branch tips is the youngest. If zooxanthella acquisition from the environment can occur, zooxanthella genotypes might preferentially enter colony areas where zooxanthella density is lowest, i.e. new tips. Thus, niche partitioning may occur between tips (found throughout the colony) and branch tissues. If timing of infection affects zooxanthella genotypic distribution, then recently formed branch tips would host different zooxanthella genotypes than older branch tissue, formed under potentially different conditions. Consequently, zooxanthellae may be distributed throughout a host colony in response to microhabitat differences or differences in timing of infection. Niche partitioning will result in a mosaic of different symbiont populations, each of which may have different physiological requirements. Alternatively, a zooxanthella population, or a single zooxanthella genotype, might uniformly populate an entire host coral. Such questions can be addressed using multilocus DNA fingerprinting.

We studied the gorgonian *P. kuna*, a branching octocoral commonly found throughout the Caribbean from shallow water to 17 m depth (Lasker et al. 1996). *P. kuna* reproduces sexually, and asexually by fragmentation (Lasker 1990; Lasker and Kim 1996). *P. kuna* colonies, therefore, may be genetically distinct or clonemates (Coffroth and Lasker 1998). In the present study, we first determined if zooxanthellae exhibit within- and between-host variation, at the currently widely used cladal level, using RFLP (restriction fragment length polymorphism) analysis of the ssRNA gene. We examined zooxanthella variation within a *P. kuna* colony, and as a function of colony depth and geographic location. Second, we examined zooxanthella variation at the genotypic level by using multilocus DNA fingerprinting. Using this level of resolution, we examined niche partitioning of symbionts by sampling different parts of a *P. kuna* colony within colonies found in different depths. Finally, we determined if different *P. kuna* colonies harbored different zooxanthella genotypes.

Materials and methods

RFLP analysis for distinction between zooxanthella clades

Plexaura kuna (Lasker, Kim, Coffroth) was sampled from four locations in the Caribbean: Bahamas ($n = 19$), Florida Keys ($n = 16$), Panama ($n = 29$), and St. Croix, USVI ($n = 6$), with samples collected from 1988–1998. Within each location, several reefs were sampled: Bahamas (4), Florida Keys (3), Panama (5), and St. Croix (2). A 2 cm long branch tip from the top portion of each sampled colony was either frozen in liquid nitrogen or preserved in 95% ethanol. For 16 colonies from Panama, we also sampled tissue from branch tips at the bottom of the colony.

The extraction of DNA from zooxanthellae followed the protocol of Coffroth et al. (1992). Using the polymerase chain reaction (PCR), we amplified the DNA encoding for the ssRNA using the "universal" primer ss5 and the zooxanthella-biased primer ss3Z (Rowan and Powers 1991b). We used 10 ng of DNA in a 30 µl PCR reaction volume, with 10× PCR buffer, 1.5 mM $MgCl_2$, 100 µM of each dNTP, 0.25 µM of each primer, and 1 U *Taq* polymerase. The PCR profile consisted of 2.5 min at 94°C; 1 min at 56°C; 2 min at 72°C followed by 30 cycles of 1 min at 94°C; 1 min at 56°C; 2.5 min at 72°C; and ending with 8 min at 72°C. The amplified DNA was digested with either the restriction enzyme

Taq I (MBI Fermentas) or *Dpn* II (New England Biolabs). We then ran the product on a 2% (w/v) agarose (Eastman Kodak) gel and stained it with ethidium bromide for UV visualization. Resulting bands were compared to RFLP patterns of cloned ssRNA genes run as zooxanthella standards. In addition, we scanned negatives of the RFLP gels into a computer and determined the molecular weight of the RFLP bands using the program NCSA GelReader 2.0.5 (NCSA, University of Illinois). The molecular weights of the RFLP bands were compared against known standards (Rowan and Powers 1991a,b).

DNA fingerprinting analysis for distinction between zooxanthella genotypes

We used multilocus DNA fingerprinting to compare zooxanthella genotypes found at the top and bottom of 16 *P. kuna* colonies collected in Panama. We cut 6 cm of tissue from branches at the top and near the base of eight shallow-water colonies (< 5 m), four from Tiantupo Reef (back reef) and four from Sail Rock Reef (fore reef), and eight deep-water colonies (> 10 m) from Sail Rock Reef (there are no *P. kuna* colonies in deep water at Tiantupo Reef). All tissue samples were frozen in liquid nitrogen.

To further test for differential distribution in response to environment or timing of infection, we sampled tissue from five different areas within three of the 16 colonies. These included: (1) tissue from a branch tip at the top of a colony, (2) tissue 2–4 cm below the tip of the top branch, (3) tissue from a branch tip near the base of the colony, (4) tissue 2–4 cm below the tip of the bottom branch, and (5) tissue from the base (holdfast).

DNA extraction and fingerprinting followed the protocols of Coffroth et al. (1992), with the modification of using a nonradioactive, chemiluminescence detection procedure (Boehringer Mannheim DIG nonradioactive nucleic acid labeling and detection system). The bands were visualized using Lumi-Phos 480 (Life Codes). When we compared the zooxanthella DNA fingerprints generated using this protocol (''standard'' zooxanthella fingerprint) with the DNA fingerprint of the host, we found that some bands appeared both in the host and in ''standard'' zooxanthella lanes. We further cleaned at least one zooxanthella DNA sample per host genotype by centrifuging the zooxanthella pellet through a 20% and 80% Percoll step gradient (Stochaj and Grossman 1997). This procedure generated a ''cleaned'' zooxanthella DNA fingerprint that either did not share bands with the host or shared a few co-migrating bands with the host. Bands were assigned to the host or symbiont by running ''cleaned'' zooxanthellae side by side with the ''standard'' zooxanthella and host lanes of the same *P. kuna* colony. Bands in the ''standard'' zooxanthella lane that also appeared in the host colony lane were not counted in subsequent analysis of zooxanthella fingerprints. All bands in the ''cleaned'' zooxanthella lane were used for comparisons with the host colony and zooxanthellae from other *P. kuna* colonies.

We distinguished fingerprints by all pairwise comparisons of fragment patterns and only compared samples run on the same gel. We compared the DNA fingerprint of the zooxanthellae sampled from different regions within each *P. kuna* colony, as well as the DNA fingerprint of a host colony with its zooxanthellae. In addition, to obtain an estimate of zooxanthella variability, we compared zooxanthella DNA fingerprints from different *P. kuna* colonies. We scored all bands between 3 and 23 kb and calculated the similarity index {SI = 2×(no. of shared bands)/[(no. of bands in individual A) + (no. of bands in individual B)]; Lynch 1988}. The similarity index ranges from 0, for genetically different individuals (i.e. no bands shared), to 1, for genetically identical individuals (i.e. all bands shared). Genetically different individuals, however, may share some bands due to co-migration and background levels of population bandsharing.

DNA fingerprinting can resolve individual genotypes (Jeffreys et al. 1985a,b). If, however, multiple genotypes are present, it is possible to obtain a composite DNA fingerprint. To determine our ability to resolve multiple zooxanthella genotypes within a DNA fingerprint, we performed a serial dilution experiment. We extracted DNA from two isoclonal zooxanthella cultures isolated from different *P. kuna* colonies. Both cultures belonged to clade B. We mixed the DNA from the two cultures in: 100:1, 10:1, 2:1, 1:1, and 1:2 proportions. We ran the original cultures next to the mixed DNA from the cultures and scored the resulting fingerprints.

Results

RFLP analysis of zooxanthellae

All *Plexaura kuna* colonies contained zooxanthellae exhibiting the same main RFLP pattern when digested with either *Taq* I or *Dpn* II (Fig. 1). These RFLP patterns are characteristic of clade B zooxanthellae (Rowan and Powers 1991a,b). Branch tips from the top and bottom of a *P. kuna* colony harbored only zooxanthellae from clade B. Furthermore, colonies both in shallow

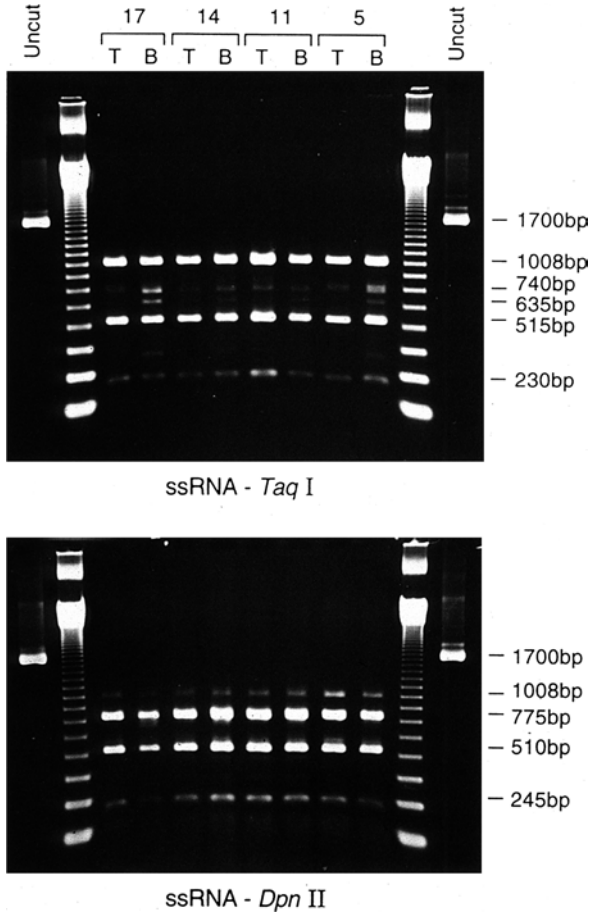

Fig. 1 *Plexaura kuna.* Restriction fragment length polymorphism gels of zooxanthella DNA encoding for the small subunit ribosomal RNA (*ssRNA*). The PCR amplified product was digested with restriction enzymes *Taq* I (*upper gel*) and *Dpn* II (*lower gel*). Zooxanthella DNA was collected from the top (*T*) and bottom (*B*) of *P. kuna* colonies located at 17, 14, 11, and 5 m depth. Uncut amplified segments were run in the outer lanes. Using the *Taq* I restriction enzyme, the fragments at 1008 bp and 515 bp are characteristic of zooxanthellae in clade B. The 740, 649, and 340 bp fragments in lanes 17B and 5B are from a putative apicomplexan and do not represent a zooxanthella clade. A 123 bp marker was used as the size standard

and deep water only contained clade B zooxanthellae. Finally, all *P. kuna* colonies sampled, on various reefs in Florida, Bahamas, USVI, and Panama, had only clade B zooxanthellae. When using the restriction enzyme *Taq* I, we did find a novel RFLP pattern that occurred in addition to the clade B RFLP pattern (seen, for example, in the 17B and 5B samples in Fig. 1). This novel RFLP pattern contained three fragments: 740, 649, and 340 bp long. After cloning and sequencing, we determined that the DNA generating the unknown banding pattern aligned most closely (BLAST search, 95% base sharing) with *Sarcocystis aucheniae*, an apicomplexan (Holmdahl et al. 1999).

DNA fingerprinting analysis of zooxanthellae

After correcting for host band contamination, the zooxanthella DNA fingerprints had 18.2 ± 2.9 bands (mean $\pm$ SD, range 13–25). Fingerprints of the host DNA produced an average of 9.9 ± 2.4 bands (range 8–15). The similarity index (SI) between "cleaned" zooxanthellae and the host was only 0.16 ± 0.07 (2.25 ± 1.18 bands shared, range 1–5). The zooxanthella DNA fingerprints of branch tips from the top and bottom of the same colony were always identical (Fig. 2), both in shallow and deep colonies, with SI = 1 in all 16 samples. Furthermore, in each of the three colonies where five different areas were sampled, all five areas within a specific colony displayed the same DNA fingerprint. Comparison of zooxanthella DNA fingerprints between different *P. kuna* colonies yielded low band sharing with an average SI of 0.28 ± 0.19 (5.11 ± 3.44 bands shared, range 2–10, $n = 9$) indicating there were different zooxanthella genotypes in different host colonies (Table 1).

The two isoclonal zooxanthella cultures used to test the resolution of the DNA fingerprinting technique contained 22 (isoclone X) and 21 (isoclone Y) bands, respectively. When the two isoclonal zooxanthella cultures were mixed, the resulting mixed DNA fingerprint had 38 bands (Fig. 3). The two cultures shared only five bands (SI = 0.23). In the mixed DNA fingerprint, we were able to detect bands from each of the isoclonal genotypes at 2:1, 1:1, and 1:2 ratios of X to Y. In the 2:1 and 1:2 dilutions, the bands of the more dilute zooxanthella genotype were fainter compared to the other genotype (Fig. 3). We could not detect the DNA fingerprint bands of the diluted zooxanthella genotype at the 10:1 and 100:1 dilutions.

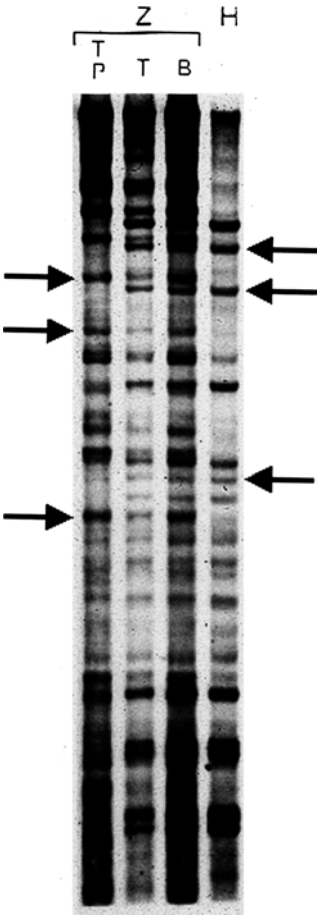

Fig. 2 *Plexaura kuna*. Autoradiograph of DNA fingerprints of a colony (*H*) and its zooxanthellae (*Z*). Zooxanthella DNA fingerprints were obtained from the top (*T*) and the bottom (*B*) of the *P. kuna* colony. In addition, zooxanthella DNA isolated from the top of the colony was further cleaned using a percoll gradient, and the resulting DNA was run on the gel (*TP*). Percoll cleaning enabled us to factor out host bands from zooxanthella lanes. For example, *arrows* point to representative zooxanthella bands (*left*) and representative host colony bands (*right*)

Table 1 *Plexaura kuna*. DNA fingerprinting band sharing scores between zooxanthellae from different host *P. kuna* colonies (non-clonemates) from Sail Rock shallow (*Sh*, < 5 m) and deep (*Dp*, > 10 m). Pairwise comparisons were only performed on samples run side by side on the same DNA fingerprinting gel

Colonies compared	Similarity index
Dp1 vs. Dp2	0.21
Dp2 vs. Dp3	0.43
Dp4 vs. Dp5	0.38
Dp6 vs. Dp7	0.11
Dp7 vs. Dp8	0.24
Sh2 vs. Sh3	0.12
Sh1 vs. Dp4	0.69
Sh2 vs. Dp8	0.13
Sh4 vs. Dp1	0.24

Discussion

All *Plexaura kuna* colonies sampled harbored only zooxanthellae belonging to clade B, regardless of the area sampled within the colony, host colony depth, or geographic location. This finding demonstrates host–symbiont specificity at this level of genetic resolution. Our study, which analyzed zooxanthellae from 70 *P. kuna* colonies, extends Rowan and Powers (1991a) finding of only clade B zooxanthellae in four shallow-water *P. kuna* samples from the Bahamas. Lack of cladal

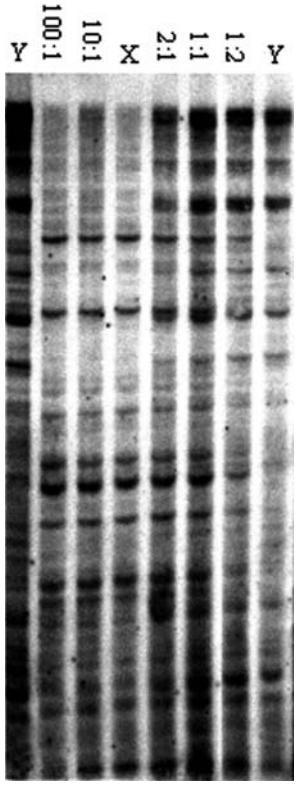

Fig. 3 *Plexaura kuna*. Autoradiograph of DNA fingerprints of a mixture of two zooxanthella cultures (cultures X and Y) originally isolated from *P. kuna*. The two cultures were mixed in various proportions. The ratios, listed at the top of each lane, represent the proportion of X to Y. Lanes denoted either X or Y, represent a DNA fingerprint of a single culture (undiluted), X or Y, respectively

variation with depth has been reported for the scleractinian corals *Montastrea cavernosa* (Billinghurst et al. 1997) and *Madracis mirabilis*, *M. decactis*, *M. formosa*, *M. senaria*, and *M. pharensis* (Diekmann et al. 2002). Loh et al. (2001) and Rodriguez-Lanetty et al. (2002) reported cladal variation with geographic location in three scleractinian corals in the Indo–West Pacific. LaJeunesse and Trench (2000) reported latitudinal cladal variability of zooxanthellae in the sea anemone *Anthopleura elegantissima*. The current study is the first to look at an octocoral over a range of depths and geographical locations. Hosting a single zooxanthella clade, as was found in *P. kuna*, occurs in 84% of cnidarians studied to date ($n = 199$ species, see "Introduction").

In addition to clade B zooxanthellae, some of the amplified *P. kuna* samples digested with the restriction enzyme *Taq* I generated an unknown banding pattern. When sequenced, this banding pattern aligned most closely with an apicomplexan (Holmdahl et al. 1999). Apicomplexans are closely related to dinoflagellates (McNally et al. 1994). Amplification of apicomplexan DNA with the PCR primers used in this study has also been documented in the scleractinian corals *Montastraea* spp. (Toller et al. 2001b) and in *Dichocoenia* sp. (A. Hannes, personal communication).

Although *P. kuna* hosts zooxanthellae in a single clade, there is no lack of zooxanthella genetic diversity in this symbiosis. Using multilocus DNA fingerprinting, we found that each genetically distinct *P. kuna* colony (i.e. non-clonemate) displayed a unique zooxanthella DNA profile, indicating that each genetically distinct host harbored a unique zooxanthella genotype(s). This finding supports the suggestion that sexual recombination occurs in zooxanthellae (Goulet 1999; Baillie et al. 2000; LaJeunesse 2001).

A zooxanthella DNA fingerprint obtained from a colony could represent one zooxanthella genotype or a mixture of zooxanthella genotypes. The results of this study support the former possibility for two reasons. First, the number of bands in a "cleaned" zooxanthella DNA fingerprint was lower than the number of bands in fingerprints obtained from isoclonal zooxanthella cultures of clade B zooxanthellae. If a zooxanthella DNA fingerprint were a composite of several genotypes, we would expect it to have more bands compared to an isoclonal zooxanthella culture (e.g. as when we mixed two isoclonal cultures). We may not see more bands if zooxanthellae shared many bands. Comparison of zooxanthellae from different colonies, however, revealed a low average similarity index among zooxanthellae (0.28 ± 0.19), negating a band-sharing possibility. This finding is further supported by the low similarity index (0.23) between two isoclonal cultures.

Second, if a single host colony harbored multiple zooxanthella genotypes, which varied throughout the colony, we would expect to see variation in the banding pattern or the band intensities of the zooxanthella DNA profiles from different areas of the same coral colony. For example, when we experimentally mixed two isoclonal cultures in varying proportions, the band intensity of the dilute genotype was fainter compared to the more concentrated zooxanthella genotype. We did not see such an effect, i.e. zooxanthellae from each region of a colony had the same DNA banding pattern and intensity (Fig. 2). Thus, either multiple zooxanthella genotypes were distributed and maintained in the same proportions throughout the colony or the DNA fingerprint represents a single prominent genotype. The latter interpretation is the more parsimonious, and we therefore conclude that the zooxanthella DNA fingerprints obtained in this study represent a single zooxanthella genotype in each colony. Furthermore, maintaining the same zooxanthella DNA fingerprint within a colony was also documented in a long-term study (Goulet 1999).

We therefore conclude that the *P. kuna* symbiosis is dominated by a single zooxanthella genotype throughout each host colony. Other zooxanthella genotypes may exist in a *P. kuna* colony, but they are less common and consequently not visualized in the DNA fingerprint. DNA fingerprints of zooxanthellae isolated from a frozen branch differed from fingerprints of cultures obtained from the same branch (Goulet and Coffroth 1997). Culturable dinoflagellates, however, may be rare clones or clones that have undergone genomic changes

due to selection and/or mutation processes (Rowan et al. 1996; Baillie et al. 1998; Carlos et al. 2000; LaJeunesse 2001; Santos et al. 2001).

There are several possible explanations for the prominence of a single zooxanthella genotype in the *P. kuna* symbiosis. First, if a single zooxanthella genotype entered an asymbiotic primary polyp and no turnover occurred after this initial acquisition, there would be no intra-colony zooxanthella genotypic variation. Newly settled *P. kuna* polyps, however, can initially acquire multiple algal genotypes (Coffroth et al. 2001). Second, if *P. kuna* hosted a prominent zooxanthella genotype along with other less common genotypes, differences in abiotic parameters, such as light levels and water flow between various areas of the same *P. kuna* colony, may not be sufficient to cause zooxanthella zonation. Lack of zonation will lead to the prominent zooxanthella genotype occurring throughout the coral colony. Third, maintaining a single prominent symbiont throughout the host colony may prevail in situations where the symbiont can move within the host. For example, in the octocoral *Rhytisma fulvum fulvum* (previously *Parerythropodium fulvum fulvum*), zooxanthellae contained within coral cells circulate freely in two canal networks (Gateño et al. 1998). Fourth, symbiont interaction and competition could result in the maintenance of a single prominent zooxanthella genotype. Conversely, fifth, the host, as a live habitat, may dictate the distribution and survival of zooxanthella genotypes. Frank (1996) proposed that a host would derive a greater benefit from a genetically uniform symbiont population compared with a symbiont population composed of multiple genotypes. Multiple symbiont genotypes may lead to competition between the symbionts for resources within the host, which, in turn, may lead to a reduction of host fitness. Thus, selection against less effective symbionts should occur. Maintaining a single prominent zooxanthella genotype may be the result of optimizing the symbiotic population within the host.

Regardless of the mechanism by which the symbiosis is established and maintained, each *P. kuna* colony hosted one prominent zooxanthella genotype throughout the colony. This finding, at the level of the individual genotype, leads to questions regarding the dynamics of the host–symbiont genotypic association. Since *P. kuna* does not transmit its symbionts to its sexually produced offspring, when does the prominent zooxanthella genotype establish itself? Does the prominent zooxanthella genotype change over the course of the host's life? Answering these questions will enable us to better predict the response of host corals to remediation efforts, such as coral transplanting, and to preservation measures in case of a reef perturbation that may lead to a loss of zooxanthellae, as in coral bleaching.

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