The Acquisition of Exogenous Algal Symbionts by an Octocoral After Bleaching

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Episodes of coral bleaching (loss of the symbiotic dinoflagellates) and coral mortality have occurred with increasing frequency over the past two decades. Although some corals recover from bleaching events, the source of the repopulating symbionts is unknown. Here we show that after bleaching, the adult octocoral *Briareum* sp. acquire dinoflagellate symbionts (*Symbiodinium* sp.) from the environment. Uptake of exogenous symbionts provides a mechanism for response to changes in the environment and resilience in the symbiosis.

A diverse array of cnidarians form symbioses with photosynthetic dinoflagellates in the genus *Symbiodinium*. These are true mutualisms, in that the symbiont receives inorganic nutrients from the host and the host obtains translocated photosynthetic products from the symbionts (1–3). Symbiont species within the diverse genus *Symbiodinium* are classified into broad groups or clades (i.e., A, B, C, etc.) on the basis of sequence variation in the small-subunit ribosomal gene (4–6). Most cnidarians preferentially establish and maintain a stable symbiosis with either a specific clade of *Symbiodinium* (7–10) or a subset of the clades that vary with environmental gradients such as light intensity (11–14). Environmental perturbation (e.g., increased temperature, increased solar radiation) can result in the breakdown of the symbiosis (i.e., coral bleaching) that can lead to coral death and subsequent reef degradation. However, some corals recover, and bleaching has been posited as a mechanism whereby hosts acquire new, potentially better-adapted symbionts (15, 16). The source of the symbionts that repopulate a host colony following bleaching is poorly understood (11, 16, 17). Are the symbionts derived from *Symbiodinium* populations remaining in the host at very low levels or from an exogenous pool of potential symbionts (12, 17–19)? To determine whether adult corals can acquire exogenous symbionts from the environment after a bleaching event, the Caribbean octocoral *Briareum* sp. was bleached and then exposed to exogenous *Symbiodinium* containing rare variants of the chloroplast 23S ribosomal DNA (rDNA) domain V region (cp23S-genotype) (20). The potential symbionts were derived from isoloncal lines of *Symbiodinium* clade B initially isolated from newly settled octocoral polyps (cp23S-genotypes B211 and B223) and an adult colony of *Plexaura flexuosa* (cp23S-genotype B224). Because these variants are not commonly found in adult *Briareum* sp., they served as markers for uptake of exogenous *Symbiodinium*. The markers B211 and B223 cp23S-genotypes were not detected in any of 255 *Briareum* sp. colonies collected from the field; one colony harbored *Symbiodinium* B224 (21); 254 colonies harbored either *Symbiodinium* B178 and/or B184, the cp23S-genotypes typically found in *Briareum* sp. (21). The cp23S-genotypes used as markers in the experiment were not found in *Symbiodinium* isolated from the experimental colonies before or immediately after bleaching (Fig. 1 and Fig. 2A, lanes P and B; table S1).

Cell counts of *Symbiodinium* within *Briareum* colonies immediately after bleaching confirmed a decrease in symbiont density to less than 1% of the original population density (Fig. 2B). Molecular analysis detected residual populations of B178 and/or B184 in 27 of the 39 colonies after bleaching (table S1). During the subsequent 6-week exposure to exogenous symbionts, cell densities within the hosts increased 9- to 31-fold, demonstrating that the symbiosis had begun to reestablish itself (Fig. 2B). Molecular analysis of the symbiont population within these hosts after 3 and 6 weeks of exposure to exogenous *Symbiodinium* cultures identified the marker cp23S-genotypes in 58% and 45% of the samples, respectively (Fig. 2A, lanes R). This demonstrates repopulation of adult *Briareum* by exogenous symbionts and thus establishes a potential exogenous source of symbionts following bleaching events (22). Furthermore, 37% of the colonies that initially harbored *Symbiodinium* B178 and/or B184 contained only *Symbiodinium* with the marker cp23S-genotypes when sampled after 3 weeks of exposure to the exogenous algal source ("switching" sensu (11)). In contrast, six colonies, which initially contained *Symbiodinium* B178 and/or B184, did not acquire symbionts with the marker cp23S-genotype. This may be due to physiological differences between the different *Symbiodinium* strains.

Supporting Online Material

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Figs. S1 to S5

References

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and/or host selectivity or the ability of Symbiodinium B178 and/or B184 to competitively exclude some Symbiodinium phylogenotypes.

Although octocoral colonies acquired Symbiodinium containing the marker cp23S-genotype during the exposure period, the symbiotic relationship did not always persist. In three colonies that acquired Symbiodinium with the marker cp23S-genotypes, the marker genotype was later “lost” or replaced with Symbiodinium B184. This suggests that these variants of Symbiodinium clade B could not maintain a stable symbiotic relationship or were competitively displaced by Symbiodinium B184. Perhaps new symbionts can infect hosts and provide the coral with an interim benefit until the original complement is reestablished (11, 23).

The repopulation of the symbiont community involved residual populations within Briareum sp., as well as symbionts from the surrounding water (Fig. 2C and table S1). As a result, some recovering colonies simultaneously harbored Symbiodinium with B178 and/or B184, as well as the marker cp23S-genotypes (Fig. 2A, colonies 1, 2, and 4, and table S1). Changes in the relative abundance of algal types also occurred during bleaching and recovery (“shuffling” sensu (11)). Before bleaching, only 31% of the colonies (12 of 39) harbored Symbiodinium B184. During the 6 weeks after bleaching, the number of colonies containing Symbiodinium B184 increased to 79% (27 of 34) at 3 weeks and 75% (18 of 24) at 6 weeks. Colonies that harbored B178 cp23S-genotypes were more likely to harbor B184 after bleaching, whereas those that initially harbored B184 Symbiodinium were more likely to retain B184 symbionts after bleaching (X² test of independence, df = 2: week 3, P < 0.01; week 6, P < 0.025). The appearance and predominance of B184 after bleaching could be due to the proliferation of a resident, but cryptic population of Symbiodinium B184 that was resistant to the bleaching stress or B184 colonization during recovery.

This study shows that recovery of coral-algal symbioses after a bleaching event is not solely dependent on the Symbiodinium complement initially acquired early in the host’s ontogeny. These symbioses also have the flexibility to establish new associations with symbionts from an environmental pool. Furthermore, changes in the relative incidence of the original symbionts, as well as the subsequent loss of acquired genotypes, suggest that
Flexibility in Algal Endosymbioses 
Shapes Growth in Reef Corals 
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The recent discovery of the genetically diverse nature of the dinoflagellate genus Symbiodinium (zooxanthellae) that forms symbiotic associations with stony corals raises the possibility that physiological properties and tolerances of reef corals may vary according to the association established. The genus Symbiodinium consists of at least seven clades (A to G) based on sequence analysis of the internal transcribed spacer (ITS) region (1–5), as well as many genetic types within each clade, referred to as subclades or strains (e.g., C1, C2) (4–6). In most broadcast spawning corals, zooxanthellae are acquired from the environment in early ontogeny by horizontal transmission and become established in the endodermal cells of coral hosts as an endosymbiosis. This creates an opportunity for the host to establish an association with a variety of symbionts. Indeed, adults of some coral species form associations with more than one Symbiodinium strain according to the local environment (7, 8) or microhabitats within a coral (6, 9, 10). Such polymorphic symbioses suggest that corals within a species may not be physiologically uniform (11) and that the taxonomic identity of the Symbiodinium partner(s) may be as significant as that of the host in determining the physiology of the holobiont (host-symbiont partnership). A recent review (12) highlights our limited understanding of the influence of symbiont type on physiological performance of the holobiont and the importance of understanding potential flexibility in Symbiodinium symbioses in an era of global coral reef deterioration. 

Acropora tenuis and A. millepora are broadcast spawning corals with horizontal transmission of symbionts (13) that, as adults, express different specificities for Symbiodinium strains at Magnetic Island (an inshore reef in the central section of the Great Barrier Reef, Australia), where adult colonies of A. millepora contain a Symbiodinium D strain, whereas A. tenuis adults contain Symbiodinium strain C1 and occasionally strain C2 (6, 10). The production of larvae free of zooxanthellae by both species provides the opportunity to observe natural patterns of zooxanthella infection and also to manipulate the strains offered for uptake in controlled experimental conditions to determine the impact of known strains on juvenile growth. 

Larvae of A. tenuis were raised from spawned gametes (14) and settled onto tiles (15). Positions of juveniles on the tiles were mapped, and the tiles were then attached to the reef (Nelly Bay, Magnetic Island) in a zone where adult A. tenuis colonies were abundant (15). Thirty juvenile corals were sampled at about 1, 2, 4, and 9 months after settlement. Total DNA (both coral and algal) was extracted from the polyps, and the polymerase chain re-