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The Acquisition of Exogenous Algal Symbionts by an Octocoral After Bleaching

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33. The nucleation rate is more precisely defined as the rate at which particles of critical size are produced. In this study, we detect only particles larger than 3 nm, and hence the value estimated from the rate of observed particle formation may be smaller than that of cluster formation.
34. *S* is defined as the ratio of the partial pressure of the organic acid to its equilibrium vapor pressure at a given temperature. For example, the equilibrium vapor pressures are 7.0×10^{-3} torr for benzoic acid and 1.9×10^{-3} torr for *p*-toluic acid at 298 K, corresponding to saturation mixing ratios of 9.2 and 2.5 ppm (parts per million) at 1 atm, respectively.
35. For benzoic and *p*-toluic acids and the experimental conditions similar to those in Fig. 2, the measured particle concentrations were slightly higher (by about 10 to 20%) when the water flow in the aerosol chamber was turned off.
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39. The bonding energy of complexes represents one of the factors that determine particle nucleation. Other physicochemical parameters, such as the surface tension and equilibrium vapor pressures of the nucleated single or multicomponent system, also influence new particle formation. For example, our experimental results indicated a negligible effect of glutaric acid ($C_5H_8O_4$) on sulfuric acid nucleation, although the bonding energy of this organic acid with sulfuric acid is 19.2 kcal mol^{-1}, comparable to those of the aromatic acid–sulfuric acid complexes.
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Figs. S1 to S5
References

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The Acquisition of Exogenous Algal Symbionts by an Octocoral After Bleaching

Cynthia L. Lewis and Mary Alice Coffroth*

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 (*4*, *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 23*S* ribosomal DNA (rDNA) domain V region (cp23S-genotype) (*20*). The potential symbionts were derived from isoclonal 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

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

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*).

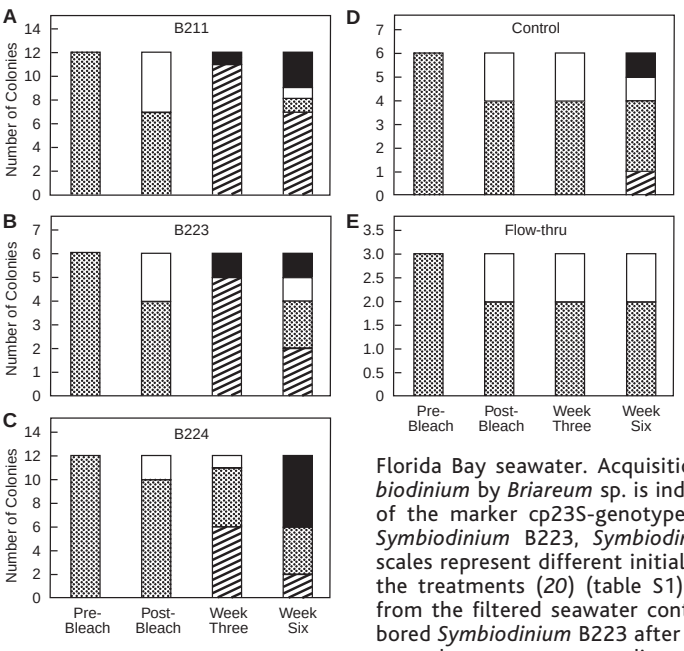


Fig. 1. Number of *Briareum* sp. colonies with *Symbiodinium* cp23S-genotypes at the four sampling times. Prebleaching, postbleaching (i.e., after 12 weeks of darkness), and after exposure to the marker cp23S-genotype cultures at 3 and 6 weeks. Treatment conditions: 1000 cells/ml of (**A**) *Symbiodinium* B211, (**B**) *Symbiodinium* B223, (**C**) *Symbiodinium* B224 and no algae added to (**D**) filtered seawater control, and (**E**) unfiltered Florida Bay seawater. Acquisition of exogenous *Symbiodinium* by *Briareum* sp. is indicated by the presence of the marker cp23S-genotype (*Symbiodinium* B211, *Symbiodinium* B223, *Symbiodinium* B224). Different scales represent different initial number of colonies in the treatments (*20*) (table S1). One colony sampled from the filtered seawater control aquarium (D) harbored *Symbiodinium* B223 after 6 weeks. However, the control treatment was adjacent to the B223 treatment, and this result was likely due to contamination. Over this same time period, symbionts with the marker cp23S-genotypes were not detected in any other control colonies or in colonies in the flow-thru tank [17 out of 18 samples, (D) and (E)]. Key: diagonal lines, marker cp23S-genotype present; stippling, marker cp23S-genotype not detected; white, no symbiont detected; black, colony died.

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 (X^2 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

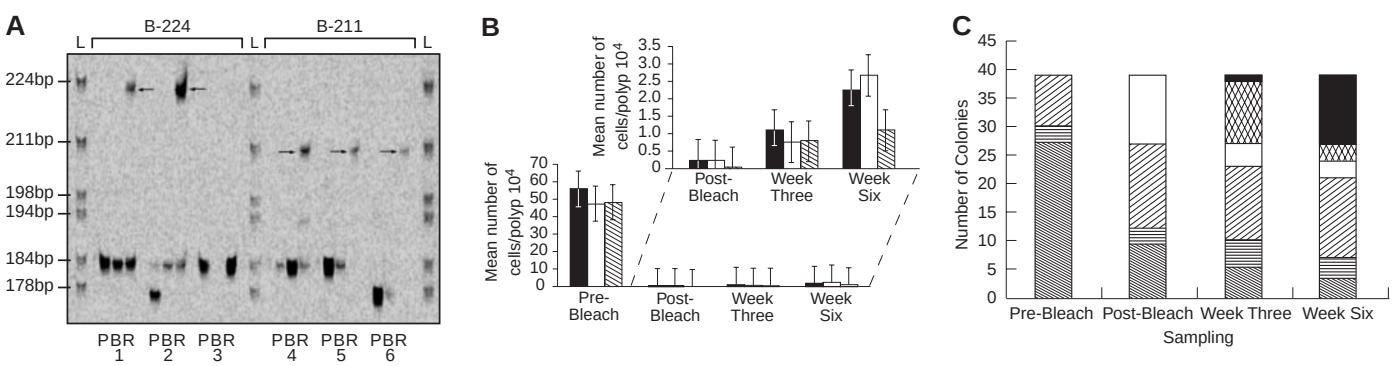


Fig. 2. (**A**) Typical polyacrylamide electrophoresis gel demonstrating the cp23S-genotype of symbionts isolated from representative *Briareum* sp. colonies. Isolates from six colonies (1 to 6) were monitored through time: (P) Prebleaching; (B) bleached, after 12 weeks of darkness; and (R) recovery, exposed to exogenous *Symbiodinium* (i.e., the marker cp23S-genotype). Arrows designate the presence of the marker cp23S-genotype. Cp23S-genotype ladder (L) was used for molecular size comparisons. (**B**) Mean *Symbiodinium* densities per *Briareum* polyp before bleaching and immediately after bleaching, and after 3 and 6 weeks of exposure to cultures containing the marker cp23S-genotypes (note the expanded scale). Mean cell densities for control colonies maintained in filtered seawater and in unfiltered seawater are also shown. Error bars indicate standard deviation. There was no significant difference between the algal growth rates of the colonies exposed to cp23S-genotype cultures and those maintained in circulating filter seawater not exposed to algal cultures [Kruskal-Wallis nonparametric test (SPSS version 11.0.1), $X^2 = 8.362$, df = 4, $P = 0.079$]. Key: black, colonies exposed to exogenous marker cp23S *Symbiodinium*; white, control group (not exposed to exogenous *Symbiodinium*); diagonal lines, flow-through colonies in unfiltered seawater. (**C**) Shift in frequency of *Symbiodinium* B184 cp23S-genotype within *Briareum* sp. colonies after bleaching (see text). Key: narrow diagonal lines, B178 only (or with marker cp23S-genotype); horizontal lines, B178 and B184 (or with marker cp23S-genotype); wide diagonal lines, B184 only (or with marker cp23S-genotype); white, no symbiont detected; hatched, marker cp23S-genotype alone; black, colony died.

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selection can occur between symbionts in adults in a manner similar to that observed in early ontogeny (*24*)

Corals have survived global changes since the first scleractinian coral-algal symbioses appeared during the Triassic, 225 million years ago (*25*). In recent decades, we have come to appreciate and better understand the fragile nature of coral reefs. The survival of individual colonies and populations should not be confused with the health of ecosystems. However, the ability of octocorals to reestablish symbiont populations from multiple sources provides a mechanism for resilience in the face of environmental change. The task ahead is to access the presence of similar processes in scleractinian corals and to determine if symbionts are available in hospite or exogenously that can allow corals to respond to future environmental changes.

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22. Although it is possible that symbionts containing the marker cp23S-genotypes were initially present as cryptic populations [detection threshold ~1000 cells (*21*)], in many cases the B178 and/or B184 cp23S-rDNA genotypes were detected after bleaching, whereas the marker cp23S-rDNA genotypes were not detected until the host colony was exposed to the isoclonal cultures. The symbionts with the marker genotypes subsequently appeared, replacing these initial genotypes. Furthermore, with one exception (see legend to Fig. 1), a given marker cp23S-rDNA genotype was only detected in colonies exposed to that culture.
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Materials and Methods

Table S1

References and Notes

1 March 2004; accepted 29 April 2004

Flexibility in Algal Endosymbioses Shapes Growth in Reef Corals

Angela F. Little,[1] Madeleine J. H. van Oppen,[2] Bette L. Willis[1*]

The relation between corals and their algal endosymbionts has been a key to the success of scleractinian (stony) corals as modern reef-builders, but little is known about early stages in the establishment of the symbiosis. Here, we show that initial uptake of zooxanthellae by juvenile corals during natural infection is nonspecific (a potentially adaptive trait); the association is flexible and characterized by a change in (dominant) zooxanthella strains over time; and growth rates of experimentally infected coral holobionts are partly contingent on the zooxanthella strain harbored, with clade C–infected juveniles growing two to three times as fast as those infected with clade D.

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-

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