Multispecies Microbial Mutualisms on Coral Reefs:
The Host as a Habitat

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abstract: Reef-building corals associate with a diverse array of eukaryotic and noneukaryotic microbes. Best known are dinoflagellates in the genus *Symbiodinium* ("zooxanthellae"), which are photosynthetic symbionts found in all reef-building corals. Once considered a single species, they are now recognized as several large, genetically diverse groups that often co-occur within a single host species or colony. Variation among *Symbiodinium* in host identities, tolerance to stress, and ability to colonize hosts has been documented, but there is little information on the ecology of zooxanthellar free-living stages and how different zooxanthellae perform as partners. Other microbial associates of reef corals are much less well known, but studies indicate that individual coral colonies host diverse assemblages of bacteria, some of which seem to have species-specific associations. This diversity of microbial associates has important evolutionary and ecological implications. Most mutualisms evolve as balanced reciprocations that allow partners to detect cheaters, particularly when partners are potentially diverse and can be transmitted horizontally. Thus, environmental stresses that incapacitate the ability of partners to reciprocate can destabilize associations by eliciting rejection by their hosts. Coral bleaching (the loss of zooxanthellae) and coral diseases, both increasing over the last several decades, may be examples of stress-related mutualistic instability.

Keywords: coral, microbe, zooxanthellae, reef, mutualism, bacteria.

Tropical environments are renowned for the diversity and intricacy of their mutualistic associations, and coral reefs are no exception. Fishes that live with sea anemones, shrimps and fishes that clean ectoparasites from fishes, and a host of specialized associations between mobile and sedentary organisms are common in these ecosystems. The most ecologically important mutualisms on reefs are thought to be those of dinoflagellates (zooxanthellae) with corals. These obligate photosynthetic symbionts provide much of the energy that powers the growth of all reef-building corals (Muller-Parker and D'Elia 1997), in turn creating the reef environment upon which so many other organisms depend (Paulay 1997). Bacterial and archaeal associates of corals may well play equally crucial roles, but studies of these symbionts are much less extensive.

Over the last several decades, our understanding of these associations has been transformed by the recognition that the microbial partners of corals are much more diverse than previously recognized. In the case of zooxanthellae, for example, it was initially believed that only one symbiont species associated with multiple species of corals (and other hosts). Later, it was recognized that zooxanthellae are genetically diverse, with different symbionts associated with different hosts. Most recently, the discovery of multiple types of zooxanthellae living with one host species or even a single colony (the polyps derived from a single fertilized egg) has changed our conception of these symbioses again. Because models of pairwise mutualisms may not necessarily apply to multipartner mutualisms (Stanton 2003), these discoveries fundamentally change how we view the evolution and maintenance of coral-microbial associations.

Microbial associates of corals are of more than academic interest because coral reefs are increasingly threatened by many factors that could destabilize the partnerships. One example is the phenomenon known as "coral bleaching." This breakdown of the partnership between corals and zooxanthellae, with potentially lethal results for the coral host, appears to be increasing in both severity and frequency since the 1980s (Glynn 1993; Brown 1997; Hoegh-Guldberg 1999). The incidence of diseases in reef corals has also increased (Richardson 1998; Harvell et al. 1999) over the same time interval. These concordant patterns suggest that the microbial associations of corals may be changing in response to anthropogenic stress.

Here we summarize what is known about these coral-microbial associations and how this information contributes to our understanding of multipartner mutualisms more generally. We begin with a description of the diversity of microbial associates that has been documented, much of it recently, with the application of molecular techniques. This is followed by two sections describing how this diversity is distributed in space (with respect to host, environment, and biogeographic region) and time (especially the mode of acquisition of zooxanthellae and the response of zooxanthellar communities to disturbance). At the end, we consider the implications of these patterns for theories of mutualisms more generally and pose questions for future study.

Who Are the Players, and What Roles Do They Play?

Understanding the evolutionary and ecological dynamics of mutualisms depends on knowing what organisms are involved and how they perform as partners. In the case of microbial mutualisms, this more often than not requires the tools of molecular biology, which is why so many basic features of the natural history of these associations have been elucidated relatively recently.

Zooxanthellae

Zooxanthellae are by far the best-understood microbial associates of corals, and the physiological nature of the association has been studied for decades. The nutritional benefits to the host (photosynthetic carbon) and symbiont (inorganic nutrients) have been reviewed extensively elsewhere (e.g., Muller-Parker and D'Elia 1997) and will not be considered in detail here. The relationship is clearly of benefit to the coral (and is indeed obligatory for reef-building species), but the degree of benefit and dependence to the zooxanthellae is less clear (Douglas and Smith 1989), since almost nothing is known about the ecology and physiology of free-living zooxanthellae in the natural environment (LaJeunesse 2001). Although most hosts acquire zooxanthellae from the environment, free-living strains have only rarely been isolated (Loeblich and Sherley 1979; Carlos et al. 1999).

The ecological and physiological roles of zooxanthellae have long been understood, but it is only recently that their true diversity has been appreciated (reviewed in Trench 1997; Rowan 1998). For many years it was assumed that a single species, *Symbiodinium microadriaticum*, associated not only with all reef-building corals but also with giant clams and other invertebrates. This view began to change with the pioneering work of Trench et al. (e.g., Schoenberg and Trench 1980*a*, 1980*b*, 1980*c*; Blank and Trench 1985), who showed that morphological and en-

zymatic differences could be observed in algae cultured from different hosts. This was followed by the application of DNA-based techniques by Rowan and Powers (1991), which elucidated the genetic diversity suggested by previous work and led the field into a new era. Their analyses of small subunit rDNA genes showed clearly for the first time that there is enormous genetic divergence within the genus *Symbiodinium*, whose major groups are distinguished by what would typically be considered family- or order-level differences in free-living dinoflagellates, and that closely related hosts often have distantly related zooxanthellae, indicating substantial evolutionary flexibility of host associations over time.

Subsequent taxonomic work initially focused on documenting the major branches of the *Symbiodinium* tree, including a number of lineages associated with other organisms, such as foraminiferans, sponges, and clams (Rowan 1998; Wilcox 1998; Carlos et al. 1999; Pochon et al. 2001). These phylogenetic analyses suggest that all *Symbiodinium* descended from a symbiotic ancestor, although a few lineages may have secondarily lost the symbiotic habit (Wilcox 1998; LaJeunesse 2002). Because these clades also contain genetic variation and are found across a wide array of hosts and habitats, it is very likely that much taxonomically and ecologically significant variation exists within each clade. However, elucidating the fine-scale taxonomy of *Symbiodinium* in natural samples was initially challenging because the small subunit rDNA genes first used to study *Symbiodinium* are relatively conservative, occur in multiple copies, and probably vary within a single genome (Toller et al. 2001*b*; LaJeunesse 2002). Until recently, the latter property made it difficult to distinguish within- from between-genome variation in environmental samples of taxa from a single clade. Nevertheless, a number of new techniques and new genes (e.g., denaturing gradient gel electrophoresis, microsatellites, more variable portions of nuclear rDNA genes, and chloroplast small subunit rDNA; Baker 1999, 2001; LaJeunesse 2001, 2002; Santos et al. 2001, 2002; Santos and Coffroth 2003) have begun to illuminate the finer taxonomy of zooxanthellae.

Only four of the major clades of *Symbiodinium* are known to associate regularly with corals (Pochon et al. 2001)—A, B, C, and D (D is also referred to as clade E by Toller et al. [2001*a*, 2001*b*] and Brown et al. [2002*a*]; table 1). Recently, clade F, typically associated with foraminiferans, has been reported from the coral *Alveopora japonica* in Korea (Rodriguez-Lanetty et al. 2000). In the Caribbean, where all four clades are abundant in corals, LaJeunesse (2002) identified 24 distinct types of *Symbiodinium* from 38 species of scleractinian corals in the Bahamas and Mexico (four types in clade A, five in clade B, 14 in clade C, and one in clade D; table 1). Because over half of the hosts were sampled only once, these numbers

Table 1: Characteristics of the four clades of *Symbiodinium* commonly associated with scleractinian corals

	Clade A	Clade B	Clade C	Clade D
Caribbean	Abundant	Abundant	Abundant	Common
No. types	4 (1 common)	5 (1 common)	14 (4 common)	1
Eastern Pacific	Absent	Absent	Abundant	Common
No. types	…	…	5 (2 common)	1
Indo-West Pacific	Very rare	Very rare	Abundant	Common
No. types	1	1	16 (3 common)	2 (1 common)
Colonization ability	Very fast	Slow	Slow	Fast
Stress resistance	High light	Cold temperature	Variable	Extreme temperature, sedimentation

Note: Ecological characteristics are tendencies suggested by some studies but are not necessarily universal across all members of a clade. Current estimates of numbers of types within clades and their abundance are drawn from Baker (1999), van Oppen et al. (2001), LaJeunesse (2002), and LaJeunesse et al. (2003).

may be substantial underestimates (although ongoing work suggests that much of the variation was captured in the initial surveys; T. C. LaJeunesse, personal communication). Seven of the 24 zooxanthellar types found by LaJeunesse were relatively unspecialized, each being associated with three to 17 species of corals (table 1).

Comparable studies are beginning to emerge for the Pacific, where almost all corals associate either with clade C or with clades C and D (table 1). In the eastern Pacific, Baker (1999) identified five types within clade C and one type within clade D associated with 13 species of corals from Panama, the Galapagos, and Mexico. In the Indo-West Pacific, Baker (1999) and van Oppen et al. (2001) analyzed a total of 32 species in the genus *Acropora* and between them characterized three types in clade C (one very rare) and one type in each of clades A and D. An additional five types of clade C and one of clade D were identified by Baker (1999) in his survey of other Indo-West Pacific coral species (55 in total). LaJeunesse et al. (2003), using a more variable region of rDNA, identified 16 types from 73 scleractinian coral species, 15 of which belonged to clade C and one to clade D. Of the clade C symbionts, four types were relatively unspecialized, each being associated with six to 42 host species. Surprisingly, the diversity of *Symbiodinium* associated with corals appears to be higher in the Caribbean than in the Indo-Pacific, in striking contrast to patterns observed in other organisms (LaJeunesse et al. 2003).

Where the characterization of zooxanthellar diversity will end is still unclear. Santos and Coffroth (2003), for example, note that two species of gorgonians appear to associate with different species of zooxanthellae, based on microsatellite evidence. The fact that the zooxanthellae from the two hosts are identical or nearly so at the ITS locus, on which the most fine-grained zooxanthellar taxonomy currently rests (LaJeunesse 2001, 2002), suggests that host specificity (and perhaps other forms of specificity) may be greater than even recent reports indicate.

Other Eukaryotes

In comparison to zooxanthellae, other microalgal, protozoan, and fungal associates of corals are scarcely studied. Most emphasis has been placed on potential pathogens (particularly among the fungi) and skeletal borers (which weaken the skeleton and are thus unlikely to be mutualists). For example, fungi are clearly involved in diseases of sea fans (Harvell et al. 1999), and corals lay down dense skeletal layers when endolithic fungal hyphae approach living coral tissue (Bentis et al. 2000). A recently discovered protozoan that is very common in corals of the genus *Montastraea* may also be a pathogen because it is related to the apicomplexans, a parasitic group (Toller et al. 2002).

Some endolithic algae may be genuine mutualists, however (Fine and Loya 2002). The benefits to the algae are relatively clear, in that the coral provides protection against predators and also shields the algae from most ultraviolet radiation (Shashar et al. 1997). Some endolithic algae are bioeroders, and in normal circumstances, the costs associated with the weakening of the coral skeleton probably outweigh the benefits of any translocated fixed carbon. However, when zooxanthellae are lost during coral bleaching associated with high temperature and other stresses, the photosynthetic products of endolithic algae may play a crucial role in increasing the probability of the coral's survival until a normal complement of zooxanthellae is restored (Fine and Loya 2002).

Bacteria and Archaea

Noneukaryotic microbes play important ecological roles (including as mutualists) in all ecosystems, but until recently, research on their associations with corals has been fairly limited. Interest in these microbes has increased substantially, however, with the realization that diseases on coral reefs, including of corals themselves, are having a devastating impact (Richardson 1998; Harvell et al. 1999).

By growing out coral-associated bacteria in culture, a number of investigators have shown that corals harbor diverse and abundant bacterial communities (Ducklow and Mitchell 1979; Shashar et al. 1994; Ritchie and Smith 1995, 1997; Santavy 1995; Rohwer et al. 2001). Specificity of associations is suggested by Ritchie and Smith's (1997) analysis of carbon source utilization patterns by bacteria cultured from the mucus layer of healthy corals, which demonstrated that Caribbean coral species have unique, species-specific, mucus-associated microbial communities.

Culture-free, DNA-based techniques have revolutionized our understanding of bacterial and archaeal diversity in the sea because many of these microbes cannot be cultured easily or at all (Giovannoni and Rappe 2000). Only a few such studies have examined the bacterial communities of healthy corals, however. Frias-Lopez et al. (2002) documented a total of 62 distinct bacterial ribotypes in single samples from each of three coral species in Curaçao, Rohwer et al. (2002) documented 430 bacterial ribotypes in 14 samples from three species in Bermuda and Panama, and Cooney et al. (2002) observed 23 bacterial ribotypes from two coral species in St. Croix and Barbados. The majority of the ribotypes in these studies were observed only once, and for two of these studies, about 50% of the sequences differed from the most similar sequences in GenBank by at least 7%, a level often used to justify recognition as a new genus (Frias-Lopez et al. 2002; Rohwer et al. 2002). Together these studies identified representatives from 12 bacterial divisions associated with corals. Rohwer et al. (2002) also calculated statistically that the true number of distinct bacterial ribotypes in the 14 samples they analyzed was approximately 6,000. Similarly, work in progress has shown that coral-associated Archaea are also abundant and diverse (F. Rohwer and L. Wedley, unpublished data). With so much diversity within so few samples, it is clear that we are only scratching the surface in terms of understanding how many bacteria and Archaea are normally associated with corals.

We know almost nothing about the role of noneukaryotic microbes in healthy corals, but as in other organisms that have been studied, mutualists must play important roles. However, unraveling the nature of these associations will be difficult because the biochemical capabilities of bacteria and Archaea are so diverse by comparison with dinoflagellates. A number of different bacterial types known to fix both nitrogen and carbon have been observed in coral-associated communities (Williams et al. 1987; Shashar et al. 1994; Cooney et al. 2002; Rohwer et al. 2002). It is also possible that coral-associated bacteria scavenge limiting nutrients (e.g., iron, vitamins) that are then harvested by the coral; this hypothesis is supported by the observations that many corals eat their own mucus (Coles and Strathmann 1973) and that bacteria are much better than corals at assimilating nutrients at very low concentrations. Finally, it is possible that some of these organisms protect corals from pathogens by blocking their entry or by producing antibiotics or enhance the ability of corals to defend themselves against predators or competitors. In sum, there are a multitude of possibilities and almost no data. Indeed, we do not even know where these microbes live, information that could have important implications for the ability of corals to regulate the numbers or activities of their microbial associates.

Patterns of Coral-Microbial Associations in Space

Documenting the amount of diversity in coral-microbial associations is but the first step in understanding them ecologically and evolutionarily. To evaluate the importance of multispecies associations, we need to know how common they are and whether multiple partnerships nevertheless exhibit patterns of specificity with respect to environment or host that constrain the types of associations that are possible. We document that multispecies associations are common and ecologically structured.

Zooxanthellae as Communities of Coexisting Taxa

For decades, it was assumed that corals contained one species of zooxanthella. The pioneering work of Trench and Rowan revealed the enormous genetic diversity of zooxanthellae, but the assumption that only one type of zooxanthella is found in any single host species remained widespread (despite the fact that a limited amount of diversity within host species, although not within colonies, had been detected early on; Schoenberg and Trench 1980*c*). This assumption was shattered, however, with the discovery that species in the *Montastraea annularis* complex often host multiple clades of zooxanthella, even within a single colony (Rowan and Knowlton 1995). Subsequent studies have shown that multiple types of zooxanthellae occur in a substantial number of coral species, although not in the majority (Baker 1999, 2001; van Oppen 2001; Chen et al., in press). Moreover, in a number of cases, multiple symbiont types are found within single coral colonies. This finding is particularly interesting evolutionarily because symbiont mixtures within a single host (especially within host individuals) are believed to set the stage for competition among symbionts and thus potentially threaten the stability of a mutualism (Herre et al. 1999; Hoeksema and Kummel 2003; Palmer et al. 2003; Stanton 2003).

The patterns of coassociation have been best studied in Caribbean corals, where the abundance and ready detectability of the four coral-associated *Symbiodinium* clades have made it relatively easy to assess mixtures. At one end of the spectrum are the three species belonging to the *M.*

annularis complex; these corals associate with all four *Symbiodinium* clades, often as mixtures within single colonies (Toller et al. 2001*b*). Overall, over 40% of the 43 assayed species of scleractinian corals in the Caribbean associate with more than one zooxanthellar clade, and 25% have been documented to have multiple clades within a single colony (compiled from Baker 1999; Diekmann et al. 2002; LaJeunesse 2002). Although this does not negate the significance of the fact that a majority of corals appear to host just one symbiont clade (Diekmann et al. 2002), it is worth noting that many of the dominant reef builders host multiple clades of zooxanthellae. It is less common to find host species with multiple symbionts belonging to a single clade (eight of 43 species, five of which exhibit multiple types of a single clade within individual colonies; Baker 1999; LaJeunesse 2002).

In the Pacific, most corals host clade C symbionts, and species or colonies associated with more than one clade are comparatively rare (Baker 1999; van Oppen et al. 2001; Chen et al., in press). In the eastern Pacific, four of 13 species studied thus far host clade D in addition to clade C, and in each case, single coral colonies containing both clades were observed. In the tropical Indo-West Pacific, 38 species in the genus *Acropora* have been examined; of these, only five were observed to host more than one clade (four with clade D and a single colony with clade A; Baker 1999; van Oppen et al. 2001; Chen et al., in press). Among the other 82 species examined, all but one hosted clade C. One species hosted only clade D, while seven hosted clade D in addition to clade C. LaJeunesse et al. (2003) sampled 73 scleractinian species and found similar results, with all but three species hosting only clade C.

Because clade C dominates Pacific corals and contains the largest number of variants, intraclade diversity is likely to be more important in the Pacific than in the Caribbean, although few surveys address this point. Both van Oppen et al. (2001) and Baker (1999) divided clade C into a number of types. In the eastern Pacific, the four species hosting clade C and clade D, plus one additional species with only clade C, also hosted multiple types within clade C (Baker 1999). No individual colonies were observed with multiple types of clade C, however. In the tropical West Pacific, 10 of 32 species in the genus *Acropora* and 16 of 82 other coral species hosted multiple types of clade C (Baker 1999; van Oppen et al. 2001). In the nonacroporid corals, within-colony diversity was reported in 12 of the 16 cases with multiple types of clade C. LaJeunesse et al. (2003) found even lower within-species and within-colony diversity; only nine of 73 coral species (and only two of 168 sampled colonies) hosted more than one type of *Symbiodinium*.

In summary, many Caribbean corals are capable of hosting more than one type of zooxanthella, and a substantial subset of those corals do so within individual colonies, while in the Pacific, the percentage of species and colonies observed with multiple types of zooxanthellae is lower. The ability of corals to host multiple types of symbionts does not imply that associations between corals and zooxanthellae are promiscuous, however. Indeed, even in the case of the *M. annularis* complex, corals associate with all four of the major clades, but only a fraction of the diversity within each of these clades. The specificity of associations between corals and *Symbiodinium* is the topic to which we now turn.

Environmental Specificity among and within Clades of Zooxanthellae

Because zooxanthellae are photosynthetic, zonation by light is an obvious possibility, and it was the first form of niche partitioning among coral symbionts to be clearly documented. In the Caribbean, the zooxanthellae associated with the *M. annularis* complex show clear evidence of zonation, with clades A and B in shallower (more brightly lit) water and clade C in deeper water (Rowan and Knowlton 1995). In habitats where clade D is abundant, it is often shallower than clade C, although it has also been documented in very deep water at the coral/sediment interface (Toller et al. 2001*b*). In a broader survey of the Caribbean, LaJeunesse (2002) documented that clade A is restricted to hosts in less than 3–4 m of water. Moreover, Coffroth et al. (2001) showed that transient populations in newly settled juveniles of a Caribbean octocoral contained clade A in shallow water but not in deeper water. In the Caribbean, for every case where a single coral species hosts more than one clade, clade C is typically found deeper than clades A and B (although many shallow water corals host only clade C). In *Montastraea*, these patterns are reiterated at small spatial scales across the surface of some colonies, with clade C restricted to lower light microenvironments in shallower depths (Rowan et al. 1997). In other coral hosts, such as *Acropora cervicornis*, the zonation patterns are only seen across colonies, not within them (Baker et al. 1997).

Although these patterns strongly suggest differences in the physiology of zooxanthellar clades with respect to light, there is comparatively little experimental and physiological data to support this interpretation. In *M. annularis*, tipping colonies on their sides resulted in a new pattern of zooxanthellar zonation that reflected the new orientation, indicating that zonation is dynamically maintained (Rowan et al. 1997). Physiological studies also suggest that only clade A is capable of producing microsporine amino acids (Banaszak et al. 2000), which are believed to be useful in protecting against ultraviolet damage. Production of these compounds may enhance the competitive ability of clade

A in high-light environments and conversely may decrease the competitive ability of clade A in environments where protection against ultraviolet wavelengths is not needed.

Given the prevalence of clade C within the Indo-Pacific across wide depth ranges, one might expect clear depth zonation patterns among the types within the clade. It is particularly easy to identify light-tolerant types in those corals that host different types of zooxanthellae at different depths. Evidence for this does exist for nine species of Indo-West Pacific *Acropora*. Both Baker (1999) and van Oppen et al. (2001) found evidence that one type (C1) consistently inhabits lower light environments, relative to a second type (C2 of van Oppen et al. [2001] or C4 for Baker [1999]).

Other potential factors affecting the distribution of *Symbiodinium* clades are much less studied. Several studies suggest that clade B may be somewhat resistant to cool temperatures. The only scleractinian coral in the Pacific to host clade B does so only in the temperate waters off Australia (Baker 1999; Rodriguez-Lanetty et al. 2001). Similarly, in a comparison of Bermudan and Caribbean corals, Savage et al. (2002a) found that clade B was relatively more common (28% vs. 67% of coral species in the Caribbean and Bermuda, respectively) and clade C was relatively less common (53% vs. 22% in the cooler Bermudan waters). Clade B, as well as clade A, was also found to be resistant to high temperature stress in members of the *M. annularis* complex (Rowan et al. 1997).

Clade D appears to be resistant to an especially wide array of stresses, including low temperature, high temperature, and sediments, in both the Caribbean and the Pacific. Chen et al. (in press) document that the only coral that associates exclusively with clade D can live on reefs where temperatures routinely range from 12° to 35°C and sometimes fall below freezing. In the *M. annularis* complex, this clade is found consistently in very near-shore waters of Caribbean Panama, where it appears to replace clades A and B, as well as in very deep water where the reef interfaces with soft sediments (Toller et al. 2001b). A nearly identical pattern of distribution was found for clade D in Taiwan (Chen et al., in press). Clade D is also found in reef-flat environments in Thailand that have lots of sediment and considerable temperature stress (Brown et al. 2002a). Finally, clade D was more resistant to high temperature stress than was clade C during a bleaching event in the eastern Pacific (Baker 1999; Glynn et al. 2001).

Finally, it bears emphasizing that apparent differences in ecological characteristics among clades may be an artifact of the limited number of studies done to date. For example, although clade C was shown to be more sensitive to high temperature in the eastern Pacific (Glynn et al. 2001) and in the Caribbean (Rowan et al. 1997), some types within clade C have been shown to be resistant to bleaching in the western Pacific (LaJeunesse et al. 2003). Similarly, Savage et al. (2002b) found little evidence of cladewide characteristics.

Zooxanthellar Host Specificity and Cospeciation

Host specificity may or may not result in cospeciation, as evidenced by concordant phylogenies of hosts and symbionts (Herre et al. 1999). At the level of zooxanthellar clades, there is no evidence for either host specificity or cospeciation—distantly related zooxanthellae occupy closely related (indeed the same) hosts, while closely related hosts often harbor distantly related zooxanthellae (Rowan and Powers 1991). The common types of clade C are also widely distributed, with no evidence of concordance between their phylogeny and that of their hosts (van Oppen et al. 2001; LaJeunesse 2002; LaJeunesse et al. 2003).

There are, however, a number of examples of apparently specific associations. Baker (1999) identified a type of clade B that is unique to, and the sole associate of, *Colpophyllia natans* in the Bahamas. He also described a common type of clade C that is uniquely associated with deeper water *A. cervicornis* in the Caribbean, a unique type of clade C common in *Montastraea cavernosa*, several types of clade C uniquely associated with several species of *Porites* in the Caribbean and eastern Pacific, and a unique type of clade D commonly associated with the coral *Diploastrea heliopora* in Australia. Similarly, LaJeunesse (2002) and LaJeunesse et al. (2003) document a number of types of *Symbiodinium* with narrow host distributions. In the Caribbean, for example, 16 of the 24 types recognized by LaJeunesse (2002) had distributions limited to either a single species or a single genus of coral. Because sampling effort per coral species was generally limited in this study, further work will be required to determine how many of these zooxanthellae are truly specialists at the level of host species or host genus. Nevertheless, his proposition that the diversity of *Symbiodinium* can best be understood as falling into two groups—widely distributed generalists and more narrowly distributed specialists—seems reasonable. Surprisingly, there is no obvious connection between vertical transmission and the presence of specific associations.

Parallels and Differences between Zooxanthellae and Other Coral Associates

Our knowledge of the nature of the diversity of bacterial and archaeal associates of corals is so sketchy that it is difficult to draw general conclusions about how their distribution patterns vary biogeographically and across ecological gradients. Nevertheless, hints of spatial patterns are beginning to emerge. Rohwer et al. (2001) demonstrated

that *Montastraea franksi* has a diverse bacterial community that was similar in samples from five reefs, up to 10 km apart. In a related study (Rohwer et al. 2002), they found that different coral species living in close proximity to each other had distinct bacterial communities but that the same coral species separated by hundreds of kilometers had similar microbiotas. Together, these observations suggest that coral species have some characteristic bacterial associates. Specific patterns of host association are also suggested by the fact that of the 34 bacteria found in more than one sample, 29 were found in the same coral species, a significant departure from a random distribution, since the three coral species they studied were sampled in approximately equal numbers. Examples include a γ-proteobacteria always associated with *Porites astreoides* (Rohwer et al. 2002) and a *Rickettsia* sp. associated with *A. cervicornis* (F. Rohwer and V. Casas, unpublished data). In the case of *P. astreoides*, this associate made up between 27% and 92% of the 419 clones sequenced from five colonies.

Essentially nothing is known about distributions along environmental gradients for these bacteria, but there are tantalizing hints of what might emerge with further study. In an analysis of the coral *Porites furcata*, one bacterial type was consistently present in the tips and absent from the middle portions of the branches. This pattern suggests that bacteria, like zooxanthellae, may be spatially structured within individual colonies.

Patterns of Coral-Microbial Associations over Time

Most studies of the microbial associates of corals represent snapshots in time. Our understanding of how such associations vary temporally is quite limited, but of potential importance. Some multipartner associations may be transitory over the normal course of events and thus rare at any particular point in time yet nevertheless may be influential in terms of evolutionary dynamics. Here we review two situations where such transitions are likely: the initial acquisition of zooxanthellae and the recovery of zooxanthellar communities following disturbance.

Acquisition of Zooxanthellae

Of fundamental evolutionary interest is the question as to whether mutualists are transmitted vertically or horizontally. Although there are many exceptions, in general vertically transmitted symbionts are more uniform genetically within hosts and thus less likely to engage in competitive tactics that are detrimental to the host (Herre et al. 1999). In corals, however, *Symbiodinium* transmission is often horizontal, with zooxanthellae being acquired anew at every generation. In order to understand why, it is useful to consider the characteristics associated with vertical ver-

sus horizontal transmission. Different patterns of coral reproduction are generally correlated with the mode of *Symbiodinium* transmission. Most scleractinian corals that release unfertilized eggs or recently fertilized embryos into the water column have horizontal transmission, while most corals that brood their offspring and release them as planular larvae (a trait found in several coral families) have vertical transmission (e.g., Fadlallah 1983), although there are conspicuous exceptions (Hirose et al. 2001; Weis et al. 2001).

There are several reasons why selection might favor vertical transmission in brooded offspring or horizontal transmission in broadcast offspring. The presence of zooxanthellae in brooded larvae might increase their ability to disperse to distant sites or may increase survivorship probabilities following short-distance dispersal. In contrast, the presence of maternally derived zooxanthellae might be disadvantageous in broadcast larvae if it inhibited the ability of recruits to pick up locally appropriate zooxanthellae at sites far from the maternal colony. Moreover, it might be difficult for positively buoyant, broadcast eggs floating near the surface to maintain healthy zooxanthellae in a regime of high irradiance. Alternatively and unrelated to selection, there may be phylogenetic constraints on the ability of corals to transfer zooxanthellae to unfertilized eggs.

No evidence clearly distinguishes among these hypotheses, and they are not mutually exclusive. Indeed, the costs of having or not having zooxanthellae in larval propagules have not been documented, making it difficult to evaluate the patterns with respect to potential benefits. Recent work suggests that other cnidarians are capable of rapidly and efficiently picking up zooxanthellae from the environment in the field (Coffroth et al. 2001), so that the risk of being unable to locate zooxanthellae seems slight. Because theory suggests that, all else being equal, vertical transmission is typically beneficial to both host (by reducing intersymbiont conflict) and symbiont (by guaranteeing transmission), the fact that most corals spawn their gametes and acquire their symbionts horizontally suggests the presence of either very strong evolutionary constraints or substantial costs to vertical transmission. This in turn makes multipartner mutualisms more likely for corals and their endosymbionts.

Variation among Zooxanthellar Clades in Their Recovery from Coral Bleaching

Coral-algal symbioses break down under environmental stress, causing the phenomenon of coral "bleaching"—the loss of zooxanthellae and/or their photosynthetic pigments (Brown 1997; Fitt et al. 2001). A variety of stresses has been implicated, including cold water, high light, and fresh

water, but the most commonly studied stress is high temperature. Coral "bleaching" is a phenomenon of considerable concern because bleaching episodes have increased substantially over the last several decades (Glynn 1993; Hoegh-Gulberg 1999), and it has been argued that corals are the canary in the coal mine with respect to global warming. During the most recent El Niño event, mortality associated with coral bleaching was extensive, reaching 80%–90% at some sites (Hoegh-Guldberg 1999).

Because both corals and zooxanthellae benefit from the association, the expulsion of zooxanthellae and subsequent coral death has long puzzled reef biologists. In retrospect, however, coral bleaching is best viewed as an extreme manifestation of normal processes regulating zooxanthellar densities (Warner et al. 2002). In particular, because mutualisms are better looked at evolutionarily as reciprocal parasitisms, both partners probably have mechanisms to respond to potential cheaters—individuals that receive benefits but do not reciprocate (Herre et al. 1999). Indeed, LaJeunesse (2002) suggests that discrepancies in the composition of cultured zooxanthellae and natural samples may reflect the presence of nonmutualistic *Symbiodinium.* Thus, when stress damages the photosynthetic apparatus of zooxanthellae, as it is known to do (Warner et al. 1999), it makes evolutionary sense for corals to discard nonperforming or poorly performing algae, provided that the ability to acquire new ones is high enough to offset the costs of temporarily living without them (see above for a related discussion of horizontal transmission). One recent study indicates that zooxanthellae expelled during temperature stress were not seriously impaired in their photosynthetic abilities (Ralph et al. 2001), suggesting that monitoring by coral cells of zooxanthellar performance could be quite subtle. Selective responses to poorly performing partners would also be facilitated by the fact that most coral cells contain only a few zooxanthellae (Muscatine et al. 1998). Massive bleaching resulting in substantial death could thus be viewed as an adaptive mechanism gone awry under conditions not typically experienced by corals in the past. This interpretation could be characterized as a weak form of the adaptive bleaching hypothesis.

More recently, however, a much stronger form of the adaptive bleaching hypothesis has emerged. First fully elaborated by Buddemeier and Fautin (1993), it states that corals expel their symbionts under conditions of stress because it is easier to acquire new, better-adapted ones when the original associates are gone. Many of the basic assumptions underlying this model are valid (Kinzie et al. 2001). Baker (2001) explicitly tested it by transplanting shallow-water corals to deep water and deep-water corals to shallow water. As expected, deep-water corals transplanted to shallow water bleached because of extreme light

stress, while shallow-water corals transplanted to deep water did not. Unexpected, however, was the fact that the unbleached, shallow-to-deep transplants survived slightly worse than the bleached, deep-to-shallow transplants. This, together with the observation that changes in symbiont communities occurred in bleached colonies (but not in unbleached ones), was interpreted as evidence in support of the strong version of the adaptive bleaching hypothesis.

Toller et al. (2001*a*) also monitored *Symbiodinium* recovery from experimental bleaching, but bleached colonies were not moved from their original habitat. They found that while moderately bleached colonies recovered with their original zooxanthellar communities, strongly bleached colonies had very different zooxanthellae upon recovery, even in the absence of environmental change. As in the case of Baker's (2001) study, clades A and D (as well as an atypical type of clade C) dominated these strongly bleached corals, and clade A densities were also much higher than normal. Clade A also dominated corals that bleached in response to yellow blotch disease. Thus, the higher mortality rates of deeper, unbleached corals observed by Baker (2001) could be due either to the lack of a bleaching response (as predicted by the adaptive bleaching hypothesis) or to the slow colonization and growth rates that appear to characterize clade C, the characteristic symbiont in deeper water for the corals and reefs studied.

Overall, these results suggest that some members of clade A, at least for the *Montastraea annularis* complex, can be viewed as weedy symbionts (Rowan et al. 1997), with greater ability to invade and/or exploit tissues largely devoid of other zooxanthellae. Their weediness is also suggested by the fact that clade A dominates cultured material to a greater extent than would be predicted from the zooxanthellar communities in hosts from which cultures were derived (LaJeunesse 2002). However, the situation is complicated, because clade A also forms apparently stable associations with many Caribbean corals, including members of the *M. annularis* complex. Clade D also appears to have weedy characteristics, not only in its resistance to stress but also in its ability to colonized bleached corals relatively rapidly (Baker 2001; Toller et al. 2001*a*). Clades B and C, in contrast, might best be interpreted as zooxanthellae typical of "climax" communities, with low rates of recruitment and growth. In Toller's (2001*a*) study, clades B and C failed to return even after 9 mo following bleaching, and work in progress suggests that return to prebleaching zooxanthellar communities may take several years (T. C. LaJeunesse, personal communication). The ecological parallels with plant communities with respect to potential competition-colonization trade-offs and

tolerance-colonization trade-offs are striking (see also Palmer et al. 2003; Hoeksema and Kummel 2003).

Broader Implications for Multipartner Mutualisms and Questions for the Future

The explosion of recent discoveries has answered many long-standing uncertainties concerning coral-microbial symbioses and has shaped our interpretation of how these mutualisms behave in ecological and evolutionary time. Most critically, these discoveries have moved us from viewing corals as single-partner mutualisms to a realization that many hosts and symbionts have more than one potential partner.

From the perspective of the microbes, corals represent a diverse array of reactive landscapes (Rowan et al. 1997). Some hosts are far more favorable than others depending on the intrinsic characteristics of host cells, the presence of other symbionts, and the environment in which the host occurs. From the host's perspective, microbial associates offer an array of partnerships ranging from parasitic to strongly mutualistic and intracellular to extracellular.

Having come this far in our understanding, we still have far to go. The discoveries of the last decade have suggested entire areas of inquiry not imagined before the diversity of these partnerships was revealed. Here we close with our personal choices of areas well worth pursuing:

1. How much ecologically significant variation in zooxanthellae remains to be discovered? Are there broadly generalist species or does variation yet to be discovered map finely onto host species or environmental characteristics (e.g., Santos and Coffroth 2003)?

2. Where are the free-living zooxanthellae, and how important is the free-living stage to our understanding of coral-zooxanthella symbiosis? In particular, does spatial variation in community composition of symbionts within hosts reflect to some extent the local community of free-living symbionts?

3. Why do some coral species host a single type of zooxanthella, while other coral species host multiple types? In the latter group, why do some species exhibit within-colony diversity and others only between-colony diversity?

4. Why are the Caribbean and Pacific so different with respect to the relative abundance of different zooxanthellar clades, and do these differences have implications for how these reef systems function (e.g., for their vulnerability to bleaching; LaJeunesse et al. 2003)?

5. What is the history of the diversification of *Symbiodinium*? How old are the groups of zooxanthellae, and was diversification a response to past environmental variation (e.g., in the Caribbean; LaJeunesse et al. 2003)?

6. How do zooxanthellar clades and types within clades differ in their physiology and ecology? Does the host also play a major role in determining the ecology of the resulting partnership, as suggested by some studies (Loya et al. 2001; Brown et al. 2002*b*)?

7. Do different types of zooxanthellae differ in their mutualistic behavior, and are there cheating zooxanthellae?

8. What are the relative roles of the host (through selectivity) and symbiont (through differences in competitive ability, colonization ability, and stress resistance) in modulating zooxanthellar distributions? In coral bleaching, for example, do corals selectively expel nonperforming zooxanthellae and/or do zooxanthellae abandon corals under stress?

9. What is the nature of the biochemical "conversation" (e.g., see Hirsch and McFall-Ngai 2000) that modulates coral-zooxanthella interactions? Can compartmentalization of one or a few zooxanthellae within individual host cells help the host maintain the mutualistic character of the association, as suggested by theory (e.g., Hoeksema and Kummel 2003)? Could the absence of such compartmentalization (e.g., the bacteria in the coral mucous layer) make associations more unstable as mutualisms?

10. What is the scope of diversity for coral-associated bacteria and Archaea, and how is it patterned in space and time?

11. Which of the many bacterial and archaeal associates of corals are true mutualists, and what roles do they play?

12. How important are bacterial and archaeal communities to the health of coral reefs, and are they being disrupted by anthropogenic stress?

Answers to these questions are of intrinsic interest to any student of mutualisms and may also help to ensure that coral reefs survive into the next century (Knowlton 2001).

Acknowledgments

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