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Genetic Diversity of Symbiotic Dinoflagellates in the Genus *Symbiodinium*

The Biological Relevance of *Symbiodinium*

Coral reef ecosystems, one of the most biodiverse habitats in the world, owe their success to obligate mutualistic symbioses involving invertebrates and photosynthetic dinoflagellate symbionts (Hallock 2001). These single-celled algae, commonly referred to as zooxanthellae and predominantly belonging to the genus *Symbiodinium* (Fig. 1), establish relationships with numerous hosts, including representatives of the Protists, Porifera, Cnidaria and Mollusca (Glynn 1996; Lobban et al. 2002; Rowan 1998; Trench 1993). In most cases the algae are intracellular, residing in complex host-derived vacuoles (Colley and Trench 1983; Wakefield and Kempf 2001), but some invertebrates (e.g., bivalves in the genera *Hippopus* and *Tridacna*) harbor their symbionts intercellularly in an elaborate tubular system (Norton et al. 1992). Given the oligotrophic nature of waters surrounding coral reefs, it comes as no surprise that the basis of the symbiosis is nutritional, with the dinoflagellates playing a significant role in host nourishment and physiology (Muscatine and Porter 1977). For example, photosynthetically fixed carbon, typically in the form of glycerol and other simple molecules, can be translocated from the algae at a rate and volume capable of meeting the hosts' respiratory demands (Falkowski et al. 1984; Muscatine 1990; Muscatine et al. 1984). Furthermore, the presence of *Symbiodinium* facilitates the assimilation and conservation of nitrogen (Ambariyanto and Hoegh-Guldberg 1996; Burris 1983; Lewis and Smith 1971), a limiting resource in these ecosystems (Muller-Parker and D'Elia 1997; Muscatine and Porter 1977). For the scleractinian corals, whose skeletons comprise the physical structure of reefs, the presence of algal symbionts also significantly enhances calcification rates (Barnes and Chalker 1990; Pearse and Muscatine 1971).

While vertical ("closed" system) transmission, in which *Symbiodinium* is passed directly from parent to progeny, is common, a vast majority of invertebrates (~85%; Schwarz et al. 2002) produce offspring that must be infected from environmental pools of *Symbiodinium* (horizontal, or "open" system, transmission). Given the more-or-less obligate nature of *Symbiodinium*—invertebrate symbioses (exceptions do exist; for example, some anemones, such as *Aiptasia* sp., can prosper without their symbionts when alternative sources of nutrition are available), it is surprising that horizontal transmission of algae to each new host generation is so prevalent. However, horizontal transmission potentially offers host progeny an opportunity to associate with *Symbiodinium* better adapted to local environmental conditions (van Oppen 2004). This hypothesis assumes functional and, by extension, genetic diversity exists within the genus *Symbiodinium*. But until the advent of culturing techniques, electron microscopy and molecular analyses, there was scant evidence supporting this assumption.

A Historical Prospective on *Symbiodinium* Diversity

In marine environments, intimate relationships (i.e., endosymbioses) between unicellular algae and invertebrates have been recognized since the 19th century. Brandt (1881) coined the term "zooxanthellae" to classify the yellow-brown algae associated with animal cells. Subsequent work by Klebs (1884), Brandt (1885), Chatton (1923) and Hovasse (1924) proposed the inclusion of these algae in the Dinoflagellata, a group

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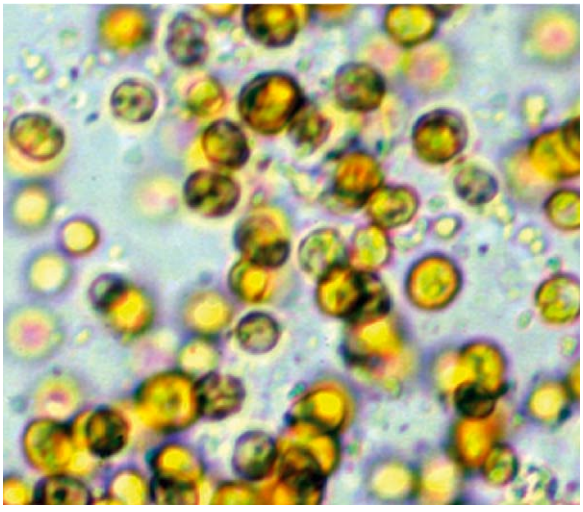

Figure 1. Photomicrograph of *Symbiodinium kawagutii*, isolated from *Montipora verrucosa*. The cells are approximately 10 μm in diameter (photograph originally appeared in Santos and Coffroth 2003a).

of single-cell protists common to aquatic environments. Support for this idea came from the successful culturing of algae from a cnidarian host (Kawaguti 1944). Kawaguti's observation of motile "swarmers", possessing the characteristic morphology of gymnodinoid dinoflagellates, along with similar reports by McLaughlin and Zahl (1959), proved unequivocally the dinoflagellate identity of these algae. Freudenthal (1962) formally described the taxonomy, life cycle and morphology of these dinoflagellates and erected the genus *Symbiodinium* to encompass the symbiotic dinoflagellates associated with a phylogenetically diverse range of invertebrate hosts (see above).

Until the 1970s, all symbiotic dinoflagellates were considered members of a single pandemic species, *Symbiodinium microadriaticum* Freudenthal (Taylor 1974). Experimental evidence, however, including behavioral, infectivity, physiological and ultrastructural, subsequently challenged that view (Schoenberg and Trench 1980a—c). Molecular genetic studies, in particular, have revealed a tremendous level of diversity within the genus *Symbiodinium* (reviewed by Baker 2003; LaJeunesse 2001 and Santos et al. 2001). Below, we focus on the genetic diversity of *Symbiodinium*, examine the distribution of this diversity over time and space, speculate as to its source and discuss future directions for research on these organisms. For consistency, we use the term "type(s)" to represent a member of the genus *Symbiodinium*, which possesses a unique genetic sequence at any particular locus.

Levels of Diversity within *Symbiodinium*

Over the last two decades, the analysis of various molecules has been used to elucidate genetic diversity within *Symbiodinium*. This diversity is evident not only at highly variable loci such as microsatellites, but also at more conserved molecules. The first molecular genetic studies of *Symbiodinium* utilized DNA/DNA hybridization and allozymes (Blank and Huss 1989; Schoenberg and Trench 1980a). In the hybridization studies (Blank and Huss 1989), the degree of binding among DNAs from some *Symbiodinium* isolates differed as much as DNA from algae in different classes. This diversity was placed in an evolutionary context using sequences derived from nuclear small subunit ribosomal DNA (18S-rDNA) (Rowan and Powers 1991a). As before, the sequence variation of the examined *Symbiodinium* isolates was unexpectedly high and comparable to that of orders of free-living dinoflagellates (Rowan and Powers 1992). This discovery led to the development and adoption of a classification scheme for *Symbiodinium* that divides the genus into one of several large groups, or clades (i.e. *Symbiodinium* clades A, B, C [Rowan and Powers 1991a], D [Carlos et al. 1999], E [*S. californium*; LaJeunesse and Trench 2000; LaJeunesse 2001], F [LaJeunesse 2001], G [Pochon et al. 2001] and the newly erected H [Pochon et al. 2004]). Within this context, *Symbiodinium* belonging to clade A clusters into one group with clade E intermediate between clade A and members of the other clades (B/C/D/F/G/H), which form a second, closely related complex (Fig. 2). Among these clades, D/G are basal to B/C/F/H, clades C and H are sister clades, and are closely related to clade F. Phylogenies inferred from chloroplast (Santos et al. 2002; Takashita et al. 2003) and mitochondrial (Takabayashi et al. 2004) genes have provided additional support for these relationships. Within any given clade, additional genetic diversity has been identified (see below), supporting the idea that each clade is comprised of multiple strains or "species" (Rowan 1998) (Table 1).

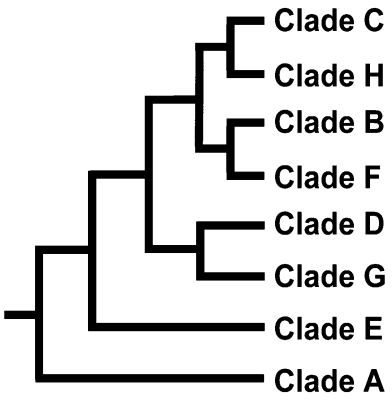

Figure 2. The phylogenetic relationships between the major clades of *Symbiodinium*. The topology is a consensus cladogram synthesized from Pochon et al. (2004) and other references cited in this review. The positioning of *Symbiodinium* clades B, C, F and H varies depending on the method of tree generation and the molecules analyzed. All the clades shown except clades E and H have been identified within scleractinian corals, with clades A, B, C and D being the predominant symbionts within scleractinians. Clade B is the dominant clade within the Caribbean octocorals, while clades A and C are also common in Red Sea and Pacific octocorals, respectively. Clade E has been found in a sea anemone while clades F, G and H are common in foraminifera.

The within-, or intra-, cladal diversity of *Symbiodinium* has primarily been explored using gene sequences of the ribosomal operon (18S, 5.8S and 28S rDNA). Although an appreciable level of variation has been identified with these molecules (Baillie et al. 2000; Baker and Rowan 1997; Barneah et al. 2004; Belda-Baillie et al. 1999; Burnett 2002; Carlos et al. 1999; Darius et al. 1998, 2000; Karako-Lampert et al. 2004; Lee et al. 1995; Loh et al. 2001; McNally et al. 1994; Wilcox 1998), the less-conserved ribosomal internal transcribed spacer (ITS) regions have recently seen widespread use in quantifying *Symbiodinium* diversity within the clades (Diekmann et al. 2003; LaJeunesse 2001, 2002; Santos et al. 2001; van Oppen et al. 2001). Hunter et al. (1997) first showed, by using the entire region (ITS1-5.8S-ITS2), the value of ITS in distinguishing among *Symbiodinium* isolates. LaJeunesse (2001) used sequence variation within the ITS to resolve 6, 4, 2 and 2 groups within clades A, B, C and F, respectively. Within these groups, up to 22 sequence differences have been observed between some clade B isolates and as many as 76 sequence differences can occur among clade A samples (LaJeunesse 2001). Although sequence and length variability of ITS makes it difficult to align this region between the clades, thus limiting its use to within-clade phylogenetics, LaJeunesse (2002) determined that the ITS2 region alone provided resolution of many "types" within *Symbiodinium*. To rapidly assess this variation and to examine how it is distributed among host taxa and geographic location, disparities in electrophoretic mobility due to differences in the primary sequence of ITS2 (i.e. denaturing gradient gel electrophoresis [DGGE]) have been utilized (see below). This approach has allowed sampling of *Symbiodinium* populations from a diverse array of hosts over many regional scales. Such surveys have uncovered over 23 and 35 "types" in cnidarians from Australia and the Caribbean, respectively (LaJeunesse et al. 2003), and extrapolation of these values suggest that 100s of *Symbiodinium* "types", as defined by ITS2 sequences, potentially exist in the world's oceans.

Host—*Symbiodinium* Relationships: Specificity vs. Flexibility

Our current understanding of diversity within *Symbiodinium* and their association with host taxa reveals a complex set of interactions suggesting varying degrees of host—symbiont specificity among partnerships. A basic question is how *Symbiodinium* diversity is distributed among host phyla (cnidarian, mollusk, foraminifera, etc.) and among species. A survey of host—symbiont pairings shows that symbiont types are not randomly distributed among hosts: i.e., members of the same host species *generally* harbor the same *Symbiodinium* taxa or species (Baker et al. 1997; Rowan and Powers 1991a, b; Schoenberg and Trench 1980a, b, c; but see exceptions below). Sampling multiple host taxa of a single reef, LaJeunesse (2002) identified 69 "types" with the majority of host species associating with a single symbiont "type". Although specific *Symbiodinium* "types" associate with particular host species in nearly all instances, the algae naturally found within a given host are a subset of the taxa that can actually infect that host (Coffroth et al. 2001; Colley and Trench 1983;

Table 1. A summary of the recognized *Symbiodinium* clades, with representative cultures and GenBank accession numbers for characteristic sequences for the most frequently used genetic markers.

Clade	Representative cultures	Host origin	18S-rDNA	ITS-rDNA	28S-rDNA	23S-rDNA (chloroplast)	Synonymous nomenclature	References
A	Cx	*Cassiopea xamachana*	AF427442	AF427466	AF427454	AY035406		Santos et al. (2002)
	FLAp#4	*Aiptasia pallida*	AF427441	AF427465	AF427453	AY035404		
B	HIAp	*Aiptasia pulchella*	AF427445	AF360564	AF427457	AY035421		Santos et al. (2002)
	Pk13	*Plexaura kuna*	AF427446	AF360559	AF427458	AY055231		
	Pk702	*Plexaura kuna*	AF427447	AF360575	AF427459	AY035419		
C	Mp	*Mastigias* sp.	AF427449	AF427469	AF427461	AY035424		Santos et al. (2002)
	Ua#31	Unknown anemone[a], Okinawa Japan	AF427452	AF427470	AF427463	AY035425		
D	A024	*Acropora bruegemanni*	AF396624	AF396630	AF396627	AY035429	Same as E of Brown et al. (2000, 2002), Chen et al. (2003), Goodson et al. (2001), Savage et al. (2002a, b), Toller et al. (2001a,b)[b]	Carlos et al. (1999), Santos et al. (2002)
	PSP1-05	*Halicona koremella*	AB016578		AF427464	AY055241		
E	#383	*Anthopleura elegantissima*	AF225965	AF334659				LaJeunesse (2001), LaJeunesse and Trench (2000), Santos et al. (2002), Tchernov et al. (2004)
	CCMP 421	Free-living *Symbiodinium*			AY684264	AY055240		
F	Mv	*Montipora verrucosa*	AF427450	AF360577	AF427462	AY035422		LaJeunesse (2001), Santos et al. (2002)
	135	*Montipora verrucosa*		AF333517				
G	*In hospite*	*Marginopora vertebralis*			AJ291538		Fr6	Pochon et al. (2001)
H	*In hospite*	*Sorites* sp.			AJ621148		Fr1	Pochon et al. (2004)

We have noted cases of synonymous nomenclature in the literature and indicated the accepted nomenclature and references.
[a]Tentatively identified as *Entacmaea quadricolor* (D. Fautin, University of Kansas, pers. comm.).
[b]See discussion in Baker (2003).

Fitt 1984; Kinzie 1974; Kinzie and Chee 1979; Santos et al. 2001; Schoenberg and Trench 1980a, c; Schwarz et al. 1999 and reviewed in Baker 2003). The fact that the subset of the *Symbiodinium* taxa that a host species may harbor can vary in different habitats suggests an interaction between specificity and local environments (LaJeunesse and Trench 2000; Rodriguez-Lanetty et al. 2001; Rowan and Knowlton 1995; Rowan et al. 1997; Secord 1995). In many cases, external environmental conditions promote certain pairings between partners. These include combinations that change with regard to depth, irradiance or temperature gradients, latitude and longitude and host ontogeny. We now turn our attention to the host—symbiont specificity and environmental flexibility that is observed within this group of dinoflagellates in order to examine how *Symbiodinium* genetic diversity is distributed among host phyla.

Specificity in Host—*Symbiodinium* pairings

Specificity in host—*Symbiodinium* pairings is well documented. Isozyme and morphological studies first suggested that the same symbiont was found within a host across broad geographic ranges while symbionts differed between host species (Schoenberg and Trench 1980a, b, c). That conclusion was confirmed with molecular genetic data. In many cases, a *Symbiodinium* "type" associates with only one or a few host species. In other instances, one of the two partners is more flexible so that some host species associate with a number of symbiont "types" and some symbionts "types" are found within a range of hosts (reviewed in Baker 2003). For example, the majority of Caribbean gorgonians harbor specific members of clade B symbionts (B184 [cp-genotype] and B1 [ITS2]; Santos et al. 2003b and LaJeunesse 2002, respectively).

On the Great Barrier Reef (GBR), "types" C1, C3 and C21 (based on ITS2) are common among a wide range of hosts, but LaJeunesse and co-workers (2003) found many host-specific symbiont "types" as well. For example, "type" C17 was found only in *Montipora* spp., C22a in *Turbinaria* spp., C8a in *Stylophora pistillata* at 10 m and C27 in *Pavona varians* at 10 m. Host—symbiont specificities are also seen among phyla; foraminifera harbor mainly *Symbiodinium* "types" within clade F (Pochon et al. 2001) and clade B "type" B1/B184 appears to be specific to cnidarians (Rodriguez-Lanetty 2003).

The life stage at which symbiont specificity is manifested varies among host taxa. As noted above, within cnidarians, *Symbiodinium* are acquired in early development either by direct transmission from the mother to her offspring (vertical or closed) or anew each generation from the environment (horizontal or open). How the specific symbiosis is re-established in the latter case is still an active area of research. Within Caribbean octocorals, initial symbiont acquisition is promiscuous and non-selective, with host polyps taking up multiple symbiont clades (i.e., clades A, B and/or C). The specificity seen in the adult host is then established as the newly settled polyp develops (Coffroth et al. 2001). In contrast, studies of scleractinians with horizontal transmission of symbionts have revealed a different scenario. Newly settled *Acropora* polyps appear to be more selective, only taking up "types" belonging to clades C or D (Little et al. 2004), although other clades are less prevalent on these reefs (LaJeunesse et al. 2004a). Larvae of the solitary coral *Fungia* exhibit a preference for the symbiont "type" of the adult (Rodriguez-Lanetty et al. 2004). Furthermore, at least in some host species, the mode of transmission appears to be correlated with the symbiont type. In the Red Sea (Gulf of Eilat), scleractinians and alcyonaceans with direct transmission generally harbor clade A "types" while those with horizontal transmission tend to harbor clade C symbionts (Barneah et al. 2004; Karako-Lampert et al. 2004). This trend has not been observed in other regions where hosts contain algae within clades B, C or D despite having direct transmission of symbionts. Finally, similar levels of symbiont diversity are found within acroporids that have vertical (*Montipora* spp.) or horizontal (*Acropora* spp.) transmission of symbionts, suggesting that mode of symbiont transmission does not affect the levels of symbiont diversity within some host species (van Oppen 2004). However, LaJeunesse and co-workers (2004a, b) contend that vertical transmission selects for host—symbiont specificity, leading to symbiont diversity and thus a greater overall *Symbiodinium* diversity on the reef.

Although coevolution can be an outcome of, and a driving force in, the evolution of specificity in a symbiosis, it has not been substantiated

among host—*Symbiodinium* systems (LaJeunesse 2002; LaJeunesse et al. 2003; Langer and Lipps 1995; Rowan and Powers 1991a; van Oppen et al. 2001). Genetic markers that can address this question at a finer level of resolution than has been used in the past are now becoming available (e.g., microsatellites and their flanking regions) and evidence in support of it may be forthcoming. If and when coevolution is documented, it will greatly advance our understanding of why particular "types" of *Symbiodinium* are distributed among host taxa as well as identifying one of the forces underlying the generating of *Symbiodinium* diversity over evolutionary time (but see below).

Flexibility in Host—*Symbiodinium* Pairings

Numerous host—*Symbiodinium* pairings are conserved over time (Goulet and Coffroth 2003b) and space (Diekmann et al. 2003; LaJeunesse 2002), suggesting the existence of specific host—symbiont recognition systems. However, in some cases, the pairings may vary in response to the environment of the holobiont. The two most common environmental parameters that correlate with the distribution of specific host—*Symbiodinium* pairings are irradiance and temperature. Rowan and Knowlton (1995) found variation in the symbionts associated with Caribbean *Montastraea* species over a depth/irradiance gradient. Clade A and B symbionts were found in shallow waters (less than 6 m) while clade C symbionts were restricted to deeper depths. In a study of *Symbiodinium* diversity within scleractinians on the GBR, LaJeunesse et al. (2003) identified 9 host species where the symbiont type varied between deep (10 m) and shallow (<3 m) sites, including *Stylophora pistillata*, which at depths of less than 3 m harbored C1, while those colonies collected at 10 m harbored C27. In fact, *Symbiodinium* genetic identity may influence the vertical distribution of a host species, as illustrated by *Pocillopora verrucosa* and *Pavona gigantea* on eastern Pacific reefs. In this case, shallow (0—6 m) reef communities are dominated by *P. verrucosa* while *P. gigantea* dominates at deeper (6—14 m) depths. *Symbiodinium* of "type" D1 (based on ITS2) is exclusive to *P. verrucosa* while C1c occurs in *P. gigantea* (Iglesias-Prieto et al. 2004), suggesting that symbiont identity plays a role in this distribution. The results of photosynthetic measurements and transplant experiments supported this hypothesis, with *P. verrucosa* D1 and *P. gigantea* C1c exhibiting "sun-loving" and "shade-adapted" qualities, respectively (Iglesias-Prieto et al. 2004). Other studies examining the distribution of symbionts along the surface of a single host colony corroborate the idea that symbiont distribution can respond to changes in irradiance levels (Rowan et al. 1997). Within single colonies of *Montastraea annularis* and *M. faveolata*, clades A and B symbionts resided in areas of the host that were under highest irradiance while clade C occurred in shaded areas (Rowan et al. 1997). van Oppen et al. (2001) also observed within colony structuring of symbiont populations consistent with differential light intensity. In their study, "type" C2 (based on ITS1) was found in areas of *Acropora tenuis* exposed to the sun, while "type" C1 was found in the shaded portions of the same colony.

Ulstrup and van Oppen (2003) have also reported intracolony variation in *Acropora* spp. In this latter study, symbiont location within an *A. tenuis* individual varied at some sites, while in other locations, this trend was not observed. Regional and latitudinal differences in symbiont availability as well as differential responses to environmental factors were given as a possible explanation. In addition to irradiance, temperature differences were proposed to explain the diversity in host—*Symbiodinium* pairings (see below).

In contrast, in a survey of 35 Caribbean octocoral species, Goulet and Coffroth (2003a, b; 2004) did not find any variation among *Symbiodinium* clade B "types" within a colony or over a range of depths. In these studies, portions of colonies were transplanted to a range of environments with variable light levels and the symbiont communities were monitored over a 20-month period. This analysis examined the symbiont complement using DNA fingerprinting, a sensitive method for detecting genetic variation. Surprisingly, the symbiont populations within individual host colonies did not change during this time span (Goulet and Coffroth 2003b). Few studies have monitored symbiont diversity temporally over normal environmental fluctuations. In those that have, most have not detected any variation over time (Goulet and Coffroth 2003b; Hannes and Coffroth unpubl. data; Rodriguez-Lanetty et al. 2003).

Flexibility in the *Symbiodinium* "type" harbored is also seen at the level of different host individuals, with variation in symbiont type observed over large, as well as small, biogeographic regions. Latitudinal variation in host—symbiont pairings has been observed for a number of host species. For example, hosts found in tropical northeastern Australian tended to harbor symbionts within clade C while host conspecifics in temperate latitudes in southeastern Australia harbored symbionts in clade B (Rodriguez-Lanetty et al. 2001). Similarly, LaJeunesse et al. (2004a) observed dramatic differences in dominance of "type" C3 h, with this particular symbiont being absent or rare on high latitude reefs but having high prevalence on a mid-latitude reef. The latitudinal distribution of specific host—*Symbiodinium* pairings such as these have been attributed to temperature and other environmental clines that occur along these transects. Variation in host—symbiont pairings has also been documented at finer genetic levels within some *Symbiodinium* "types" (Santos and Coffroth 2003a). Along a ~450 km transect in the Bahamas, Santos et al. (2003b) sampled 575 individuals of *Pseudopterogorgia elisabethae* and identified 23 unique "types" (based on allele size variation at two microsatellite loci) of *Symbiodinium* B1/B184 in association with these colonies. Added to this, striking population structure was observed since most of these *Symbiodinium* clade B "types" were either unique to a reef or found infrequently on other reefs (Santos et al. 2003b).

The correlation of environmental parameters with symbiont distribution suggests that physiological differences between the symbiont "types" may generate the variability in host—symbiont pairing. Characterizing the physiological response of the various *Symbiodinium* taxa to different environmental parameters is in its infancy, but it is clear that physiological variation exists and is responsible for at least some of the variation in host—symbiont pairings. *Symbiodinium* "types" vary in their physiological response to environmental changes (Iglesias-Prieto and Trench 1997; Kinzie et al. 2001; Rowan et al. 1997; Warner et al. 1996) as well as their thermal tolerance (Bhagooli and Hidaka 2004; Kinzie et al. 2001; Perez et al. 2001; Rowan 2004). *Montipora digitata*, which is resistant to bleaching, harbors "type" C15 (based on ITS2), while other *Montipora* that bleach more readily harbor other "types" of clade C (LaJeunesse et al. 2003). Several researchers have reported on the thermal tolerance of clade D *Symbiodinium* (Baker et al. 2004; Chen et al. 2003; Fabricius et al. 2004; Glynn et al. 2001; Rowan 2004). Those studies report that symbionts in clade D dominated the symbioses at sites with routinely elevated temperatures (Fabricius et al. 2004) and on reefs that had previously experienced temperature-related bleaching (Baker et al. 2004; Glynn et al. 2001). Physiological measurements confirm the thermal tolerance of clade D that associates with *P. damicornis* and *P. verrucosa* (Rowan 2004). Although there have been attempts to broadly assign the various clades to functional groups such as "sun loving", "shade adapted" or "stress tolerant", studies have demonstrated that clade level identity does not always correlate with physiological function (Kinzie et al. 2001; LaJeunesse et al. 2003; Savage et al. 2002a; Tchernov et al. 2004). Tchernov et al. (2004) found that when exposed to elevated temperatures, algae from different clades responded similarly while isolates from the same clade, including most closely related sister "types", exhibited significantly different responses (Tchernov et al. 2004). Besides contributing to an area of *Symbiodinium* research in which data are sorely lacking, the work of Tchernov and co-workers demonstrates that in the absence of explicit data, the assignment of specific physiological attributes to particular *Symbiodinium* taxa should be made using a measure of caution.

Sources of *Symbiodinium* Genetic Diversity

Diversity in *Symbiodinium* is reminiscent of an onion; as new genetic markers are utilized, a novel level of variation is discovered below those that were previously recognized (Fig. 3). This idea is exemplified by *Symbiodinium* clade B found in the Caribbean. Although *Symbiodinium* B1/B184 represents the most prevalent symbiotic dinoflagellate in the Caribbean (LaJeunesse 2002) and could be considered a "generalist", sequence variation in microsatellite flanking regions has identified at least five unique "types" within this group and found them associating specifically with particular hosts (Santos et al. 2004). This situation raises questions regarding the processes creating this diversity and how such a high level of variation is maintained in natural populations. Below, we

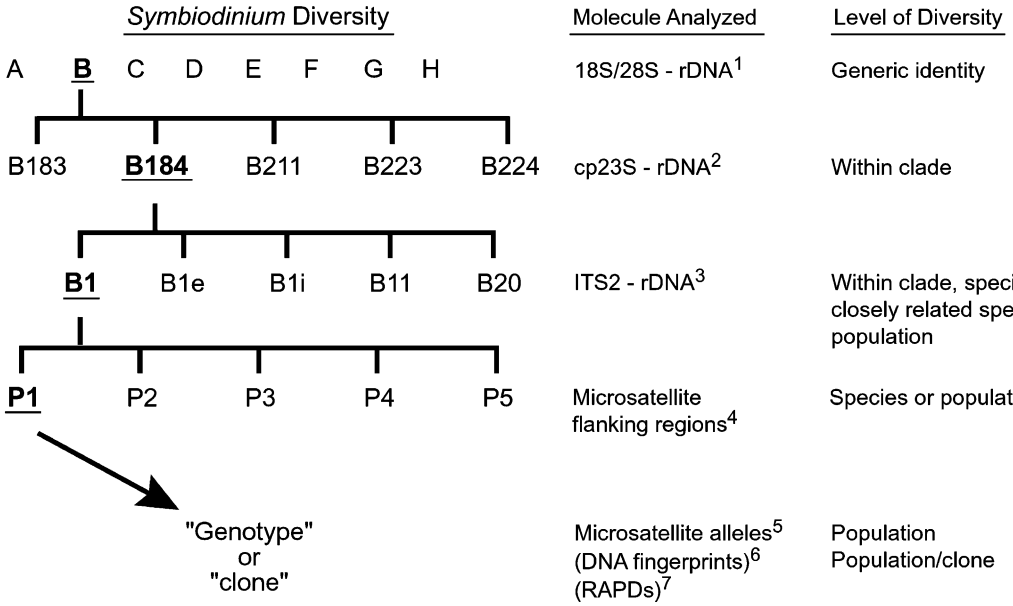

Figure 3. A schematic representation of the multiple levels of diversity found within *Symbiodinium*. Understanding the diversity of zooxanthella populations has important ramifications for studying the symbiosis and understanding how the symbiosis responds to environmental changes. This figure illustrates the levels of diversity that have been identified in *Symbiodinium* and the methods used to quantify this diversity, using particular members of clade B (underlined in the figure) as an example. Analysis of *Symbiodinium* using relatively conserved molecules such as the 18S- and 28S-rDNA distinguishes the broad subgeneric groups or clades. Sequence analysis of these markers is useful in distinguishing *Symbiodinium* from other dinoflagellate groups and placing the taxa in a phylogenetic context (see Fig. 2). As noted in the text, although these clades are phylogenetically distinct, adaptive radiation has lead to much diversity within each group so that "clade-specific" characteristics can be identified in only a few cases (i.e., thermal/stress tolerance appears to be a trait of Clade D and C17 appears resistant to bleaching). This within-clade variation is detected using more variable molecules such as chloroplast 23S-rDNA and the ITS regions. Primers developed to study variation in these molecules can be applied across clades. These markers can be used to compare closely related symbiont taxa and have been used to examine finer scale pattern within and among host species and across biogeographic regions. Within the ITS "types", additional diversity is recognized through the sequence analysis of microsatellite flanking regions. These regions are variable and tend to be unique to a subset of a given ITS2 "type". For example, analysis of microsatellite flanking regions within symbionts harbored by the host family Gorgonicea revealed the existence of five phylotypes (Santos et al. 2004). Using allelic variation at these microsatellite loci, symbiont diversity at the population level was then characterized within one of these host species (*Pseudopterogorgia elisabethae*). Between 8 and 10 alleles per loci were detected, and when combined, corresponded to 23 unique genotypes (Santos et al. 2003b). Other techniques, such as DNA fingerprinting, have been used to further resolve the genetic diversity at the level of the individual symbiont clones. Representative studies that used these molecules include: [1]Baker et al. (1997), McNally et al. (1994), Rowan and Powers (1991a,b), Wilcox (1998). [2]Santos et al. (2002). [3]LaJeunesse (2001). [4]Santos et al. (2004), [5]Santos et al. (2003b). [6]Goulet and Coffroth (2003a). [7]Belda-Baillie et al. (1999).

discuss two hypotheses proposed in the literature to explain these phenomena.

Symbiodinium phylogenies inferred from different molecules typically have a similar appearance; each of the major clades is separated from each other by a relatively long branch and is punctuated by a cluster of highly similar (and thus, closely related) sequence types. LaJeunesse (2005) proposed that this pattern is consistent with the occurrence of *Symbiodinium* radiations over the last 5—6 million years. Furthermore, his interpretations suggest that corresponding genetic bottlenecks in diversity have also occurred through

geological time in response to major climate shifts. Rapid diversification through host specialization and allopatric differentiation proceeds following episodic radiations of a few opportunistic (generalist) "types". Repeated iterations of this process eventually give rise to the numerous "types" with the distinct host, geographic and environmental attributes identified today (LaJeunesse 2005). Interestingly, based on biogeographic and paleotological evidence (i.e. molecular clock estimates), it appears that the progenitors of the *Symbiodinium* clade C radiation spread to numerous host taxa in response to climate changes occurring at the Miocene/Pliocene boundary, corresponding to a period of major global ecological change (reviewed by LaJeunesse 2005).

Diversification of *Symbiodinium* in coral reef communities by the above process will lead to an increase in the level of genetic diversity of the genus *Symbiodinium* as a whole. But how is this variation maintained over space, as well as time, once it is created? By applying the idea that the "host is a habitat" from the viewpoint of *Symbiodinium* (and other microbes) (Goulet and Coffroth 1997; Knowlton and Rohwer 2003; LaJeunesse et al. 2004b), one avenue by which genetic diversity is maintained in these algae becomes apparent. For example, as discussed in the Introduction, *Symbiodinium* typically resides within the host's cells. It is reasonable to assume that subtle biochemical differences exist between identical cell types in congener host species. Thus, each can be considered distinct niches for unique *Symbiodinium* to occupy. This appears to be the case for *Pseudopterogorgia bipinnata* and *P. elisabethae*, Caribbean gorgonians that acquire their symbionts horizontally and occur sympatrically, yet harbor distinct *Symbiodinium* "types" (Santos et al. 2004). Another layer can be added to this: morphological and structural complexity within a given host creates additional niches, which other "types" of *Symbiodinium* can exploit. An example of this is *Montastraea* sp., in which members of *Symbiodinium* clades C and A predominately occur on the sides and top, respectively, of the same colonies (Rowan et al. 1997). A similar pattern is seen in *A. tenuis*, where variants of clade C are found in light and shaded portions of the same colony (van Oppen et al. 2001). Lastly, the environment in which a host occurs will also define the niche particular symbionts can occupy. For members of the *M. annularis* complex, colonies found at inshore habitats hosted members of clade D while colonies of the same species at offshore sites harbored *Symbiodinium* clade C (Toller et al. 2001a). Thus, the host is by no means a static entity and should be considered a strong selective force in the creation and maintenance of genetic variation in *Symbiodinium*.

Directions for Future Research within Cnidarian—*Symbiodinium* symbioses

We have presented a summary of the extensive genetic diversity within the genus *Symbiodinium* that has been revealed over the past decade through the application of molecular techniques. However, one vexing question remains unresolved: which molecule(s) differentiates a species within this genus ? The "species problem" is also relative to studies of their hosts and our inability to discriminate species for either partner is particularly troublesome. For instance, if we cannot make a distinction between species, questions such as "How does the diversity within *Symbiodinium* compare with that of the host?" are difficult or impossible to answer. Pochon and co-workers (2001) presented an in-depth view of symbiont diversity within the soritid foraminifera, but this level of detail is lacking for other hosts. The symbiont "types" that we label "Clade B", or chloroplast 23S-rDNA type B184, or ITS2 type B1, are not equivalent (Fig. 3). Within the B184/B1 group, analysis of microsatellites and the regions flanking the repeats reveal high levels of variation, but it is unclear whether this variation characterizes the population genetics of a single species or variation between independently evolving taxa (Santos et al. 2004). Thus, what is a "species" in this enigmatic group? Until the "species" question is resolved for *Symbiodinium*, it will remain difficult to compare levels of diversity and fully address questions of host specificity and coevolution.

As we make great strides in understanding this complex system, each discovery leads to new questions. For example, one area that is important to understanding the system, but is as yet relatively unstudied, is the availability and nature of free-living *Symbiodinium.* Viable *Symbiodinium* are routinely released into the environment by a range of cnidarians (Hoegh-Guldberg and Smith 1989; Hoegh-Guldberg

et al. 1987; Steele 1977; Stimson and Kinzie 1991) and predators feeding on the host (Augustine and Muller-Parker 1998). In addition, *Symbiodinum* have been isolated from the water column as well as coral sands (Carlos et al. 1999; Chang 1983; Gou et al. 2003; Loeblich and Sherley 1979). Although these isolates fall within the genus *Symbiodinium*, work is still required to establish that these dinoflagellates are able to colonize a host and establish a viable symbiosis. Other questions also need to be considered, including how large are these populations and whether their physiology differs relative to when they are sequestered within a hosts' tissues. Identifying free-living *Symbiodinium* and characterizing their potential as symbionts is a necessary first step in determining the environmental pool of symbionts that are available for initial colonization or recolonization after a disturbance.

Coral bleaching and mortality has increased steadily over the last two decades (Gardner et al. 2003; Hoegh-Guldberg 1999; Hughes et al. 2003; Pandolfi et al. 2003; Wilkinson 2000) and many argue that corals reefs are now threatened on a global scale. Variation in symbiont diversity, as seen over depth and geographic range, may offer important insights to understanding the tolerance and plasticity of coral species to natural and anthropogenic perturbations. Does *Symbiodinium* diversity provide these symbioses with mechanisms to survive these changes? It has been proposed that bleaching is an adaptive mechanism allowing hosts to obtain symbionts better suited to the changing environment (Buddemeier and Fautin 1993; Buddemeier et al. 2004; Rowan and Powers 1991a). Tests of this hypothesis offer varying levels of support. Studies have shown that corals recovering from bleaching can harbor *Symbiodinium* that differ physiologically from the pre-bleaching populations (Baker 2001; Baker et al. 2004; Toller et al. 2001b), with the source of symbionts arising from residual populations within the host or exogenously from environmental pools (Lewis and Coffroth 2004). However, although there is evidence suggesting corals have the potential to shuffle or switch partners, there is little real indication that this occurs in an unmanipulated setting (see LaJeunesse et al. 2004a, 2005). In fact, most studies find that in the field, host—*Symbiodinium* pairings are stable (Goulet and Coffroth 2004; LaJeunesse et al. 2005). Glynn et al. (2001) documented that during a bleaching episode in the Eastern Pacific, those corals with a C "type" symbiont suffered bleaching, while those corals with the "more stress tolerant" D "type" symbiont did not bleach. In the Persian Gulf/Indian Ocean, Baker et al. (2004) report an increase in the number of corals with "type" D symbionts 2—4 years following a bleaching event. Although these studies suggest important physiological variation among *Symbiodinium* "types", it is not clear that individual corals "switched" or "shuffled" symbionts. In studies where individual corals are followed, there is a fundamental need for evidence demonstrating that the "new" symbiosis is stable and that these particular corals survive and proliferate in the future before bleaching can be truly labeled "adaptive". Additional questions also need to be addressed: Will those corals harboring new dominant symbionts following a bleaching event be able to sustain reefs on a global scale? If not, what impact will this have on the continual existence of reefs? For holobionts that are selected against, will their loss be temporary or permanent, and how will this affect overall coral reef diversity? Quantifying and characterizing *Symbiodinium* diversity both at the genetic and physiological levels will be important in predicting holobiont responses to the forecasted global warming trend as well as prescribing remedial action that should occur.

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