

The evolutionary history of *Symbiodinium* and scleractinian hosts—Symbiosis, diversity, and the effect of climate change

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Abstract

Marine invertebrates representing at least five phyla are symbiotic with dinoflagellates from the genus *Symbiodinium*. This group of single-celled protists was once considered to be a single pandemic species, *Symbiodinium microadriaticum*. Molecular investigations over the past 25 years have revealed, however, that *Symbiodinium* is a diverse group of organisms with at least eight (A–H) divergent clades that in turn contain multiple molecular subclade types. The diversity within this genus may subsequently determine the response of corals to normal and stressful conditions, leading to the proposal that the symbiosis may impart unusually rapid adaptation to environmental change by the metazoan host. These questions have added importance due to the critical challenges that corals and the reefs they build face as a consequence of current rapid climate change. This review outlines our current understanding of the diverse genus *Symbiodinium* and explores the ability of this genus and its symbioses to adapt to rapid environmental change.

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Introduction

Dinoflagellates are alveolate protists, comprising around 2000 extant species with a monophyletic origin closely related to apicomplexans and ciliates (Lenaers et al., 1989, 1991; Zhang et al., 2000; Coates, 2002; Baldauf, 2003). Dinoflagellates share several unique features in their genome structure. The DNA is permanently condensed in the nucleus (Rizzo, 1991), which contains an atypical histone complement that does not support a nucleosome structure (Herzog and

Soyer-Gobillard, 1981; Rizzo, 1981, 1991). Dinoflagellate DNA displays features of archaeobacterial-like folded rRNA (Rae, 1970; Herzog and Maroteaux, 1986) and suggests evidence for the use of RNA to maintain DNA packaging as seen in prokaryotes (Soyer-Gobillard and Herzog, 1985). In addition, dinoflagellates are the only eukaryotes that contain 5-hydroxymethyluracil as well as thymidine within the genome (Rae, 1970).

The dinoflagellates that are symbiotic with many invertebrate hosts are phototrophic unicellular protists and form mutualistic relationships with a wide variety of marine invertebrates and protists, in both tropical and temperate waters (Trench, 1979, 1997; Banaszak et al., 1993). There are currently eight genera in four or five

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orders with symbiotic dinoflagellate representatives. These have been referred to by the non-taxonomic term ‘zooxanthellae’, which describes coccoid yellow-brown endosymbionts of both dinoflagellate and diatom origin (Trench, 1979, 1997; Blank and Trench, 1986; Trench and Blank, 1987; Banaszak et al., 1993). *Symbiodinium* is the most commonly found symbiotic dinoflagellate in symbiosis with marine invertebrates and protists including Cnidaria, Platyhelminths, Mollusca, Porifera, and Foraminiferans (Trench, 1979; Pawlowski et al., 2001). *Symbiodinium* isolated from the jellyfish *Cassiopeia xamachana* was first characterised by Freudenthal (1962), and is now placed in the family Symbiodiniaceae, order Suessiales, and class Dinophyceae (Freudenthal, 1962; Taylor, 1974; Fensome et al., 1993; Steidinger and Tangen, 1997).

Morphology and life cycle of *Symbiodinium*

Symbiodinium can interchange between a vegetative cyst and a motile zoospore (Fig. 1; Freudenthal, 1962;

Schoenberg and Trench, 1980a). The vegetative cyst ranges in size from 5 to 15 μm and contains a typical eukaryotic complement of cytosolic compartments, with the addition of peripheral irregular disk-shaped chloroplasts containing multilobed parallel thylakoid membranes, assimilation products, starch grains, and one or two vacuoles (Freudenthal, 1962; Schoenberg and Trench, 1980a; Blank, 1987). The haploid vegetative cyst (Santos and Coffroth, 2003) is the dominant form when endosymbiotic with invertebrates and has been shown to be under host control in at least one symbiosis (Octocoral, *Simularia lochmodes*; Mitsuru et al., 2000). Control of the cell division of *Symbiodinium* appears to be exerted by arresting the algae in the cell-dividing non-motile stage via chemical signalling (Koike et al., 2004), with population density maintained by host-controlled dinoflagellate division, dinoflagellate cell digestion, and expulsion (Trench, 1987; Baghdasarian and Muscatine, 2000).

The vegetative cyst of *Symbiodinium* can follow one of several developmental pathways. Asexual reproduction producing two or three (in culture) daughter cells can

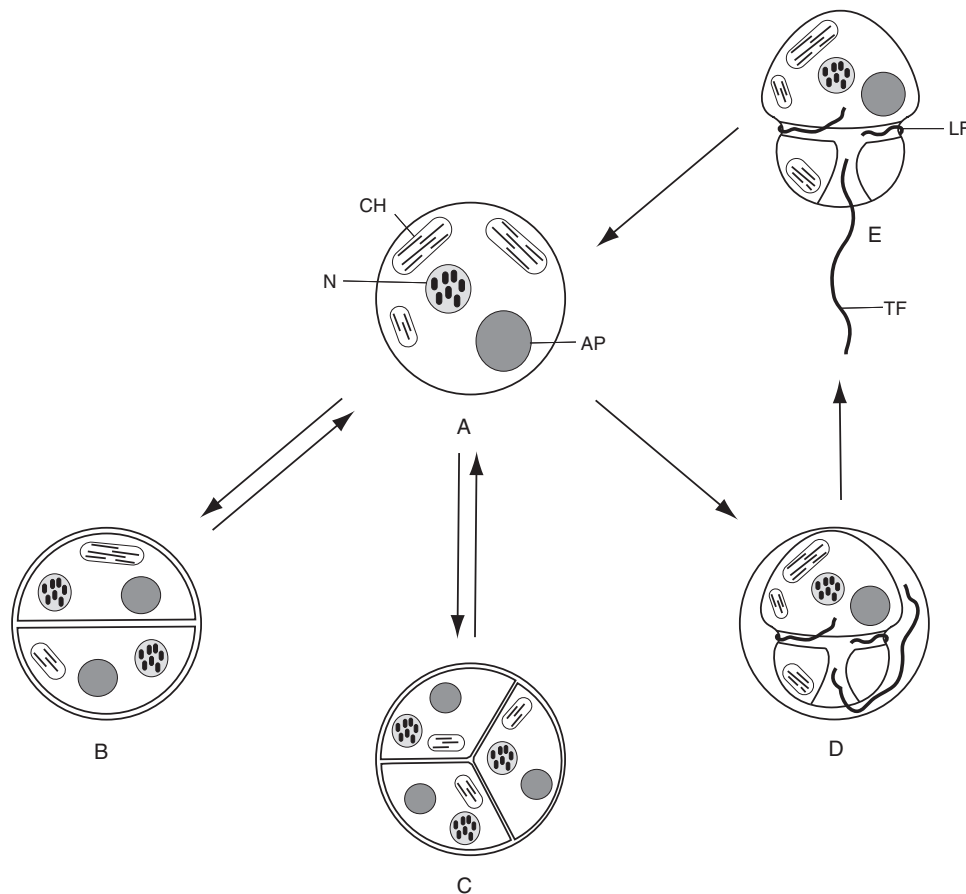


Fig. 1. Life cycle of *Symbiodinium*. (A) vegetative cyst, (B) dividing vegetative cyst producing two daughter cells, (C) dividing vegetative cyst producing three daughter cells, (D) developing zoospore, and (E) zoospore. CH: chloroplast; N: nucleus; AP: accumulation product; LF: longitudinal flagella; TF: transverse flagella. Adapted from Schoenberg and Trench (1980a) and Freudenthal (1962).

occur. Alternatively, *Symbiodinium* cysts can also form singular or tetrads of motile zoospore with the addition of thecal plates and a transverse and longitudinal flagellum (Freudenthal, 1962; Schoenberg and Trench, 1980a; Fitt and Trench, 1983). The motile zoospore is active under illumination (Freudenthal, 1962) and motility is regulated in a diel cycle between periods of darkness and light (Yacobovitch et al., 2003). The development of gametes has been proposed but sexual reproduction and identification of gamete formation has not been confirmed (Freudenthal, 1962; Trench, 1997). However, there is growing molecular evidence that indicates that sexual recombination occurs in the genus (Baillie et al., 1998, 2000a; Belda-Baillie et al., 1999; LaJeunesse, 2001; Goulet and Coffroth, 2003a; Santos et al., 2003a), most likely during the free-living stage (Trench, 1997). Free-living *Symbiodinium* sp. have recently been isolated from the environment (Carlos et al., 1999; Gou et al., 2003), completing the identification of free-living representatives in all genera of symbiotic dinoflagellates (Rowan, 1998).

***Symbiodinium*: the diverse yet non-descript brown cell**

All members of the genus *Symbiodinium* have the same characteristic dominant phenotype *in symbio*, which under the light microscope appears as a brown coccoid cell of 5–15 µm diameter. This lack of morphological variation led early researchers to conclude that all symbiotic algae living with marine invertebrates belong to a single pandemic species, *Symbiodinium microadriaticum* (Freudenthal, 1962; Taylor, 1974). In contrast to these early conclusions, later work has shown that the dinoflagellate symbionts of invertebrates are highly diverse organisms. Investigation into the composition and ultrastructure of *Symbiodinium* has revealed a number of significant differences in characters that include chromosome number (Blank and Trench, 1985a; Trench and Blank, 1987; Blank and Huss, 1989), cell size in the vegetative phase (LaJeunesse, 2001), chloroplast number, size and arrangement (Blank and Trench, 1985b; LaJeunesse, 2001), mycosporine-like amino acid production (MAA) (Banaszak et al., 2000), isoenzyme profiles (Schoenberg and Trench, 1980b; Blank and Trench, 1985a), fatty acids and sterol composition (Blank and Trench, 1985a), and also photoadaptive physiology (Blank and Trench, 1985b; Iglesias-Prieto and Trench, 1994) and host infectivity (LaJeunesse, 2001). Despite these advances in characterising *Symbiodinium*, further complication arises due to the observed phenotypic plasticity of *Symbiodinium*. Morphology is affected by the culture phase, the nutrient exposure *in hospite*, the concentration of lipids

and starch, and also irradiance, which can affect the cell size, and chloroplast size (Rowan and Powers, 1991a; Muller-Parker et al., 1996; LaJeunesse, 2001). In addition, culturing of *Symbiodinium* from the host generally leads to different strains dominating the culture medium representing cryptic types (at very low levels) within the host, or a surface contaminant, hindering the identification of the dominant and ecologically important symbiont (Trench, 1979, 1997; Goulet and Coffroth, 1997; Santos et al., 2001).

Despite these complications, 11 species have been allocated to the genus including *S. microadriaticum*, *S. pilosum*, *S. kawagutii*, *S. goreau*, *S. californium*, *S. corculorum*, *S. meandrinae*, *S. pulchrorum*, *S. bermudense*, *S. cariborum*, *S. muscatinei*, and two *Gymnodinium* species *G. linucheae* and *G. varians* that have been identified as *Symbiodinium* (Table 1; reviewed in Baker, 2003). Given the diversity that appears to be characteristic of the *Symbiodinium* group, it is apparent that these few species that have been allocated severely under-represent the true diversity within *Symbiodinium*.

The mutualistic symbiosis between *Symbiodinium* and its invertebrate hosts

Symbiodinium lives within the cells of at least five phyla including the Cnidaria (corals, jellyfish, and anemone), Mollusca (snails and clams), Platyhelminthes (flatworms), Porifera (sponges), and Protista (e.g. single-celled ciliates, Foraminifera; Table 1). The focus of this review however, will examine the symbiotic association between *Symbiodinium* and the Scleractinia (stony corals), with occasional reference to the symbiosis with other marine hosts.

In most cases, *Symbiodinium* are located in the endodermal cells of the animal host that line the gastrovascular cavity, with the exception of bivalve clams where they are extracellular although located within specialised channels within the mantle (Lewin and Cheng, 1989; Norton et al., 1992; Farmer et al., 2001). The symbiosis between invertebrates and dinoflagellates appears to be quite ancient. Most evidence points toward the mutualistic symbiosis between coral and symbiotic dinoflagellates originating in the mid-Triassic (Trench, 1997). The successful association achieved by nutrient cycling accounts for the growth and development of coral reefs in the nutrient-poor ocean environment (Muscatine and Porter, 1977). The photosynthetic algae pass 95% of their photosynthetic products to their animal host primarily as glycerol but also in the form of peptides, amino acids, sugars, and complex carbohydrates (Muscatine, 1967; Trench, 1979; Muscatine et al., 1984; Sutton and Hoegh-Guldberg, 1990; Swanson and Hoegh-Guldberg, 1998;

Table 1. *Symbiodinium* clades, species designation, and associated marine invertebrate hosts

<i>Symbiodinium</i> clades	Species designation	Clade reference	Hosts	
			Phylum	Order
A	<i>S. pilosum</i>	Rowan and Powers (1991b)	Cnidaria	Scleractinia
	<i>S. corculorum</i>			Actiniaria
	<i>S. meandrinae</i>			Zoanthidea
	<i>S. microadriaticum</i>			Alcyonacea
	<i>G. linucheae</i>			Rhizostomea
	<i>S. cariborum</i>			Hydroida
B	<i>S. muscatinei</i>	Rowan and Powers (1991b)	Cnidaria	Scleractinia
	<i>S. bermudense</i>			Actiniaria
	<i>S. pulchorum</i>			Zoanthidea
				Alcyonacea
				Rhizostomea
				Hydroida
C	<i>S. goreau</i>	Rowan and Powers (1991b)	Cnidaria	Scleractinia
				Actiniaria
				Zoanthidea
				Corallimorpharia
				Alcyonacea
				Rhizostomea
D		Carlos et al. (1999)	Cnidaria	Scleractinia
				Actiniaria
				Zoanthidea
				Alcyonacea
				Veneroida
				Haplosclerida
E	<i>G. varians</i>	LaJeunesse and Trench (2000)	Cnidaria	Actiniaria
	<i>S. californium</i>			Veneroida
F	<i>S. kawagutii</i>	LaJeunesse (2001)	Cnidaria	Scleractinia
			Protozoa	Actiniaria
G		Pawlowski et al. (2001)	Cnidaria	Scleractinia
			Protozoa	Alcyonacea
H		Pochon et al. (2004)	Protozoa	Foramniferida
			Protozoa	Foramniferida

Hoegh-Guldberg, 2004). In addition, energy from the symbiotic algae assists in calcification and formation of the reef framework (Pearse and Muscatine, 1971; Hoegh-Guldberg, 2004). In return, the algae receive inorganic nutrients from host metabolism, including ammonium and phosphate. The host also provides an environment which, although regulated by the host and its growth form, is presumably ideal for *Symbiodinium* (Pearse and Muscatine, 1971).

One of the most significant steps in the formation of endosymbiotic mutualism is the entry of one cell into the cytoplasm of another without triggering the complex series of self-non-self recognition steps that prevent organisms succumbing to invading organisms. Following recognition between the endosymbiotic partners are processes that avoid the degradation of endosymbiotic dinoflagellate cells during normal cellular metabolism and maintenance. Recognition may occur initially at the stage of cell–cell interaction via cell-surface proteins and/or after ingestion of the dinoflagellate cells by the host (Trench, 1979; Reynolds et al., 2000). While much remains to be discovered regarding the mechanisms involved in the symbiosis between *Symbiodinium* and invertebrates, there appears to be elements of the type of recognition system seen between partners in related plant–animal symbioses, such as that between Hydra and the green alga *Chlorella* (Trench, 1979). The sym32 protein was identified as a possible recognition protein between *Symbiodinium* and sea anemones (Reynolds et al., 2000), not unlike similar proteins found in the Hydra symbiosis. Sym32 appears to reside along the edge of gastrodermal cells in aposymbiotic hosts. The sym32 protein is then subsequently engulfed by infecting symbionts (Reynolds et al., 2000). The accumulation of the sym32 protein in *Symbiodinium* prevents degradation of the phagosome by preventing the fusion with lysosomes (Trench, 1979; Schwarz and Weis, 2003). Another study showed that live dinoflagellate cells exclude the vesicle trafficking protein ApRab7 from the phagosome, which performs a role in signalling the phagosome for digestion (Chen et al., 2003). In contrast, dead or photosynthetically inactive dinoflagellate cells accumulate ApRab7 in the residing phagosome signalling fusion with lysosomes leading to cellular digestion.

Symbiont acquisition in coral symbiosis

Reef-building corals reproduce by either broadcast spawning gametes or by brooding their planulae (larvae). Gametes are released by the parent colony into the ocean environment for external fertilisation in a synchronous event in broadcast spawning, which is also the dominant reproductive mechanism of corals (Harrison et al., 1984; Harrison and Wallace, 1990).

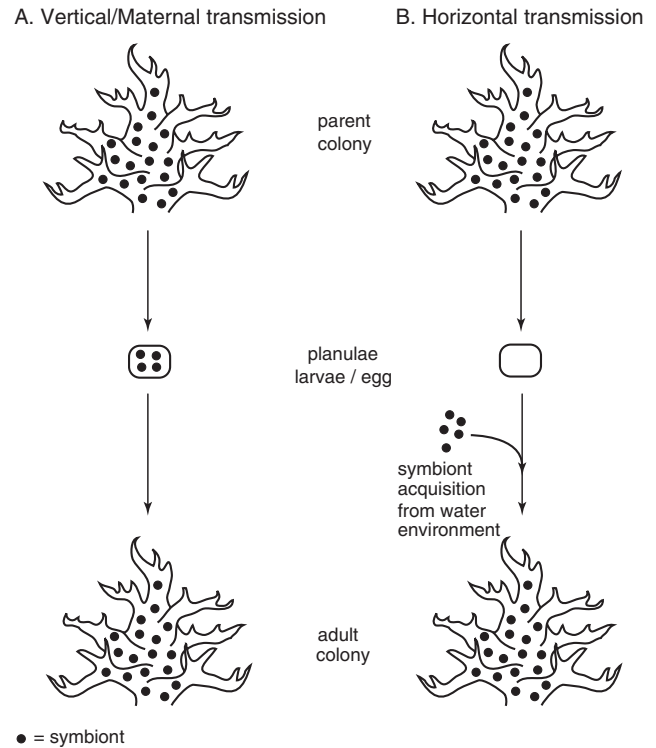


Fig. 2. The maternal/vertical and horizontal symbiont acquisition strategies employed by coral.

Brooding corals, on the other hand, have internal fertilisation and development of the planula larvae before release into the water column (Harrison and Wallace, 1990). These larvae tend to be better developed and larger when released. These contrasting reproductive and developmental systems have implications for the dispersal of coral species (Babcock and Heyward, 1986), with shorter external developmental periods translating into shorter dispersal distances. The developmental strategies are also correlated with how *Symbiodinium* is acquired by the next generation of symbiotic corals (Fig. 2). *Symbiodinium* may be acquired by vertical or maternal transmission, in which dinoflagellate cells are present in the egg or brooded planulae larvae prior to release from the parent. Alternatively, *Symbiodinium* may be horizontally transmitted, with the eggs, zygotes, or brooded larvae of the coral being released without symbionts (aposymbiotic), which are acquired anew each generation from the surrounding environment.

The eukaryotic nuclear rDNA

The development and approach of molecular methods has been critical to the current day understanding of the phylogeny of *Symbiodinium*. Eukaryotic nuclear rDNA consists of the 18S, 5.8S, and 28S rRNA genes, which are separated by the Internal Transcribed Spacers, ITS1

and ITS2. External Transcribed Spacers (ETS) flank these genes to form rDNA operons that exist in tandem arrays separated by non-transcribed spacer regions (NTS; Fig. 3; Long and Dawid, 1980; Maroteaux et al., 1985). The 18S and 28S rDNAs are divided into regions that are under varying levels of evolutionary pressure due to secondary structural constraints, and consequently contain conserved and variable domains (Gerbi, 1986; Hillis and Dixon, 1991; Muse, 1995). This means that different regions of the rDNA can be used for comparing closely related or distantly related taxa, due to variation in rates of evolution across the array (Hassouna et al., 1984; Michot et al., 1984; Sogin et al., 1986; Lenaers et al., 1989, 1991; Srivastava and Schlessinger, 1991).

The primary 45S transcript contains the rRNA, the ETS, and the ITS regions (Long and Dawid, 1980; Arnheim, 1983). A series of nucleolytic reactions and transport to the cytoplasm occurs before the folded rRNA is assembled with ribosomal proteins to produce the mature ribosome (reviewed in Raue and Planta, 1995). The 18S rRNA is incorporated into the small ribosomal subunit, while the 5.8S and 28S, as well as the 5S rRNA (located at a separate locus except in yeast and some protozoans), constitutes part of the large ribosomal subunit (Maroteaux et al., 1985; Hillis and Dixon, 1991; Srivastava and Schlessinger, 1991; Muse, 1995).

The transcribed ITS and other *cis*-acting structural features, in combination with *trans*-acting proteins, are involved in cleaving the rRNAs and ensuring they fold into the correct secondary structures (Raue and Planta, 1995; van Nues et al., 1995).

The number of rDNA genes within eukaryotes ranges between 40 and several thousand copies and appear to correlate to some extent with genome size, presumably as there is more mRNA to translate in more complex organisms (Long and Dawid, 1980; Li, 1983). The organisation of rDNA in the genome also varies between a single tandem array, as seen in the amphibian *Xenopus laevis* (Arnheim, 1983), to multiple repeats located on five chromosomes in humans (Long and Dawid, 1980; Arnheim, 1983). The dinoflagellate *Proocentrum micans* has a tandem repeat of 200 copies (Herzog and Maroteaux, 1986).

The central paradigm for rDNA in an organism is to have a single homologous sequence maintained through concerted evolution driven by unequal crossing over and gene conversion (Smith, 1976; Li, 1983; Hillis et al., 1991; Buckler et al., 1997). However, a number of organisms including bacteria (Mylvaganam and Dennis, 1992; Nubel et al., 1996; Wang et al., 1997), *X. laevis* (Wegnez and Monier, 1972; Jacq et al., 1977), the malaria-causing agent *Plasmodium* (Gundersen et al., 1987), the platyhelminth *Dugesia* (Schmidtea

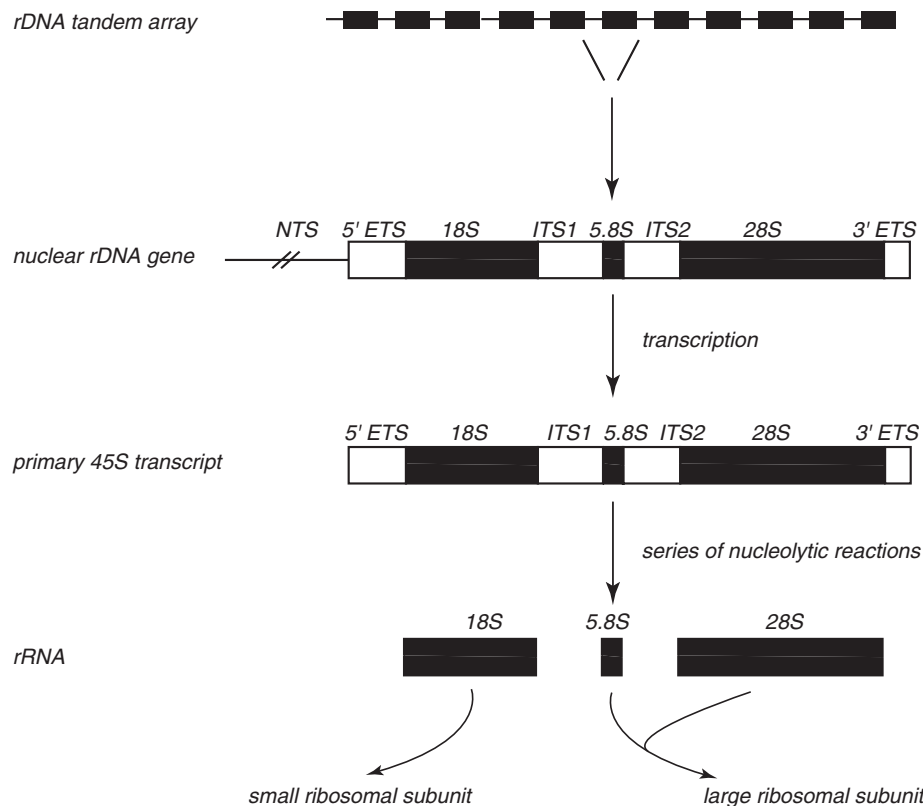


Fig. 3. The eukaryotic nuclear rDNA array. NTS: non-transcribed spacer; ETS: external transcribed spacer; ITS: internal transcribed spacer.

mediterranea (Carranza et al., 1996), yeast (Childs et al., 1981), the dinoflagellates *Alexandrium minutum* and *Gymnodinium catenatum* (Zardoya et al., 1995), *Drosophila* (Childs et al., 1981), and the coral genus *Acropora* (Odorico and Miller, 1997) show evidence of sequence heterogeneity in rDNA. The most well-known example is *Plasmodium*, where two paralogous copies are present that are differentially expressed at different stages of the parasite's life cycle (Gunderson et al., 1987) presumably as a method for post-transcriptional protein regulation. Non-concerted evolution can occur if the rate of fixation is less than the rate of mutational divergence (Long and Dawid, 1980; Arnheim, 1983).

Molecular diversity and rDNA in *Symbiodinium*

Pioneering work by Rowan and Powers (1991a, b) into the molecular diversity of *Symbiodinium* using RFLP and sequencing of the 18S rDNA revealed that the amount of molecular diversity in *Symbiodinium* is equal to that seen between free-living dinoflagellates that are placed in different orders (Rowan and Powers, 1992; Rowan, 1998). Most studies investigating the molecular diversity of *Symbiodinium* since then have focused on the rDNA array including the 18S, 28S, and ITS regions. In addition, allozyme (Bythell et al., 1997; Baillie et al., 1998; Belda-Baillie et al., 1999; Rodriguez-Lanetty et al., 2004), RAPD (Belda-Baillie et al., 1999; Baillie et al., 2000a), microsatellites (Santos et al., 2001, 2003a, 2004; Santos and Coffroth, 2003; Magalon et al., 2004), DNA fingerprinting (Goulet and Coffroth, 1997, 2003a, b), sequence data from chloroplast rDNA (Santos et al., 2002a, b, 2003b), the *psbA* gene (Moore et al., 2003; Takishita et al., 2003), and the mitochondria *cox1* gene (Takabayashi et al., 2004) have provided insight into the genetic diversity and population structure of *Symbiodinium*.

A phylogenetic nomenclature based on rDNA currently divides the genus into eight broad clades, A–H (Fig. 4; Table 1; Rowan and Powers, 1991b; Carlos et al., 1999; LaJeunesse and Trench, 2000; LaJeunesse, 2001; Pawlowski et al., 2001; Pochon et al., 2001, 2004). *Symbiodinium* clades represent divergent lineages, each of which contains multiple, closely related molecular types, revealed by the analysis of rDNA variable regions (e.g. Fig. 4, clade C). The highest number of molecular types has been identified within clade C, which is found associated with most marine invertebrate hosts that harbour *Symbiodinium*. The diversification of *Symbiodinium* types within clade C has been postulated to have occurred through a genetic bottleneck followed by radiation during the early Pliocene to Miocene followed by diversification (LaJeunesse, 2005). Comparative analysis between studies of the phylogeny and diversity

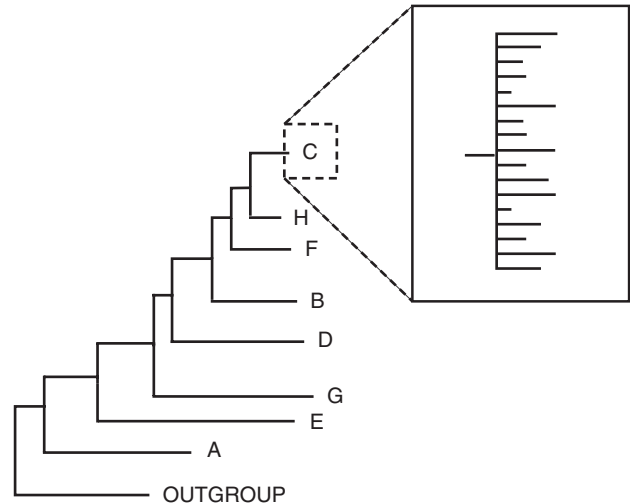


Fig. 4. *Symbiodinium* clade phylogeny. Phylogeny of *Symbiodinium* inferred using rDNA. The diversity within each divergent clade lineage is represented for clade C only. Adapted from Pochon et al. (2004).

of *Symbiodinium* beyond the clade level has been hindered due to a lack of a universal nomenclature system. However, an extensive database of *Symbiodinium* molecular types based on the ITS2 region is becoming the paradigm for molecular typing in the genus (LaJeunesse and Trench, 2000; LaJeunesse, 2001, 2002, 2005; LaJeunesse et al., 2003, 2004a, b; Pochon et al., 2004).

Despite advances in understanding the phylogeography of *Symbiodinium*, certain aspects on the utilisation of rDNA as a phylogenetic tool can lead to a misrepresentation of the evolutionary relationship between molecular types. Most notably, the occurrence of heterogeneity in the rDNA tandem repeat (Baker et al., 1997; Toller et al., 2001a; Santos et al., 2003c) can overestimate the diversity of *Symbiodinium* or incorrectly reconstruct the phylogeny of molecular types if a low copy sequence is favoured in a PCR reaction (Suzuki and Giovannoni, 1996; Buckler et al., 1997). Sequencing artefacts can occur through the production of chimeric or incorrect sequences during amplification, if cloning is used prior to sequencing (Saiki et al., 1988; Wang and Wang, 1997). As the copy number and organisation of the rDNA repeat is not known for *Symbiodinium*, these factors probably have some level of contribution to the current phylogeny of *Symbiodinium*.

The diversity of *Symbiodinium* within individual coral colonies

The majority of coral colonies are dominated by a single type of *Symbiodinium* (Baker, 2003; LaJeunesse, 2005). Of significant importance, Santos et al. (2001)

suggested that many if not all hosts contain a heterogeneous pool of symbionts that may be overlooked by standard molecular typing techniques, which generally only detect the dominant types. The presence of cryptic *Symbiodinium* in coral is also supported through culturing, whereby the in vivo symbiont is displaced in culture by a previously undetectable type (Trench, 1979, 1997; Goulet and Coffroth, 1997; LaJeunesse, 2001; Santos et al., 2001). These low abundant symbionts may be cell-surface contaminants (Trench, 1997), or remnants from initial uptake events by juvenile corals (Coffroth et al., 2001; Little et al., 2004) or may be acquired de novo by adults (Buddemeier and Fautin, 1993; Baker, 2001). It is also possible that these cryptic *Symbiodinium* are types that have overcome the initial steps of being accepted as an intracellular symbiont, but await further evolutionary steps to become a prominent endosymbiont.

The occurrence of intracolony variation in coral and other marine invertebrates resulting in a heterogeneous pool of symbionts with a homogenous spatial distribution has been shown for a number of species (Rowan and Powers, 1991a; Baker and Rowan, 1997; Baker et al., 1997; Goulet and Coffroth, 1997; Rowan et al., 1997; Carlos et al., 1999, 2000; Baillie et al., 2000a,b; LaJeunesse and Trench, 2000; Coffroth et al., 2001; Loh et al., 2001; Santos et al., 2001, 2003a; Fabricius et al., 2004; LaJeunesse et al., 2004a; Little et al., 2004; Magalon et al., 2004). The spatial distribution of *Symbiodinium* types as a result of the differential amount of irradiance on the colony is a rare circumstance that has been shown to occur in *Montastrea* sp. and *Acropora* sp. (Rowan et al., 1997; van Oppen et al., 2001a; Ulstrup and van Oppen, 2003). Rowan et al. (1997) found that in the Caribbean, *Montastrea* sp. associate with three clades (A–C), with clade A and B present in areas of high irradiance, all three clades present at intermediate levels of irradiance, and clade C in areas of low irradiance within a single colony. In addition, the spatial distribution was identified to be dynamic. After experimentally changing the orientation of colonies, and thereby changing the regions of relative light distribution, the symbionts responded by re-establishing the zonation of clades to that observed in the field (Rowan et al., 1997). The detection of a single *Symbiodinium* type in the majority of corals may be an artefact of methodology, as the majority of studies fail to sample multiple times at different locations on a coral colony, hindering the identification of all symbiont types which may be involved in the symbiosis. This is clearly an important area for future research given the importance of flexibility at the colony level in the face of rapid environmental change.

The differences seen in the level of diversity within individual colonies belonging to different host species may be due to biotic and abiotic factors including

symbiont–host specificity, microenvironments, mode of symbiont acquisition, and the biogeographic distribution of symbionts (Baker et al., 1997; LaJeunesse, 2005). A heterogeneous population of symbionts within the host may convey advantages in the event of rapidly changing environmental conditions, but could also be disadvantageous at times due to competition for nutrients resulting in a depressed host state (Douglas, 1998). These issues are potentially fascinating relative to the physiology of whole colonies and differences between species and habitats, and have largely been overlooked by early studies that did not have access to information on *Symbiodinium* diversity.

Population structure of *Symbiodinium* as a function of water depth

Coral colonies experience varying levels of irradiance as a function of depth due to the scattering and absorption of light as it passes through the water column. A number of coral species have been shown to associate with different symbionts depending on the depth of the coral colony (Baker and Rowan, 1997; Rowan, 1998; LaJeunesse et al., 2003, 2004b; LaJeunesse, 2005); however, in some cases, this environmental niche partitioning may be due to the limited sampling of each host species (LaJeunesse et al., 2003). The zonation of *Symbiodinium* according to colony depth was first demonstrated in *Montastrea* sp. located on reefs in the Caribbean (Rowan and Knowlton, 1995). Similar to the partitioning within single colonies discussed above, Rowan and Knowlton (1995) found clades A and B in colonies inhabiting shallow (0–3 m, high light) water, while clades A–C were found in colonies at intermediate depths (3–6 m), and clade C was present in colonies in deep (6–4 m, low light) water. Later, Toller et al. (2001a) found clade E (subsequently identified as clade D) was also partitioning according to colony depth in *Montastrea* sp., in both inshore and offshore reefs. Inshore reef colonies appeared to have a higher proportion of this clade E (now D), in shallow high irradiated colonies, and it appeared to be displacing clades A and B within corals on this reef. The diversity of *Symbiodinium* in offshore reef colonies appeared to be similar in pattern to that reported by Rowan and Knowlton (1995), with clade E (now D) however, present along with clade C in deep water colonies. This led Toller et al. (2001a) to postulate that clade E (now D) may be a stress-resistant symbiont capable of growth in harsh conditions at both ends of the irradiance scale.

Recently, Iglesias-Prieto et al. (2004) showed that the vertical distribution of certain coral species could be explained by their association with symbionts adapted to a particular light regime. In this study, physiological

parameters associated with the efficiency of light capture correlated with the depth at which they were found. Interestingly, their features did not change when colonies were moved from deep to shallow depths and vice versa. This suggests that the symbionts have evolved to occupy a specific environmental niche, the depth at which coral grow, specialising in the physiological attributes required to maximise light capture at that depth.

The biogeographic distribution of *Symbiodinium*

The diversity of *Symbiodinium* located at reefs between different oceans and over latitudinal and longitudinal scales, has led to adaptation of specific host–symbiont associations (Baker and Rowan, 1997; Rodriguez-Lanetty et al., 2001; Burnett, 2002; Karako-Lampert et al., 2004; LaJeunesse et al., 2004a, b; van Oppen, 2004). In some cases, one coral host species may have different *Symbiodinium* partners in different geographical regions (Rodriguez-Lanetty and Hoegh-Guldberg, 2003; LaJeunesse, 2005). The diversity of hosts, host–symbiont specificity, and the symbiont availability at each reef are factors influencing the biogeographic distribution of *Symbiodinium* (Baker and Rowan, 1997).

The population structure of *Symbiodinium* on coral reefs appears to be dominated by one or two generalists (types that are capable of forming symbiosis with a wide range of hosts) and numerous specialist (specificity towards host, depth of colony, or geographic location) symbiont types (LaJeunesse, 2001, 2002, 2005; Diekmann et al., 2002). A study performed by LaJeunesse (2005) investigated species radiation within clade C *Symbiodinium*, which contains the highest number of subclade molecular types (more than 100 based on the ITS2 region), and calculated a molecular clock using the ITS2 region calibrated with the final closure of the Isthmus of Panama (3.1–3.5 MYA). The study indicated that within clade C, the generalists have a global distribution and appear to be the ‘living ancestors’ from which evolution of all other clade C types has occurred. The numerous specialists have evolved on independent lineages extending from the ancestor types. The phylogeography of *Symbiodinium* reported by LaJeunesse (2005) gains support from other studies (LaJeunesse, 2001, 2002; LaJeunesse et al., 2003, 2004a; Ulstrup and van Oppen, 2003; Pochon et al., 2004).

The diversity between *Symbiodinium* present on reefs in the Caribbean to reefs in the Pacific (mainly the Great Barrier Reef) has received the most attention to date. Corals present in reefs in the Caribbean predominantly harbour clades A–C (Baker and Rowan, 1997; LaJeunesse, 2002), whereas corals in the Pacific mainly contain

clade C symbionts (Baker and Rowan, 1997; Baker, 2003; LaJeunesse et al., 2003, 2004a), indicating a higher diversity of symbionts in the Caribbean compared to the Pacific. Interestingly, this reveals an inverse relationship between host diversity and symbiont diversity, as the Pacific contains a richer diversity of hosts (Veron, 2000; Baker and Rowan, 1997; LaJeunesse et al., 2003).

The abrupt changes in the diversity of marine organisms between the Atlantic (including the Caribbean Sea) and Pacific has been hypothesised to have resulted from the closure of the Central American Seaway from the uplift of the Central American Isthmus 3.1–3.5 million years ago (Coates and Obando, 1996), forming a barrier to connectivity between the two Oceans (LaJeunesse et al., 2003; LaJeunesse, 2005; Pochon et al., 2004). The ensuing environmental perturbations and extinctions in the Caribbean during the Pliocene/Pleistocene transition lead to a greater impact on diversification relative to the Pacific (Vermeiji, 1987; Budd, 2000), also accounting for the differences seen in *Symbiodinium* diversity in these regions (LaJeunesse et al., 2003; Pochon et al., 2004; LaJeunesse, 2005). Furthermore, within clade C, the ancestor molecular types (generalists) are present in both oceans, and the diversity within the clade has been postulated to have resulted from a selective sweep of these types or their ancestor before the closure of the Central American Isthmus, followed by allopatric evolution resulting in different molecular types found in conspecific hosts between the two Oceans (LaJeunesse, 2001, 2005).

Some studies have found symbiont transmission to affect specificity, where hosts with a horizontal transmission strategy share a pool of symbionts (generalists), and hosts with a vertical transmission strategy display specificity with a particular symbiont (specialists; Loh et al., 2001; Rodriguez-Lanetty et al., 2001; Barneah et al., 2004). Contrary to this, van Oppen (2004) found that for some *Acropora* sp. and *Montipora* sp., there was no difference in the diversity of symbionts between hosts with different transmission strategies.

Variation in host–symbiont specificity can occur between different geographical regions. LaJeunesse et al (2004a) found that a rare symbiont displaying specificity in the southern Great Barrier Reef increased in abundance and the number of hosts it associated with at lower latitudes in the central Great Barrier Reef. This variation in abundance of *Symbiodinium* types from one location to another appears to be a consequence of host–symbiont adaptation to different environments over geographical scales (Iglesias-Prieto et al., 2004), and is characteristic of the geographical mosaic of coevolution described by Thompson (1999a). However, the complex evolutionary history of the Scleractinia, and the incongruence with coral phylogeny and morphological taxonomy between some regions, hinders the

resolution of host–symbiont associations over wide geographical regions (Veron, 1995; Fukami et al., 2004).

Evolution of scleractinian species

The evolution of species in the classical ‘Darwinian’ sense, where reproductive boundaries develop between populations to eventually create species, appears somewhat at odds with the evolutionary history of the Scleractinia. Instead, a model of reticulate evolution has been proposed, driven by wide geographical genetic connectivity and introgression, which permeates species boundaries with constant fusion of species over evolutionary timescales, similar to what is evident in some plants (Veron, 1995, 2000). In this model of evolution, genetic exchange between species generates reproductively viable hybrids with discrete coral morphologies, which are in turn capable of fertilisation with each parental species, thereby obscuring species boundaries. In addition, the evolutionary history is complicated by cyclical climate changes and associated changes in oceanic movement over geological time (Veron, 2000). This affects coral species dispersal and gene flow between reefs, where fusion of species located at different reefs occurs during one era, which then becomes isolated, only to be connected with another region later, and therefore limits allopatric speciation.

The modern genetic analysis of Scleractinian corals has focused on determining whether there is molecular evidence for reticulate evolution in coral. Molecular analysis and reproductive studies support introgressive hybridisation among Scleractinia (Kenyon, 1997; Odorico and Miller, 1997; Hatta et al., 1999; van Oppen et al., 2000, 2001b, 2002; Diekmann et al., 2001; Miller and van Oppen, 2003), which is likely to increase the adaptive evolution of coral by increasing genetic diversity and heterozygosity (Miller and van Oppen, 2003). However, Vollmer and Palumbi (2002, 2004) argue that some of the observed molecular data can be explained by incomplete lineage sorting rather than introgression, and the production of species hybrids represent asexual morphotypes that may increase genetic diversity but have little evolutionary potential.

Genetic analyses performed to date on corals have mainly utilised the ITS region (Hunter et al., 1997; Odorico and Miller, 1997; Lopez and Knowlton, 1997; Medina et al., 1999; van Oppen et al., 2000, 2001b, 2002; Diekmann et al., 2001; Rodriguez-Lanetty and Hoegh-Guldberg, 2002; Lam and Morton, 2003; Marquez et al., 2003; Takabayashi et al., 2003). Vollmer and Palumbi (2004) caution its use in coral phylogeny, however, as the rDNA in some species of Scleractinia is heterogeneous within a genome, and divergence within a species can be comparable to that between closely related coral

congeners. The use of other molecular markers (Chen et al., 2002; MacKenzie et al., 2004; Severance et al., 2004), and allozyme analysis (Adjeroud and Tsuchiya, 1999; Ridgeway et al., 2001; Goffredo et al., 2004), will allow reticulate evolution and genetic connectivity to be properly investigated in Scleractinian species.

Specificity, flexibility, and coevolution between coral hosts and their symbionts

Knowledge of the flexibility and specificity in the symbiosis between coral hosts and *Symbiodinium* is important for understanding the evolution of the coral holobiont mutualism and the potential for adaptive responses to environmental change. Research into these key areas of coral biology has gathered impetus with the realisation that climate change represents a major threat to corals, their symbionts, and the reefs they build. One of the key questions that has been posed is whether corals and the symbiosis they form have the ability to vary their symbionts to meet the challenges of increasing environmental stress.

There are some key observations that one can make from the phylogeny of the *Symbiodinium* genus. On the question of specificity, for example, an examination of host and symbiont phylogenies based on molecular analysis reveals specificity at the family level (Gast and Caron, 1996; Baillie et al., 1998, 2000b; Pochon et al., 2001, 2004; van Oppen et al., 2001a; Goulet and Coffroth, 2003b; Santos and Coffroth, 2003; Santos et al., 2003b, 2004; Barneah et al., 2004; LaJeunesse et al., 2004b; LaJeunesse, 2005). In this case, some types of *Symbiodinium* (e.g. type C17; LaJeunesse, 2005) only associate with the Poritidae. However, there are other cases where a *Symbiodinium* type associates with a broader array of coral families (e.g. type C3; LaJeunesse et al., 2003) associating with Acroporidae, Faviidae, Pocilloporidae, and other families.

Studies have shown that initial infection of a host appears non-specific, with changes in the population of *Symbiodinium* occurring with the ontogeny of the host, followed by selection of a particular symbiont as an adult (Belda-Baillie et al., 1999; Coffroth et al., 2001; Goulet and Coffroth, 2003b; Little et al., 2004). In addition, most infectivity studies show that hosts consistently accept their homologous algae, reject others, and when accepting heterologous symbiont types, display reduced fitness and growth (Kinzie, 1974; Kinzie and Chee, 1979; Schoenberg and Trench, 1980c; Trench, 1987, 1997; Weis et al., 2001; Belda-Baillie et al., 2002; Rodriguez-Lanetty et al., 2003). In the coral *Fungia scutaria*, however, the specificity of the coral host to its homologous symbiont was shown to be achieved at the stage of the developing planulae larvae

(Weis et al., 2001). This study suggests that even though initial infection appears non-specific in some hosts and competition of different types and subsequent abundance in the host environment can vary (Little et al., 2004), recognition and uptake of a specific symbiont occurs during the onset of symbiosis, which ultimately determines which symbiont will establish itself as the dominant type. These observations support the notion that forming endosymbiotic associations leading to a sustainable healthy host state requires a complex series of coevolutionary steps.

Coevolution occurs when two species interact with each other intimately enough and over sufficiently long timescales to affect each other's evolution (Ronquist, 1997). At the finer scales of *Symbiodinium* subclades and coral species, there is evidence of phylogenetic congruence between corals and their symbionts (Hunter et al., 1997; Diekmann et al., 2003). This feature of the phylogeny of *Symbiodinium* is evidence that switching between *Symbiodinium* clades or even subclades is a relatively rare event that has probably occurred over thousands of years as opposed to months or years. Cospeciation is most likely to occur in specialist symbiotic associations, particularly if the type of *Symbiodinium* is an obligate symbiont (Ronquist, 1997; Douglas, 1998; Roderick and Gillespie, 1998). Reciprocal diversification is driven by coevolution (Thompson, 1999b), and may explain the high number of specialist symbionts in hosts with a maternal transmission strategy (LaJeunesse et al., 2004a; LaJeunesse, 2005). Broadcast spawners that share a pool of generalist symbionts may display a different evolutionary history, as there is greater opportunity to encounter potential new hosts leading to host switching and subsequent phylogenetic incongruence (Roderick and Gillespie, 1998). In addition, changes in symbiont population with the ontogeny of the host will affect analysis of coevolution, depending on the developmental stage of the host when the study is performed.

Investigating population changes of *Symbiodinium* in coral hosts

Several authors have questioned the fidelity of *Symbiodinium* species to their host species and have suggested that endosymbiotic symbioses may be far more flexible in their associations. One statement of this flexibility is embodied in the Adaptive Bleaching Hypothesis which states that 'bleaching provides an opportunity for the host to be repopulated with a different type of partner' (Buddemeier and Fautin, 1993). Since the introduction of this hypothesis, many studies have been performed investigating the ability for recombination to occur between the animal host and

different *Symbiodinium* types. Changes in the *Symbiodinium* population within a host can occur either by shifts in frequency of existing *Symbiodinium* types, or by the expulsion and acquisition of a 'novel' type (Fig. 5; Buddemeier and Fautin, 1993; Hoegh-Guldberg et al., 2002). The alluring possibility in these observations lies in the potential for hosts to switch symbionts for ones that might be more tolerant to the changing environmental conditions around them. If this is a potent force, then it would overcome the potential slow pace of coral host evolution (which is a consequence of long generation times and low population diversity due to asexual reproduction) relative to rapid changes such as those being exerted by climate change.

The *Symbiodinium* population in coral has been shown to be dynamic with daily release of the dinoflagellate complement, and changes in density during different seasons (Gates, 1990; Stimson, 1997; Brown et al., 1999a; Fagoonee et al., 1999; Kinzie, 1999; Fitt et al., 2000; Warner et al., 2002). There is evidence to support changes in the symbiont population within marine hosts following artificial induction of bleaching, suggesting that hosts can switch one type of *Symbiodinium* for another (Baker, 2001; Toller et al., 2001b; Lewis and Coffroth, 2004). Similarly, colonies of *Acropora palifera* were shown to change the clade of

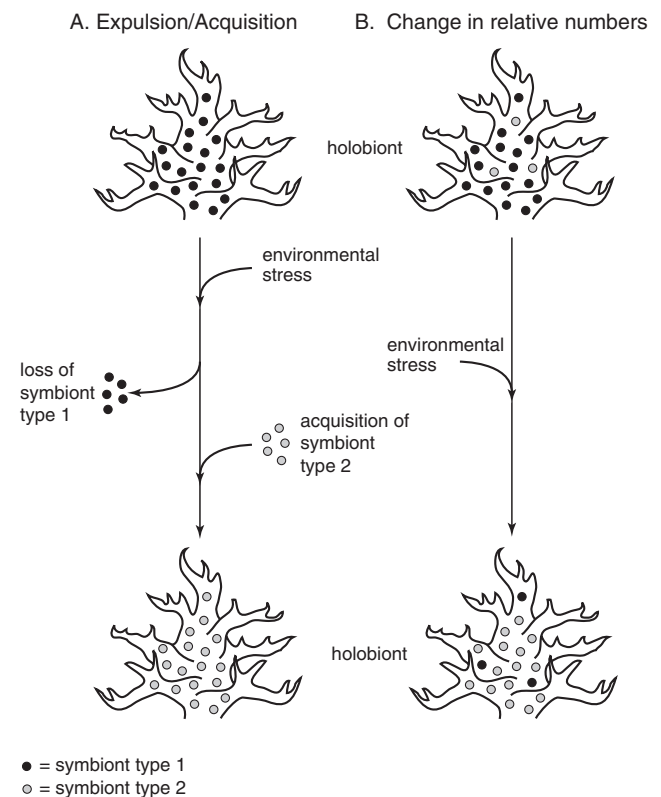


Fig. 5. The two processes that can account for changes in the population of *Symbiodinium* in a host. (A) expulsion/acquisition and (B) changes in frequency of existing types.

Symbiodinium harboured with different seasons throughout the year (Chen et al., 2005a), and post-bleached *Montastrea* sp. colonies appeared to gradually shift the dominant clade of *Symbiodinium* over several years to the host–symbiont assemblage typical for that coral (Thornhill et al., 2005). It is important to note, however, that these coral species are known to harbour multiple clades of *Symbiodinium* (Rowan et al., 1997; Toller et al., 2001a; Chen et al., 2005b). These observed shifts do not provide empirical data to support the introduction of a new symbiont type following changes in environmental conditions. Alternatively, shifts in the population of *Symbiodinium* can be explained through competition of multiple types present in the host that were in undetectable low numbers, as discussed earlier (Hoegh-Guldberg et al., 2002; Hoegh-Guldberg, 2004). The variation in symbiont heterogeneity between hosts and the number that are capable of maintaining healthy coral–symbiont assemblages may dictate which species are likely to survive environmental perturbations. Lewis and Coffroth (2004) induced bleaching in the octocoral *Briareum*, and exposed bleached colonies to a variety of *Symbiodinium* types. The study showed the uptake of a rare symbiont type that was not detected in the host prior to bleaching. Although lending support for the acquisition of a new exogenous symbiont, the symbiont type, even though rare, is one that is found in symbiosis with *Briareum* under normal conditions, and therefore does not represent the uptake of a completely new symbiont to that host species. Baker et al. (2004) reported community shifts in *Symbiodinium* populations in reefs that had been affected by bleaching events, suggesting increased abundance of a bleach-resistant symbiont in hosts from those reefs. However, the authors noted that the observed community shifts can be explained by differential mortality of hosts before and after the bleaching events as the same colonies were not sampled in the study. Contrary to this, several studies have been performed showing a stable symbiotic association between hosts and *Symbiodinium* over temporal timescales (Goulet and Coffroth, 2003b; Rodriguez-Lanetty et al., 2003). The temporal stability of the symbiont population is further supported from the phylogeography of *Symbiodinium* ITS2 types, which reveals specialisation and divergence in support of infrequent recombination of host and symbiont type, as host-specific subclades have remained for millions of years (LaJeunesse, 2005). The evolutionary history depicted in this study lends strong support for host selection and evolution with a dominant symbiont type that has persisted over evolutionary timescales. Cryptic types may still be present in these host species, but may have lost or have not yet evolved, the ability to become a dominant symbiont type over extended periods of time. Therefore, the adaptive potential of coral may be limited to the suite of existing symbiont types present in the

host, which may fluctuate in abundance (including cryptic types), but most importantly, lies in their ability to maintain a healthy host state.

Bleaching and the susceptibility of corals to environmental stress

Coral bleaching results from the loss of pigments from the symbiotic algae, which may or may not be accompanied by loss of the actual dinoflagellate cells. Loss of the brown pigment reveals the white calcium carbonate skeleton of the coral host, due to the largely transparent tissues of the host that remain behind, resulting in a ‘bleached’ appearance (Hoegh-Guldberg and Smith, 1989; Porter et al., 1989; Brown et al., 1995). Bleaching is considered a symptom of stress and may be induced by a variety of physical and biological factors, including high and low sea surface temperatures (SSTs; Jokiel and Coles, 1977; Fitt and Warner, 1995; Saxby et al., 2003; Hoegh-Guldberg and Fine, 2004), UV irradiation (Jokiel and Coles, 1977; Gleason and Wellington, 1993; Brown et al., 1994), bacterial infection (Kushmaro et al., 1996; Rosenberg and Falkovitz, 2004), lowered salinity (Goreau, 1964; Glynn, 1993), and pollution (Glynn, 1993). All of these factors have the ability to cause local-scale bleaching events; however, it is the synergistic effect of increased SST and irradiance associated with periods of El Niño Southern Oscillation that causes mass bleaching and poses the greatest challenge to coral reefs worldwide (Hoegh-Guldberg and Smith, 1989; Porter et al., 1989; Glynn, 1991, 1993; Fitt and Warner, 1995; Hoegh-Guldberg, 1999, 2004; Lough, 2000). Increases in SSTs as little as 0.5–1.5 °C sustained for several weeks above the local maximum are sufficient to induce coral bleaching, as coral reefs are biological systems that live close to their thermal threshold (Porter et al., 1989; Glynn, 1993; Hoegh-Guldberg, 1999).

The primary mechanism leading to SST-induced bleaching is thought to occur from photosynthetically damaged *Symbiodinium* (Fitt and Warner, 1995; Takahashi et al., 2004; Yakovleva and Hidaka, 2004), either by direct damage to Photosystem II (Iglesias-Prieto et al., 1992; Warner et al., 1999), or by damage to Photosystem II as a secondary effect following an initial over-reduction of the electron transport chain during the Calvin cycle (Jones et al., 1998). In either case, symbiotic dysfunction and subsequent dinoflagellate expulsion follows. Lesser and Farrell (2004) suggested both mechanisms leading to bleaching occur at the same time, with the host meanwhile undergoing DNA damage, apoptosis, and tissue necrosis. The production of active oxygen radicals leading to oxidative stress in *Symbiodinium* also appears to contribute to

photosynthetic dysfunction (Lesser et al., 1990; Lesser and Farrell, 2004). Other studies have indicated that disassociation of thylakoid membrane lipid components may also be involved in sensitivity to thermal stress through effects on the production of oxygen radicals which accumulate under high irradiance triggering death, and expulsion (Tchernov et al., 2004). However, thermal stress was examined over relatively long (days) periods, while most evidence (e.g. Jones et al., 1998) indicates that dysfunction occurs on much shorter timescales. Alternatively, infection with the bacteria *Vibrio shiloi* and the induced production of bacterial toxins with increased SST that inhibit photosynthesis has been proposed as a general model for the bleaching symptom (Rosenberg and Falkovitz, 2004). However, bleaching should not be interpreted as a disease that is induced by a particular stress or pathogen, but as a broad symptom of distress that can be triggered by a number of factors. Finally, photosynthetically active cells are also expelled during bleaching (Ralph et al., 2001; Bhagooli and Hidaka, 2004), outlining the fact that bleaching and its mechanism are still poorly understood and much remains to be learnt about both the diversity of responses within the bleaching symptom and the underlying mechanisms.

Initially, clades A and B were thought to be resistant to bleaching while clade C was more susceptible (Rowan et al., 1997). This theory has since been dismissed with the discovery of molecular subclades displaying varying levels of thermal tolerance (e.g. C15 and Poritidae display bleach resistance, and C3 and Acroporidae display bleach susceptibility; LaJeunesse et al., 2003). This emphasises the point that clades are a diverse group of organisms and should not be collectively grouped according to physiology. *Symbiodinium* clade D appears to be a bleach- and stress-resistant type, and has been postulated to have increased in abundance on post-bleached reefs (Glynn et al., 2001; Toller et al., 2001a; Baker et al., 2004; Fabricius et al., 2004; Rowan, 2004), although whether the symbiont in clade D identified as resistant (Rowan, 2004) is the same subclade indicated in all the above studies is not known.

Much of the recent work on stress susceptibility has concentrated on the abilities of the symbiont and has largely ignored the critical contribution that the host makes to the immediate environment surrounding the symbiont. This point is conveyed through the observation that the type of symbiont that is associated with susceptible symbioses in terms of stress (e.g. C3 and Acroporidae) can also associate with coral families that are relatively tolerant of stress (e.g. C3 and Faviidae; LaJeunesse et al., 2003). Therefore, if *Symbiodinium* were the principle reason determining the stress tolerance of symbiotic corals, one would expect that tolerant symbionts should only be found in tolerant associations. There is ample evidence that the host is critical to the

overall abilities of the holobiont. Coral morphology and related tissue thickness (Hoegh-Guldberg, 1999; Loya et al., 2001), protein turnover (Gates and Edmunds, 1999), dinoflagellate density (Stimson et al., 2002), and host protective pigments (Hoegh-Guldberg, 1999; Salih et al., 2000; Dove et al., 2001; Verde and McCloskey, 2002; Prescott et al., 2003; Dove, 2004), MAA and xanthophyll production (Brown et al., 1999b; Warner et al., 1999), acclimation to previous solar irradiation (Brown et al., 2000, 2002a,b), and thermal history (Coles and Jokiel, 1978) all appear to play important roles in determining coral sensitivity to light and thermal stress.

Debate still ensues as to the flexibility of the symbiosis between *Symbiodinium* and corals, with the observation of diversity within host corals supporting the notion that selection of a subset of *Symbiodinium* types from a diverse pool occurs on an on-going basis (Coffroth et al., 2001; Little et al., 2004). Some authors (e.g. Hoegh-Guldberg et al., 2002) still question whether the critical evidence has been gathered to support the hypothesis that hosts can take on truly novel symbionts in a dynamic and relatively frequent basis. These same authors also point to aspects of the cell biology and phylogeny of *Symbiodinium* symbiosis (as discussed above) that indicate that switching can occur but that it is a rare evolutionary event involving several coevolutionary mechanisms. Perhaps the most important point of this last statement is that mechanisms such as these are likely to lead to evolutionary change and continued persistence over long periods but will not prevent the rapid decline of corals and ensuing loss of biodiversity that appears highly likely on the basis of future projections of environmental stress. These projections of future stress include rates of warming and carbon dioxide concentrations exceeding most, if not all, of those seen over the last 400,000 years (Wigley et al., 1997; Lough, 2000; Coles and Brown, 2003; Hoegh-Guldberg, 2004).

The paradox between coral reef persistence since the Triassic surviving multiple shifts in climate compared to current loss in coral reef cover is poorly understood. Critical to any analysis is the timescale at which persistence is evaluated. For example, persistence in geological time has very different implications for coral reefs when compared to persistence of coral reefs on the timescale of human industries and activities. These sorts of questions impact on how one rates the potential effect of the rapid changes in the environment being wrought by human activities over the past century. Comparative analysis between past climate perturbations, and associated widespread extinction and loss in biodiversity, to current climate change also must consider the rate at which environmental perturbations occur (Glynn, 1993). The adaptive response of coral reefs to rapid climate change following bleaching events addresses the resilience of reefs over time. The loss in diversity of both

coral and *Symbiodinium* following bleaching events (Rowan et al., 1997; Fabricius et al., 2004), and the potential for reefs affected by bleaching to suffer consecutive bleaching events (Ward et al., 2000; Edmunds et al., 2003) suggests an outcome of loss in coral reef cover and diversity if current trends continue. Range shifts of species and changes in gene flow and connectivity leading to coral reef expansion to higher latitudes is questionable, as factors other than ocean temperature are required for reef formation (Walther et al., 2002; Ayre and Hughes, 2004). The combined effects of coral bleaching, overfishing, and coastal development act synergistically on reef degradation (Hoegh-Guldberg, 1999). Community phase shifts towards ecosystems dominated by macroalgae and subsequent decrease in coral cover are likely to occur (Ostrander et al., 2000; Diaz-Pulido and McCook, 2002; Bellwood et al., 2004). The concerns regarding the future of coral reefs in a period of rapid climate change justifies the need for studies on the resilience and connectivity of coral reefs, which is important for developing effective policies on marine conservation.

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