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MULTIBIONT SYMBIOSES IN THE CORAL REEF ECOSYSTEM

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1. Introduction

Symbiotic systems are ubiquitous and play key roles in a variety of ecological systems (Douglas, 1995). Symbioses involving microorganisms underpin certain biological communities (coral–algae, plant–mycorrhizal fungi, plant–bacteria), and in these systems they are important to the flux of energy and nutrients (Douglas, 1995). The classic symbiotic systems (involving two partners) usually involve one large organism (host) and one/several smaller organisms (symbionts) that are located within the body of their host (Douglas, 1994). The original definition of symbiosis: "phenomena of dissimilar organisms living together," was coined by de Bary (1879) and has been frequently misinterpreted and used as a synonym of mutualism, mainly due to the fact that de Bary was engaged in research of the mutualistic relationship between algae and fungi in lichens (Castro, 1988). Much of the literature on symbiosis presupposes that the associations are mutualistic, but this view is not fully supported by direct experimental study (Douglas, 1995). Along with the continuous study of symbiotic systems and data accumulation, much effort has been invested by researchers to supplying a better and more accurate definition of the term "symbiosis." The study of various symbiotic systems by different researchers, who employed different tools for the task, led to difficulties and confusion (Smith, 1992). Several researchers saw the ecological consequences or outcomes of interspecies intimacy as the main factor in defining symbiosis (see Saffo, 1992), while others employed the presence or absence of metabolic dependency as the main criterion (Smyth, 1962; Castro, 1988). According to Smith (1992), for a large number of associations, the facts on the interactions between the partners were still too imperfectly understood to allow safe judgments about the existence of mutual benefit, and even in cases in which the "mutual benefit"-type definition is adopted, problems still arise, because "benefit" is a very difficult concept to define and measure (Smith, 1992). Moreover, even when the dynamics of certain symbiotic associations are fairly well understood, it is sometimes, nonetheless, too complex to pigeonhole into simple categories of mutualism, commensalism, and parasitism (Saffo, 1992).

While there are many well-studied symbioses of two interacting organisms, it is only recently that scientists have discovered more complex relationships involving three or more organisms from different taxonomic groups (Douglas, 1998; Hunter, 2006). When dealing with symbiotic systems that involve more than two partners, the above- mentioned difficulties of defining, categorizing and sorting of an association type are even more pronounced; indeed the possible relations between partners can include both antagonistically and mutualistically interacting pairs (Saffo, 1992) and can involve complex metabolic pathways in which not only symbiont–host interaction but also symbiont-symbiont interactions can be engaged. Along with the ongoing efforts to categorize symbiotic interactions, one can see that the vast variety of interactions, the complexity of physical and metabolic relations, as well as the number of unknown variables in several symbioses, inevitably leads to a retreat from strict classification back to a more broad and flexible terminology. Saffo (1992) suggested 'living together' as the appropriate interpretation for symbiosis, and that is the essence of the matter and at the root of most of our questions. Using such a broader definition of symbiosis facilitates investigations unprejudiced by preconceptions about outcomes (Saffo, 1992). Castro (1988) reported in his review of symbioses in coral reefs that a growing number of investigators have chosen to overcome the difficulties of categorizing symbioses by minimizing the need to classify the association in question, and merely referring to it as an instance of symbiosis.

Several multibiont symbiotic systems are already documented from the terrestrial environment. These include: (1) systems in which two of the partners are at the same organizational level (usually two bacteria), like the mutualistic relationship between the glassy-winged sharpshooter (*Homalodisca coagulata*) and two bacteria *Baumannia cicadellinicola* and *Sulcia muelleri* (discussed later) (Wu et al., 2006), or the colonization of many plant species with multiple genotypes of mycorrhizal fungi (De La Bastide et al., 1995; Clapp et al., 1995; Perotto et al., 1996); and (2) systems that include partners at different organizational levels. An example of the latter involves the relationship between a virus, a bacterium, and an insect: All aphids require a primary endosymbiont, the bacterium *Buchnera*, in order to synthesize the nutrients missing in their xylem food source. Some aphids, however, also contain a secondary bacterial symbiont, such as *Hamiltonella defensa*, which confers defense against other bacteria (Hunter, 2006). Moran et al. (2005) have identified an associated bacteriophage virus called APSE-2, whose genome contains a gene encoding cytolethal-distending toxins, which disrupt the eukaryotic cell cycle. The authors suggest that the phage-borne toxin provides defense against eukaryotic parasites for the aphid host. From the terrestrial environment it is also known that multibiont symbioses can involve many combinations of organisms of different sizes, in which one organism is host to another, while resident in a third (e.g., nematodes, bacteria, and insects; Hunter, 2006). Moreover, some of the symbioses involve interactions other than endosymbiosis. In some cases bacteria live outside their host, and have been given the name “episymbionts” (Hunter, 2006). Interestingly, it was found that the reindeer lichen *Cladonia arbuscula* (which in itself is an association of fungi and photoautotrophs) is associated with bacterial cells of different taxonomic groups that are embedded in a biofilm-like layer (Cardinale et al., 2008). It would appear that the complexity and range of terrestrial multibiont symbioses is only just beginning to unravel. In this chapter, we focus on multibiont symbioses in the marine environment, and especially in the coral reef ecosystem.

Coral reefs are often described as the rain forest of the sea (Connell, 1978). As such, they include numerous organisms that interact with each other in a complex array of symbiotic associations (Paulay, 1997). A single coral colony may accommodate a variety of symbiotic organisms, including invertebrates and vertebrates, bacteria, archaea, viruses, fungi, protozoa, and algae, all living in close proximity and interacting with each other (Paulay, 1997; Wegley et al., 2007). The term “coral holobiont” was introduced by Rowan (1998) to describe the complex of the coral and its symbiotic algae. Later, Rohwer et al. (2002) expanded its meaning to include the complex and dynamic assemblages of the coral animal with its associating microbial eukaryotes (algae, fungi, and protozoa), bacteria, archaea, and viruses. The wealth and complexity of associations within a coral colony will serve here as the basis for our discussion of multibiont symbioses. The chapter will focus on multibiont symbioses involving corals and microorganisms such as zooxanthellae, cyanobacteria, and endolithic algae (microsymbionts), as well as associations that involve the coral and macroorganisms such as mussels, barnacles, and fish (macrosymbionts). Aspects of metabolic contribution, symbiont effectiveness, host gain/loss, and the establishment of multibiont symbioses will also be discussed.

The chapter is divided as follows:

1. Introduction
2. An overview of symbioses involving a coral colony and more than one microsymbiont type
3. An overview of symbioses involving a coral colony, its microsymbionts, and at least one type of macrosymbiont
4. Multibiont symbiosis during stress – the coral–algal adaptive bleaching hypothesis (ABH)
5. The establishment of multibiont symbioses – vertical and horizontal transmission of symbionts
6. Discussion

2. Coral Host and Microsymbionts

2.1. CORAL–ALGAL SYMBIOSIS AS A MULTIBIONT ASSOCIATION

The keystone symbiosis for coral reef existence is undoubtedly that between members of the phylum Cnidaria, such as corals and anemones, and their photosynthetic dinoflagellate symbionts *Symbiodinium* spp. (also called zooxanthellae), which together form the trophic and structural foundations of coral reef ecosystems. Since the early days of zooxanthellae research and the description of the first *Symbiodinium* species, i.e., *Symbiodinium microadriaticum* Freudenthal (1962), dozens of studies have dealt with the diversity of these algal symbionts and provided sound evidence for the occurrence of high genetic variability among them (see Baker, 2003). The advent of molecular tools has resulted in a growing number of studies dealing with partner specificity, biogeography, and ecology of coral–algal symbiosis, and it was not long after the first molecular data were obtained that it was demonstrated that coral hosts can be associated with one or more genetically distinct algal symbionts (Rowan and Powers, 1991; Rowan and Knowlton, 1995; Rowan et al., 1997; Toller et al., 2001; LaJeunesse, 2001), hence negating the idea of uniformly strict specificity (in which all hosts exclusively contain only one symbiont type; Baker, 2003).

One of the first studies that demonstrated the relationship of a single coral host with more than one symbiont taxa dealt with two of the dominant corals of the Caribbean Sea (Rowan et al., 1997). Three distinct taxa of algal symbionts were found to be associated with *Montastraea annularis* and *M. faveolata*. Both coral species host members of *Symbiodinium* groups A, B, and C, with specificity dictated by the environment: corals at shallow-to-intermediate depths host two or three taxa of symbiont assemblages that map to the "sun" (*Symbiodinium* A and/or B) and "shade" (*Symbiodinium* C) patterns on colony surfaces. When such intracolony irradiance gradients are manipulated, the symbionts reestablish correct patterns of zonation (Rowan et al., 1997).

As molecular research has progressed, various examples of scleractinian species and other invertebrates hosting multiple algal taxa in a single individual/ or colony have accumulated (see Baker, 2003). These served as the springboard for a range of new studies looking into the functional and physiological capabilities of the different symbiont types and their implications on the coral host during stress periods (see below). An implicit motivation behind much of this research has been to understand the role of symbiont diversity and/or flexibility in determining possible long-and short-term responses of coral reefs to environmental change and global warming (Baker, 2003).

The quest to understand the role of genetically different symbionts for their coral host during stress somehow preceded the search for a greater understanding of their role during "normal" times. It is only recently that this crucial question has been addressed by Loram and coworkers (2007), who explored how the nutritional function of *Symbiodinium* maps onto the molecular diversity of this genus. A thorough study was conducted on the giant sea anemone *Condylactis gigantea* that associates with members of two clades of *Symbiodinium*, either singly or in mixed infection. It was demonstrated that symbioses of *C. gigantea* with the dinoflagellate algae *Symbiodinium* of clades A and B are functionally different. The incorporation of algal photosynthetic carbon into animal lipids and amino acid pools was significantly higher in symbioses with algae of clade A than of clade B (Loram et al., 2007). In the symbioses involving mixed infections, the metabolic indices either did not significantly differ from the monomorphic symbioses or were intermediate between them. A key question driving that study was that of whether the symbionts in mixed infections are less cooperative than those in single infections. The results suggest that the proportion of fixed carbon translocated to the host is not depressed in the mixed infections and that competition between co-occurring *Symbiodinium* taxa is suppressed in the symbiosis (Loram et al., 2007). The authors suggest three potential mechanisms by which the host might suppress such competition: (1) it might control the supply of limiting nutrients to its symbionts; (2) it might control the proliferation rates of symbionts; or (3) it might impose sanctions on any algal cells that release photosynthate at low rates. Furthermore, the authors point out a structural constraint of the symbiosis (the presence of each algal cell within a symbiosome inside the host cell) that might minimize the opportunity for inter-algae competition. The example presented above demonstrates for the first time the selective metabolic contribution to the host of two genetically different symbiont taxa.

2.2. CORAL, ALGAE, AND CYANOBACTERIA

The coral *Montastrea cavernosa* displays another three-party association that includes zooxanthellae and cyanobacteria as the symbionts. Both zooxanthellae and cyanobacteria are endosymbiotic. The latter were found to be genetically similar to either *Synechococcus* sp. or *Prochlorococcus* sp. within the order Chroococcales. In this system it was demonstrated that the cyanobacteria provide a source of nitrogen that is utilized by the zooxanthellae (Lesser et al., 2007). The pattern of nitrogen fixation is diurnal and confined to those times of the day when physiological hyperoxia or anoxia does not inhibit nitrogen fixation. Moreover, the availability of this novel source of inorganic nitrogen does not appear to affect the stability of the mutualistic association between the symbiotic zooxanthellae and the coral host (Lesser et al., 2007). This association involves two distinct endosymbiont types: zooxanthellae, which contribute photoassimilates to the coral host and cyanobacteria, which supply fixed nitrogen to the zooxanthellae. The symbionts in this consortium perform their “tasks” on the basis of alternating diurnal dominance. In this association it was demonstrated that a mixed infection could be beneficial to the host via an indirect pathway, in which one symbiont type contributes to another.

2.3. CORAL, ENDOSYMBIOTIC ALGAE, AND ENDOLITHIC ALGAE

A different multibiont association is that involving the ahermatypic stony coral *Oculina patagonica*, its endosymbiotic zooxanthellae, and its endolithic algae found within the coral skeleton (Fine and Loya, 2002). Although 80–90% of the colonies of the Mediterranean encrusting coral *O. patagonica* bleach annually, surprisingly more than 90% of the bleached colonies recover (Kushmaro et al., 1996, 1998). One of the most intriguing questions being asked by coral reef researchers is that why some coral species survive bleaching events while others do not (Fine and Loya, 2002). Fine and Loya (2002) looked into the dynamics and photosynthetic pigment concentrations and biomass of endoliths in the skeleton of *O. patagonica* throughout a bleaching event, and demonstrated that during repeated summer bleaching events these endolithic algae receive increased photosynthetically active radiation (due to the loss of zooxanthellae), markedly increase in biomass, and produce increasing amounts of photoassimilates, which are transferred to the coral. It was therefore concluded that the endolithic algae serve as an alternative source of energy during coral bleaching (Fine and Loya, 2002). This case study demonstrates a situation in which stressful conditions cause a shift in symbiont abundance, which is directly followed by a shift in the energetic contribution from the symbionts to the host. It should be emphasized that this three-party association prevails throughout the coral's existence, with alternating metabolic dominance of the "algal player" as dictated by the bleaching severity.

3. Coral Host and Macrosymbionts

In the previous section, several multibiont symbioses involving corals and their unicellular photosynthetic symbionts were addressed. Additionally, a coral colony often provides shelter and food for diverse groups of macroorganisms such as polychaetes, molluscs, crustaceans, and fish (Castro 1988) (see Fig. 1). Several studies have dealt with coral macroorganism symbiosis (Goreau et al., 1970; Mokady et al. 1998; Simon-Blecher et al. 1996). This section is devoted to several case studies that demonstrate the metabolic contribution of the macroorganism to the coral colony via its photosynthetic symbionts.

3.1. BACKGROUND

Coral reefs are oligotrophic environments in which concentrations of inorganic nitrogen in the surrounding seawater are often <1 μmol/l (Muscatine and Porter, 1977; D'Elia and Wiebe, 1990). Consequently, zooxanthellae in the gastrodermal

Figure 1. (continued) The barnacles' shells are covered with the coral tissue except for the aperture, enabling the barnacle to breath and feed. Notice the deformation in the coral skeleton and the dark color of the coral tissue. (Photo by M. Fine.) Scale bar 10 mm. (**e**) The coral *Turbinaria* sp. with numerous acoel worms belonging to the genus *Waminoa* (black arrow). The worms contain both *Symbiodinium* sp. and *Amphidinium* sp. algal symbionts. (Photo by A. Shoob.) Scale bar 7 mm. (**f**) The polychaete worm *Spirobranchus giganteus* (Serpulidae) embedded in a faviid coral at Eilat (Red Sea). Notice the remains of coral tissue around the polychaete tube. (Photo by O. Ben-Tzvi.) Scale bar 18 mm.

Figure 1. Examples of coral hosts and their macrosymbionts: (**a**) A broken piece of the coral *Montipora erythrea* exposing the boring mussel *Lithophaga purpurea*. Additional burrows can be seen in the inner coral skeleton. (Photo by I. Brickner.) Scale bar 13 mm. (**b**) Open shells of the boring mussel *L. purpurea* taken from the massive stony coral *Montipora erythrea.* The arrow points towards a pair (male and female) of pea crabs *Pinotheres* sp. regularly, only one crab can be found within the *Lithophaga* mantel cavity. In cases where a pair of crabs does occur, they reside only in one side of the mussel mantel cavity, otherwise, the mussel could die due to damage caused to the gills. (Photo by I. Brickner.) Scale bar 8 mm. (**c**) *Trapezia* crab between the branches of *Stylophora pistillata*. Notice the algae on the crab's left arm, a result of grazing over the tissue-exposed area of the coral. (Photo by I. Brickner.) Scale bar 18 mm. (**d**) The massive stony coral *Porites* sp. infested with several barnacles.

cells of corals, sea anemones, and other zooxanthellate invertebrates are sometimes nitrogen-limited (Cook and D'Elia, 1987; Cook et al., 1988; Muscatine et al., 1989; Belda et al., 1993; Falkowski et al., 1993). Indirect confirmation of this comes from experiments in which increases in the concentration of external ammonia were shown to promote greater numbers of zooxanthellae (Stambler et al., 1991; Stimson and Kinzie, 1991; Belda et al., 1993; Muller-Parker et al., 1994). If zooxanthellae assist the host in meeting its energy requirements (Muscatine et al., 1981; Falkowski et al., 1993), then increased cell densities might be beneficial (Meyer and Schults, 1985). Conversely, raising the concentration of external nitrogen for prolonged periods encourages balanced growth within a population of zooxanthellae, which lowers the translocation of fixed carbon to the host (Falkowski et al., 1993).

Inorganic nutrients on coral reefs originate from several sources (Entsch et al., 1983). One of the suggested sources is the fertilization by associate organisms, e.g., coral symbionts of the waters surrounding coral colonies (Wielgus and Levy, 2006).

3.2. STONY CORALS AND BORING MUSSELS

One of the first prominent models for coral fertilization was that of highly modified mussel *Fungiacava* that lives within fungiid corals (Goreau et al., 1970). According to a model suggested by Goreau et al. (1970), this boring mussel releases nutrients (as ammonium) into the polyp coelenteron, enhancing the production of additional symbiotic algae, some of which, in turn, will be released as food for the *Fungiacava*. However, this model was never confirmed. Similarly, the boring bivalve *Lithophaga simplex* was found to inhabit the scleractinian coral *Astreopora myriophthalma* in high densities in the northern part of the Red Sea (Mokady et al., 1998). Ammonium production rate by the bivalves and its consumption rate by the coral (via the symbiotic algae) were measured in the laboratory. Ammonium production by the bivalves inhabiting the coral was found to be higher during daytime than at night. Under naturally occurring levels of ammonium, recycling of nitrogenous waste produced by the bivalves (ammonium) may supply a significant portion of the needs of the coral/zooxanthellae. Mokady et al. (1998) hypothesized that the association between *L. simplex* and *A. myriophthalma* may also represent an example of mutualistic symbiosis, contrary to the generally accepted view of boring bivalves as parasites of their coral hosts.

3.3. MASSIVE STONY CORALS AND CRUSTACEANS

Simon-Blecher et al. (1996) studied the spatial distribution of chlorophyll in three coral species carrying invertebrate symbionts, using spectral imaging techniques. The multipixel fluorescence map and the relative-intensity fluorescence ratios demonstrated a high concentration of chlorophyll A next to the pits of the pit crab

Cryptochirus coralliodytes in the stony coral *Favites halicora*. Spectral similarity maps of the fire coral *Millepora dichotoma* inhabited by the barnacle *Savignium milleporum* revealed relatively higher chlorophyll concentrations in these two corals next to the symbionts. Those researchers hypothesized that the invertebrate symbionts fertilize their immediate surroundings with their excreta, enhancing algal growth.

Cook et al. (1991) demonstrated that ^{32}P and ^{14}C ingested by the coral-inhabiting barnacle *S. milleporum* is mobilized and excreted, and subsequently taken up by the zooxanthellae of the hydrocoral host *M. dichotoma*. They suggested that uptake of excreted substances from symbiotic barnacles in the nutrient-poor waters of the Red Sea may be beneficial to *M. dichotoma*. However, the low density of *S. milleporum* on *M. dichotoma* colonies would not be sufficient to support the ammonium demands of the hydrocorals (Achituv and Mizrahi, 1996). Moreover, Achituv and Mizrahi did not find differences in zooxanthellae densities between *M. dichotoma* colonies or branches with or without barnacles.

3.4. CORALS AND POLYCHAETE WORMS

Ben-Tzvi et al. (2006) reported the presence of the polychaete worm *Spirobranchus giganteus* (Serpulidae) embedded in two faviid corals at Eilat (Red Sea; Fig. 1f). The authors observed a colony of the stony coral *Cyphastrea chalcidicum* that was almost completely dead and covered with turf algae, apart from three small area of living coral tissue that surrounded *S. giganteus* tubes. Only one of these living areas continued to grow around the polychaete tube, while the other areas died. In other instances, coral colonies belonging to the species *Favia favus* and *F. laxa* were damaged by bleaching and predation respectively. As in the former case, areas of coral tissue in close proximity to the polychaete tube showed no damage and the colonies quickly recovered. It was suggested that the corals benefit from increased availability of nutrients from waste materials excreted by the worm. *Astreopora*, *Cyphastrea*, *Echinopora*, *Leptastrea*, *Millepora*, *Montipora*, *Pavona*, and *Porites* at Eilat (northern Red Sea) have recently become infested with boring spionid polychaetes and there are indications that these infestations are correlated with anthropogenic nutrient discharges (Wielgus et al., 2006). Wielgus and Levy (2006) studied the influence of the infestation by the boring spionid polychaetes colonies on the reef-building coral *A. myriophthalma*. They used an active fluorescence technique to examine differences in the functional absorption cross-section of Photosystem II ($^{\sigma}$PSII) between areas of a coral colony that were infested with spionid worms versus areas lacking such worms.

The mean $^{\sigma}$PSII value in areas of the *A. myriophthalma* colony that were infested with spionid worms was significantly higher than in the areas that were not infested. The differences in $^{\sigma}$PSII between different areas of a coral colony reflect variations in photosynthetic activity. The researchers suggested that fertilization of the surrounding water by the boring spionid polychaetes can result in zooxanthellae proliferation. Increases in zooxanthellae abundance will lead in turn to a rise in chlorophyll levels, and can also lead to further increase in $^{\sigma}$PSII. These results were followed by morphological changes in the infested area of the coral colony, including increased roughness and bumpiness in tangential-to-radial growth. Such morphological changes reflect a higher tissue growth/calcification rate that can only occur if the symbiotic association is provided with an increased amount of nitrogen (Wielgus and Levy, 2006).

3.5. CORALS AND FISH

Meyer et al. (1983) recorded the high level of ammonium excreted by haemulid fish schools resting over coral colonies. The schools feed in sea-grass beds at night and during daytime they rest over the coral heads, where they excrete substantial quantities of ammonium and particulate nitrogen and phosphorus into the nutrient-poor waters. The percentages of these nutrients contributed by the fish were comparable to those derived from other sources. Coral heads with resident fish schools grew faster than those without such schools.

4. Multibiont Symbiosis During Stress: The Coral–Algal Adaptive Bleaching Hypothesis

It is now well documented that different strains of *Symbiodinium* (symbionts of corals) exhibit variation in thermal tolerance and photosynthetic response to irradiance (Iglesias-Prieto and Trench, 1997; Warner et al., 1999; Savage et al., 2002; Rowan, 2004; Goulet et al., 2005). This variation has ecological implications and high temperatures and irradiance are thus considered the primary causes of coral bleaching (the breakdown of the symbiotic relationship between the coral and its zooxanthellae; Glynn 1996; Brown, 1997), a major threat to coral reef existence worldwide (Hoegh-Guldberg, 1999). The increase in frequency and severity of bleaching events worldwide (Wilkinson, 1999) and the emerging physiological differences between genetically distinct symbionts, gave rise to the ABH (Buddemeier and Fautin, 1993). The ABH posits that when environmental circumstances change, the loss of one or more types of zooxanthellae is rapidly, and sometimes unnoticeably, followed by the formation of a new symbiotic consortium with different zooxanthellae that are more suited to the new conditions in the host's habitat. Empirical data aimed at reinforcing the theory which demonstrated that stony corals taken from deep water and transplanted into shallow water experienced severe bleaching that resulted in the loss of their suboptimal low-light symbionts. Consequently, the newly vacant hosts allowed the proliferation of high-light algae (Baker, 2001, 2002). Such a result tends to favor the adaptive nature of bleaching, but fails to prove that the new combination of symbionts in a host is indeed really new (Hoegh-Guldberg et al., 2002) and not just a phenotypic change caused by the increase in proportion of rare genotypes of symbionts that were always there. As the original ABH became more controversial over the last decade, new discoveries emerged and highlighted several crucial facts: laboratory cultures of zooxanthellae can become dominated by types of *Symbiodinium* that are not representative of the dominant symbiont in the host from which they were originally isolated (Santos et al., 2001; LaJeunesse, 2001). This indicates that algal symbiont communities *in hospite* include novel symbiont types whose relative numerical abundance is below the detection threshold of conventional PCR-based identification methods (Baker and Romanski, 2007; Thornhill et al., 2006). These "cryptic" symbionts may be critical in providing corals with greater capacity for symbiont "shuffling" in response to environmental change (Little et al., 2004; Baker and Romanski, 2007). Moreover, symbioses with a capacity for mixed infections/multiple genetically distinct symbionts may be at an advantage in times of rapid global climate change (Douglas, 1998; Loram et al., 2007). Therefore, although the ABH in its original context might not be accurate, experimental evidence suggests that bleaching can become adaptive in certain coral hosts that originally possessed a diverse set of algal symbionts. It should be kept in mind, however, that as molecular techniques improve (see below), the original hypothesis might be proven correct in other coral hosts.

5. The Establishment of Multibiont Symbioses: Vertical and Horizontal Transmission of Symbionts

The onset of symbiosis can occur at a variety of host life-history stages, depending on the host species. Symbionts can be transmitted horizontally, in which the host's sexual progeny acquire symbionts from the surrounding environment; or vertically being passed directly from host parent to offspring (Trench, 1987; Douglas, 1994). Horizontal transmission offers the host the opportunity to recombine with different symbiont types that are differentially adapted to the existing environmental conditions. In contrast, vertical transmission guarantees that a host is provided with a complement of symbionts, which are transmitted faithfully from parent to offspring (Douglas, 1998). Associations with horizontal transmission tend to be considered as more plastic and "ready to change," whereas those with vertical transmission are believed to be strict and inflexible, and hence are also termed "open" versus "closed" systems (Trench, 1987). When we think of multibiont associations, we are naturally drawn to think of them as open systems. However, surprisingly, this is not always the case. We present below several multibiont associations in which the symbionts were proven to be vertically transmitted, and introduce two case studies of horizontally transmitted symbionts that illustrate different symbiont makeup in juvenile versus adult hosts (of the same species).

5.1. VERTICAL TRANSMISSION

The marine sponge *Chondrilla australiensis* (Demospongiae) contains unicellular cyanobacteria with an ultrastructure resembling that of *Aphanocapsa feldmannii*, which occur in the cortex, and bacterial symbionts, which are located throughout the mesohyl. In *C. australiensis*, the developing eggs are distributed throughout the mesohyl and are surrounded by nurse cells attached to them by thin filaments. The nurse cells form cytoplasmic bridges with the eggs, apparently releasing their contents into the egg cytoplasm. The presence of cyanobacterial and bacterial symbionts inside developing eggs and nurse cells in 25% of female *C. australiensis* was confirmed using transmission electron microscopy, suggesting that these symbionts are sometimes passed on to the next generation of sponges via the eggs (Usher et al., 2001).

Waminoa brickneri, a newly discovered species from the reefs of Eilat (Red Sea; Figs. 1e and Fig. 2a) is epizoic on living corals (Ogunlana et al., 2005). Similar worms belonging to the genus *Waminoa* were detected there on 14 species of stony and soft corals at a depth range of 2–50 m (Barneah et al., 2007a). The worms possess two distinct types of dinoflagellate algal symbionts within their cells: small symbionts 5–10 µm in diameter, which were identified as belonging to the genus *Symbiodinium*; and larger symbionts 12–20 µm in diameter, which were identified as belonging to the genus *Amphidinium* (Barneah et al., 2007a; Barneah unpublished data). The initial hypothesis that the worms receive their *Symbiodinium* algal symbionts from their coral hosts was examined using denaturing gradient gel electrophoresis (DGGE) profiles of the ITS2 region of *Symbiodinium* derived from coral hosts and resident worms (Barneah et al., 2007a). However, it was found that the corals and the worms possess different phylotypes of *Symbiodinium*, thus suggesting different sources for their symbionts. Histological sections performed on sexually mature worms (Fig. 2b) showed an ovary with oocytes containing the two distinct types of algal endosymbionts within their ooplasm (Fig. 2d, e). Transmission electron microscopy corroborated the presence of algal symbionts within the developing embryos (Barneah et al., 2007b). These findings offer the first definitive evidence of simultaneous maternal transmission of two distinct taxa of dinoflagellate algal symbionts in a triploblastic organism (Barneah et al., 2007b).

The following example is taken as a comparative example from the terrestrial environment. The sharpshooter (*Homalodisca coagulate*) is an important plant parasite that feeds on the xylem fluids of the plant. It harbors two distinct symbiotic bacteria, *B. cicadellinicola* and *Sulcia muelleri*, which are responsible for the selective synthesis of vitamins and cofactors and essential amino acids, respectively (Moran et al., 2005; Wu et al., 2006; Hunter, 2006). The sharpshooter provides the bacteria with the raw carbon-based ingredients that they need. This three-party symbiosis was discovered as obligatory, and the bacteria are often found to coexist in the same cell in adult sharpshooters (Wu et al., 2006). The symbionts are vertically transmitted together in eggs and are housed in a dedicated "bacteriome" within developing sharpshooter nymphs. Phylogenetic studies have shown that the three partners are coevolving (Hunter, 2006).

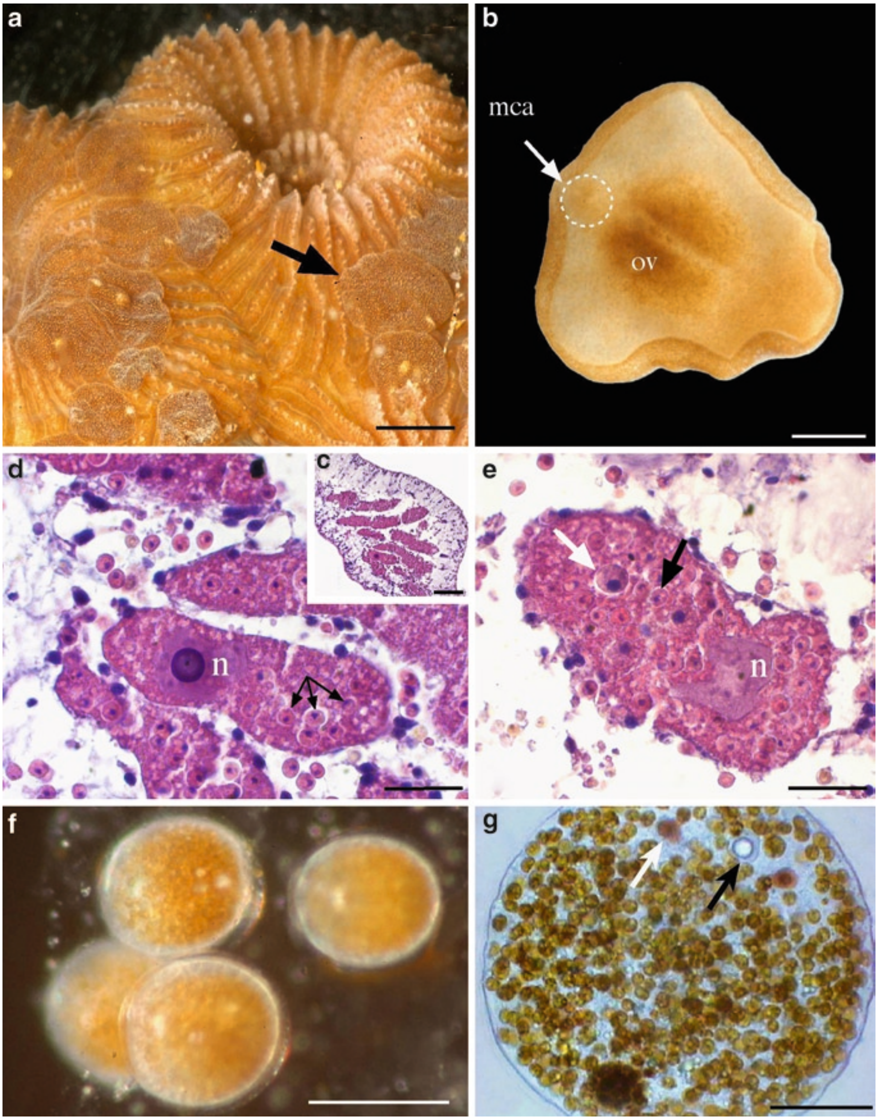

Figure 2. (**a**) The stony coral *Plesiastrea laxa* with *Waminoa brickneri* worms. Scale bar 3 mm. (**b–f**) Stages of sexual reproduction in *W. brickneri*. (**b**) Ventral view of a sexually mature specimen with paired ovary (ov) and the male copulatory apparatus (mca) circled with dashed line. Scale bar = 1 mm. (**c**) Histological section of worm containing gonads, 5 days prior egg laying, showing elongate oocytes. Scale bar = 110 µm. (**d**) Oocyte containing nucleus with prominent nucleolus and algal symbionts (arrows). Scale bar = 30 µm. (**e**) Oocyte containing two symbionts types: *Symbiodinium* sp. (black arrow) and single larger symbiont (white arrow). Scale bar= 30 µm. (**f**) Gelatinous egg mass. Scale bar = 200 µm. (**g**) Worm hatchling containing numerous algal symbionts, statocyst (black arrow), and eyespot (white arrow). Scale bar = 45 µm. (From Barneah et al., 2007b.)

5.2. HORIZONTAL TRANSMISSION

The corals *Acropora tenuis* and *A. millepora* are broadcast-spawning corals that as adults express different specificities for *Symbiodinium* strains. At Magnetic Island (Great Barrier Reef, Australia), adult colonies of *A. millepora* contain *Symbiodinium* strain D, whereas *A. tenuis* adults contain *Symbiodinium* strain C1 and occasionally strain C2 (Little et al., 2004). The production of aposymbiotic larvae by both species provides the opportunity to observe natural patterns of zooxanthellae infection and dynamics (Little et al., 2004). Larvae of *A. tenuis* were raised from spawned gametes, settled onto tiles that were attached to the reef, and were monitored up to 9 months after settlement. Contrary to expectations, it was found that the apparent specificity for strain C1 observed in adult populations of *A. tenuis* was not present in the early stages of the infection. Two distinct *Symbiodinium* strains, D and C1, were acquired by juveniles in the first month. In the subsequent 4 months, the relative abundance of these two strains within the symbiosis changed, with a clear increase in the number of juveniles harboring strain D. The opposite dominance of strains D and C1 in juveniles and adults of *A. tenuis* in the Magnetic Island populations suggests that there may be "active" selection by the host (Little et al., 2004). Similar results were reported by Weis et al. (2001), who examined host–symbiont specificity during symbiosis onset in the planula larvae of the solitary Hawaiian scleractinian *Fungia scutaria*. Such a selection was suggested as a mechanism by which to maximize the symbiont effectiveness, which varies with differences in physiological requirements between juveniles and adult corals (Little et al., 2004). For example, corals may have a higher demand for nutrients when they reach reproductive maturity, leading to a preference for one type to meet increased energy requirements. It is possible that strain C1 persists in the symbiosis at very low densities and is maintained as an undetectable "background" strain (Little et al., 2004). This case study demonstrates that the dynamics of coral–zooxanthellae associations may vary with the changing physiological needs of the host in response to life-history stage requirements (Little et al., 2004). The suggested difference in symbiont effectiveness for the host was proven correct in a different symbiotic system: that of the flatworm *Convoluta roscoffensis* and the prasinophyte algae *Tetraselmis* (Douglas, 1985). The worms reproduce sexually and produce larvae that are aposymbiotic and become infected by feeding on the algae (Douglas, 1985). At one site in the United Kingdom, each animal bears algae of either subgenus *Tetraselmis* or *Prasinocladia*. In natural populations, animals bearing *T.* (*Prasinocladia*) sp. are smaller and less fecund than animals with *T.* (*Tetraselmis*) sp. It was found that *T.* (*Tetraselmis*) sp. releases four times more photosynthate than *T.* (*Prasinocladia*) sp. to the animal (Douglas, 1985), and correlated with this is thus a less-effective symbiont than *T.* (*Tetraselmis*) sp. Under laboratory conditions, juvenile *C. roscoffensis* can form a symbiosis with any species of *Tetraselmis* (*Platymonas* and *Prasinocladius*). When exposed to a mixed suspension of different *Tetraselmis* species, all the algal species are ingested and start to proliferate. However, only one species is retained, while the others are lost from the animal over a period of up to 2 weeks. The transient mixed infection reduces animal growth over 30 days and thus appears to be costly to the animal (Douglas, 1985, 1998).

6. Discussion

Multibiont symbioses are undoubtedly more common than the few examples discovered so far both in the terrestrial and the marine environment. The variety of organisms involved in such symbioses is immense and the types of associations are assorted. A host can be associated with two symbionts at the same organizational level (e.g., coral–algal symbiosis) or different organizational levels (e.g., coral–algae–mussel). The association can be classified as endosymbiosis or can include one endosymbiotic and one ectosymbiotic partner. Furthermore, some associations represent even higher degrees of complexity, like that involving the coral *Montipora erythraea* (and its endosymbiotic algae); the burrowing mussel *Lithophaga purpurea*, found within its skeleton; and the pea crab, *pinnotheres* sp., which infests the mussel and harms its reproduction (Brickner, personal comm.). This association highlights the occurrence of simultaneous mutualistic and parasitic interaction contained in one multibiont symbiosis.

In several symbiotic systems, mainly those in which the symbionts belong to the same organizational level (bacteria, algae, etc.), such as coral–algal symbiosis, the magnitude of the presence of more than one symbiont genotype is only recently gaining gradual exposure, a process directly linked to the progress in molecular techniques. This process has undoubtedly also affected the ability of different researchers to analyze and draw conclusions regarding crucial issues such as the capacity of coral–algal symbiosis to cope with changing environmental conditions. A recent publication (Goulet, 2006) dealing with the ability of corals to change their algal symbionts stated that out of 442 coral species only 23% host multiple zooxanthellae clades, while 77% exhibit fidelity to a narrow subset of a single zooxanthellae clade or to a specific algal genotype. Baker and Romanski (2007) critically reevaluated the same data with the exclusion of coral species that were either under sampled or not adequately defined, and showed that when the analysis is restricted to the species for which sampling has been more significant, over two thirds of all coral species (and almost three quarters of scleractinian coral species) host multiple algal symbiont types at the clade level or below. Moreover, upon looking into within-clade diversity, they found that 20% of the corals that had been documented as hosting only one clade of *Symbiodinium* actually contained multiple types within that clade. These two latter publications, deriving nearly opposite conclusions from one data set, further highlight the obstacles and setbacks still occurring in the research of algal symbiont diversity in coral hosts. It is now evident that technical constraints hinder the efforts to reveal the true diversity of zooxanthellae within a host (Carlos et al., 2000; Kinzie et al., 2001; Baker and Romanski, 2007). Although extensive sampling of invertebrate host and algal symbionts (see Goulet (2006) and Baker and Romanski, (2007)) have been undertaken worldwide, it is assumed that most field surveys published to date are extremely likely to have underestimated within-colony *Symbiodinium* diversity at all taxonomic levels (Baker and Romanski, 2007). If this is indeed the case, then as molecular techniques continue to improve and the "background symbionts" become more easily detectable, we foresee that symbioses currently identified as monomorphic could be reclassified as actually being comprised of multiple genetically different algal symbionts. Thus, it is possible that multibiont associations in coral–algal symbiosis are far more widespread than previously assessed.

The multibiont symbioses that were presented in Section 3 share a common feature: the role of the macrosymbiont (fish, crustacean, polychaete, or bivalve) as an ammonium donor. The contribution of ammonium is beneficial to the coral holobiont via its microsymbionts, which are the actual recipients. Overall, it seems that such multibiont symbioses enable the recycling of nitrogen through the flow of ammonium ions in one direction (to *Symbiodinium*) and amino acids in the other (to the coral host), thus conserving nitrogen in the otherwise nutrient-poor tropical oceans (Muscatine and Porter, 1977).

Established symbioses might respond to environmental changes by switching partners (Buddemeier and Fautin, 1993; Rowan and Knowlton, 1995; Lewis and Coffroth, 2004) or by "shuffling" of existing partners (quantitative change in the relative abundance of existing symbiont communities within colonies; Baker, 2003), as was suggested for the role of bleaching in coral–algal symbiosis. Interestingly, recent literature concerning the coral holobiont and its associated microbial community suggests that the coral animal can also adapt to differing ecological niches by "switching" its microbial partners (Wegley et al., 2007). The metabolic roles of the microbial community associated with specific coral hosts are beyond the scope of this chapter. However, recent experimental data suggests that coral-associated bacteria take part in carbohydrate, protein, sulfur, and nitrogen cycling in the coral animal, and thus are important to the functioning of the holobiont (Wegley et al., 2007). Further studies concerning the metagenomic analysis of the microbial community associated with different coral species (Wegley et al., 2007) will undoubtedly shed new light on the specificity, physiology, stability, and significance of such associations to the coral reef dynamics.

The occurrence of symbioses with multiple genotypes of symbionts raises the question of whether a host with a symbiont population of multiple genotypes derives the same benefit as a host with a genetically uniform population of symbionts (Douglas, 1998; Frank, 1996). Frank (1996) assumed that in the former state, each symbiont genotype is predicted to exhibit more competitive traits, including increased proliferation rates and elevated acquisition of host-derived nutrients, resulting in a depressed performance of the host. There might not, however, be any decisive answer to this question, as every symbiotic system has its own unique characteristics (structural, metabolic, and physiologic). Moreover, it was demonstrated that in juvenile *Convoluta roscoffensis*, the presence of more than one symbiont can cause a transient state of reduced host growth for up to

30 days, by which time only one symbiont will be retained in the worm (Douglas, 1998). Such a scenario might indicate the occurrence of a competitive process. A different scenario is presented by Loram et al. (2007), who showed that the proportion of fixed carbon translocated to the sea anemone *C. gigantean* is not depressed in the mixed infections (of two co-occurring *Symbiodinium* taxa) and suggest that competition between the symbionts seems to be suppressed (Loram et al., 2007). In support of the complexity of this issue, is some experimental data that show evidence for competition between mycorrhizal fungi, resulting in reduced plant performance (Pearson et al., 1993). On the other hand, Newsham et al. (1995) have demonstrated that individual plants may derive distinct benefits from different fungal genotypes.

Browsing through the multibiont symbioses discussed in this chapter and from those taken from the terrestrial environment, the rarity of vertical transmission of symbionts is prominent. Several researchers have argued that mutualistic symbioses evolved from parasitic relationships, and that vertical transmission played a key factor in the reduction of symbiont virulence (Ewald, 1987). Surprisingly, in several mutualistic associations vertical transmission is completely absent, such as in those between plants and mycorrhizal fungi, legumes and rhizobia, and some corals and dinoflagellates. It is expected that all mutualisms must have evolved a perfect vertical transmission if the relationship is truly mutualistic, because hosts may fail to acquire symbionts if these are not vertically transmitted (Genkai-Kato and Yamamura, 1999). Douglas (1995) stated that vertical transmission of symbionts is advantageous to the host, in that the host is assured of gaining a compatible symbiont. Vertical transmission is an obvious trait during asexual reproduction of hosts, whether mediated by fragmentation, binary fission, or specialized asexual propagules. Two factors may limit the incidence of vertical transmission: structural barriers in the host and the cost of vertical transmission. The costs can be two-fold: space and nutrition. Potentially there is also a long-term cost of vertical transmission. The host, presumably, lacks access to a variety of alternative symbiont taxa. Nevertheless, a recent study has demonstrated for the first time the uptake of heterologous zooxanthellae by zooxanthellate primary polyps (with maternally-derived zooxanthellae) of the soft coral *Litophyton crosslandi* (Zurel et al., 2008). Based on a mathematical model, Genkai-Kato and Yamamura (1999) suggested that mutualistic symbiosis without vertical transmission should evolve only when (1) vertical transmission involves some costs in the host, (2) the symbiont suffers direct negative effects if it exploits the host too intensively, (3) the host establishes the ability to make use of waste products from the symbiont, and (4) the mechanism of vertical transmission is controlled by the host. Coral–dinoflagellate relationships have persisted since the Triassic period and are likely to have contributed to the longevity, diversity, and success of this group (Stanley, 2006). From the coral's perspective, horizontal transmission and complex mixtures of symbionts might provide a short-term ecological flexibility to cope with fluctuating physical conditions that outweighs the possible costs of evolutionary conflicts among symbionts (Herre et al., 1999). From all the abovementioned findings and factors, it seems that the modes of symbiont transmission can not serve as a criterion for classifying symbiotic (mutualism, parasitism, etc.) or multibiont symbiotic systems. In support of this assumption comes the study by Marlow and Martindale (2007) of *Symbiodinium* localization and mode of gastrulation in two species of scleractinian embryos: *Fungia scutaria* (with horizontal transmission) and *Pocillopora meandrina* (with vertical transmission). Those researchers determined that both species, independent of whether or not they "seed" their oocytes with symbionts, undergo a "nutritive" stage before gastrulation, wherein lipid-rich cells (*F. scutaria*) or membrane-bound cellular fragments (*P. meandrina*) are passed to the blastocoel, from where they are subsequently taken up by the definitive endoderm. This emergent property of anthozoan development appears to have been co-opted to facilitate the movement of *Symbiodinium* to the blastocoel (future site of the endoderm) in the seeded species, where they are later phagocytosed by the newly formed definitive endoderm. Unfortunately, data concerning the modes of transmission of other symbionts taxa in the coral holobiont are still lacking. Future studies focusing on morphological adaptations and the recognition process among the partners taking part in multibiont symbioses may lead to a better understanding of the true nature of symbiosis.

Multibiont symbioses constitute an extremely complex phenomenon and therefore are hard to categorize with simplified titles. Currently it would appear that "digging deep enough" could eventuate in identifying more and more partners in one symbiotic system, much like a Russian "Babushka" doll. On top of structural complexity, metabolic and physiologic aspects can not be ignored. The resolution of the search will also affect its complexity; hence if one looks for phage/virus inside bacteria, inside algae, inside a worm that is living on a coral. The intricacies of such associations appear endless. Multibiont symbioses, the puzzle of nature, are fascinating, intriguing, and challenging.

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