Chapter 10
Animal–*Symbiodinium* Symbioses: Foundations of Coral Reef Ecosystems

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Abstract A variety of animal taxa have benefited from symbioses with photoautotrophic symbionts that provide fixed carbon in exchange for nutrients and highlight habitats. Corals are one such animal lineage, harboring dinoflagellates in the genus *Symbiodinium*. This genus has remarkably high genetic diversity that translates into morphological, cellular, physiological, and even host infectivity differences. Many corals acquire their symbionts as larvae or recruits upon settlement on the reef, likely from local populations of *Symbiodinium* present in the sediment. Onset of symbiosis is initiated by host–symbiont recognition and proceeds through a winnowing process where only suitable, healthy *Symbiodinium* are retained within a host-derived symbiosome membrane. High stress, as is predicted by future climate models, can lead to symbiosis dysfunction and loss of the symbiont from host tissues with negative consequences to the host. Some corals have the ability to exchange their symbiotic partners for those that may be more thermotolerant, but the extent to which coral–dinoflagellate symbioses can acclimatize and adapt to rapid climate change remains to be determined.

10.1 Introduction to Animal–Algal Symbioses

Eukaryotes have acquired the metabolic benefits of photosynthesis multiple times throughout evolution via primary endosymbiosis of photosynthetic cyanobacteria or secondary endosymbiosis: that is acquisition of a unicellular photosynthetic eukaryote that itself had previously acquired a cyanobacterial symbiont (Venn et al. 2008). Some of these acquisitions are ancient whereby the symbionts evolved into modern plastids with extensive genome reduction and migration of genomic material to host nuclei. Many others, including the lichenized fungi, animal–algal, and protist–algal symbioses, are more recent and remain interactions between organisms with separate, but closely interacting genomes.

Animal–algal and protist–algal symbioses are highly diverse and defy simple categorization. In all cases, however, the symbiosis is centered around nutritional

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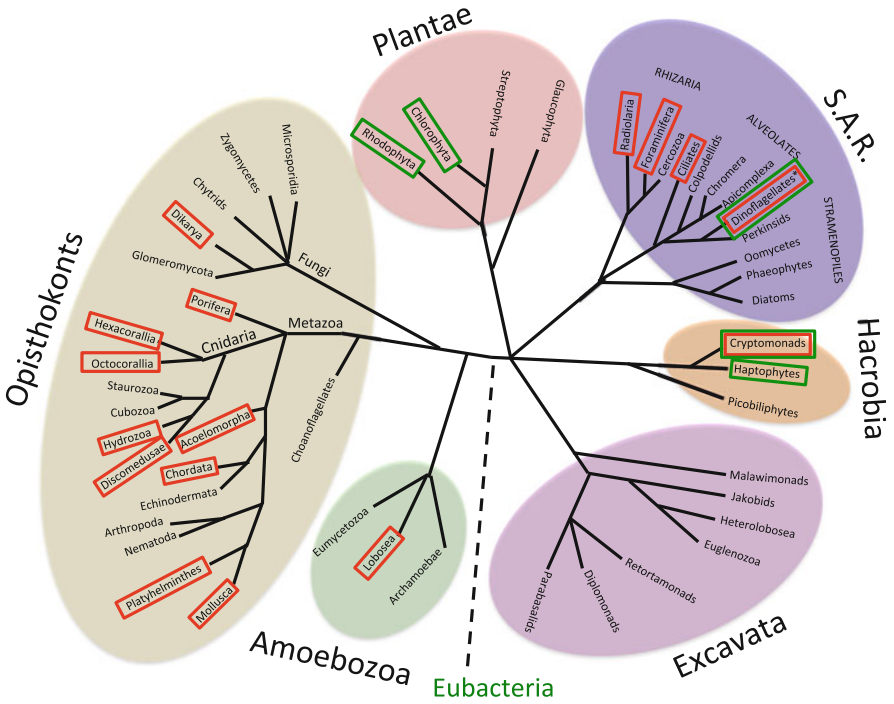

Fig. 10.1 Eukaryotic tree of life cladogram. Algal symbionts are denoted in *green text* and hosts are contained within *red boxes*

exchange, with the photosynthetic symbionts providing reduced organic carbon to the host in return for inorganic nutrients and a high-light habitat. Algal symbionts arise from a variety of prokaryotic and eukaryotic taxa (Fig. 10.1). Animal host taxa are limited to sponges, cnidarians, flatworms, and a very few mollusks and chordates (even a few amphibian species, Kerney 2011). Protozoan host groups include the Rhizaria and ciliates. Venn and coworkers (2008) note that algal symbioses develop in animal groups that can provide structures with a very high surface-area-to-volume ratio to allow for housing symbiont populations in a high-light environment. Symbionts vary from epi- to endosymbionts. Furthermore, some endosymbionts are housed intracellularly in host cell vacuoles, while others are housed in extracellular spaces.

The most prevalent, abundant, and ecologically significant animal–algal symbioses are those involving the dinoflagellate *Symbiodinium* spp. These include the reef-building corals, which form the trophic and structural foundation for coral reef ecosystems, one of the most productive, biodiverse, and threatened on the planet. This chapter will focus on symbioses involving *Symbiodinium*. There is a great deal of research on *Symbiodinium* symbioses that has been performed in the last 25 years and there are numerous reviews aimed at a variety of aspects of this large field (Table 10.1). In this chapter, we aim to provide an integrative view of *Symbiodinium* biology and its symbioses including (1) an overview of

Table 10.1 Summary list of reviews on *Symbiodinium* biology and symbiosis in the last 15 years

Topic	References
Diversity and systematics	Stat et al. (2012)
Flexibility and specificity of the partnership	Baker (2003), Coffroth and Santos (2005), Pochon and Pawlowski (2006), Goulet (2006), Stat et al. (2006), Fay and Weber (2012)
Cell biology and physiology of *Symbiodinium* symbioses	Muller-Parker and Davy (2001), Weis et al. (2008), Yellowlees et al. (2008), Venn et al. (2008), Gordon and Leggat (2010), Fransolet et al. (2012), Davy et al. (2012), Hill and Hill (2012), Lesser et al. (2013)
Genomics	Leggat et al. (2011), Meyer and Weis (2012)
Mechanisms of coral bleaching	Lesser (2006), Weis (2008), Baird et al. (2009a), Lesser (2011)
Dinoflagellate genomic organization	Wisecaver and Hackett (2011)

Symbiodinium symbioses; (2) systematics, diversity, and genome structure; (3) life history and population genetics; (4) mechanisms of inter-partner recognition; (5) host–symbiont specificity; and (6) global change biology.

10.2 Overview of *Symbiodinium* Symbioses

Symbiodinium was first described in the late nineteenth century by Brandt and is now recognized to be a highly diverse genus (reviewed in Coffroth and Santos 2005). *Symbiodinium* spp. are resident in a variety of hosts: foraminifera (Pochon and Pawlowski 2006), ciliates (Lobban et al. 2005), and members of the animal phyla Porifera, Acoelomorpha, Cnidaria, and Mollusca. In sponges, *Symbiodinium* predominantly associates with boring demosponges (Carlos et al. 1999; Hill et al. 2011) and enhances host reproduction, growth, and bioerosion rates (Hill 1996; Rosell and Uriz 1992; Schönberg 2006) by translocating photosynthetically fixed carbon to the host (Weisz et al. 2010). Acoel flatworms (Acoelomorpha), which are epibionts on corals, harbor *Symbiodinium* in their mesodermal parenchyma cells. Interestingly, the *Symbiodinium* types resident with the epibiont flatworm and hosting coral are not necessarily the same (Barneah et al. 2007). Mollusks that harbor *Symbiodinium* have evolved complex structures to support their symbionts. In the giant clam *Tridacna* spp., *Symbiodinium* cells reside within a specialized tubular system inside the mantle tissues (Norton et al. 1992). The branched tubes and the hypertrophied mantle result in a highly increased surface area, thereby providing maximum light exposure for symbiont photosynthesis. This complex tubular system atrophies in bleached (aposymbiotic) individuals (Norton et al. 1995). In nudibranchs, *Symbiodinium* cells reside in the high-surface-area digestive diverticula that extend into dorsal cerata. The slugs can derive nutrition

from the symbionts, either by selective digestion or from transfer of fixed carbon from the symbionts (Kempf 1984). For a current, in-depth review of molluscan–algal symbioses including those with *Symbiodinium*, see Vermeij (2013).

The most prevalent and best studied *Symbiodinium* symbioses occur in cnidarians and are associated with scyphozoans (jellyfish), hydrozoans (fire corals), and numerous anthozoans including all orders within the anthozoan subclass Hexacorallia (Baker 2003). We will focus primarily on the symbiosis between *Symbiodinium* spp. and stony corals (Hexacorallia: Scleractinia) which are the foundational species creating tropical reef ecosystems and soft corals (Octocorallia) which are numerically dominant in many shallow-water reefs in the Caribbean. Coral reefs are among the most productive ecosystems on the planet. They provide important ecosystem services and are critical economic anchors to many nations where they serve as reservoirs of biodiversity, habitat for fisheries, barriers against storms, and destinations for ecotourism. Because corals are the trophic and structural foundation of these ecosystems, coral fitness and health, including a balanced relationship between the host and its *Symbiodinium* partners, is critical to the overall health of the ecosystem (Weis and Allemand 2009).

Symbiodinium interactions in cnidarians range from obligate to facultative. For example, many Scleractinia–*Symbiodinium* partnerships appear to be highly obligate. When the partnership breaks down during stress, a process known as coral bleaching, failure to reacquire a complement of symbionts is often lethal (Hoegh-Guldberg et al. 2007). Many *Symbiodinium* types, especially those in clade C (see ahead), appear to be engaged in symbioses that are highly obligate and specialized for one or a very few host taxa. These types cannot be cultured outside of their host and do not appear to have a free-living life history stage. In contrast, *Symbiodinium* types facultatively associate with the North American Pacific coast sea anemone *Anthopleura elegantissima* (Actiniaria). Anemones can remain symbiont-free, in low-light environments such as caves or under rock shelves (Muller-Parker and Davy 2001). In a laboratory setting, other anemones including *Aiptasia* spp. (a commonly studied model organism) can be maintained in an aposymbiotic state (Weis et al. 2008). Likewise, many, but not all, strains of *Symbiodinium* can be cultured outside of their host.

The majority of protist–*Symbiodinium* and animal–*Symbiodinium* associations display horizontal transmission whereby the host partner acquires symbionts anew from the environment with each host generation. The inter-partner recognition mechanisms that take place during onset of symbiosis which result in host–symbiont specificity are discussed in some detail ahead. A significant minority of *Symbiodinium* symbioses display vertical transmission where symbionts are transmitted directly from host parent to offspring, most often by invasion by *Symbiodinium* into oocytes. In scleractinians, for example, 85 % of symbiotic corals display horizontal transmission compared to just 15 % that transmit symbionts vertically (Baird et al. 2009b). These contrasting transmission modes are a topic that has interested the general field of symbiosis for decades as they represent

markedly different evolutionary strategies (Douglas 2010). Horizontal transmission, thought to be the ancestral condition, allows for the potential of shuffling or switching of partnerships to adapt to changing environmental conditions (Douglas 1994). However it might come at the cost of managing potential negative interactions and cheating. In contrast, vertical transmission, considered the derived condition, allows for tight coevolution of the partnership but may result in a decreased ability to respond to environmental change and increased genetic bottlenecking.

10.3 Systematics, Diversity, and Genome Structure of *Symbiodinium*

Dinoflagellates in general and *Symbiodinium* in particular are fascinating in that they break the rules of biology in almost every category. Modern genomic approaches are adding new and very interesting information to our basic understanding of dinoflagellates and *Symbiodinium*. It is worth describing these developments, because this information enriches the understanding of the symbioses in which *Symbiodinium* engages and points to the many challenges in studying these protists.

10.3.1 Systematics

Several monophyletic supergroups have recently been constructed within the eukaryote domain (Fig. 10.1, Adl et al. 2012). Dinoflagellates fall within the supergroup *SAR* that describes the monophyly of the *S*tramenopiles (e.g., diatoms), *A*lveolata (e.g., ciliates, apicomplexans, and dinoflagellates), and *R*hizaira (e.g., radiolarians and foraminiferans) (Adl et al. 2012; Burki et al. 2008). All alveolates contain membranous sacs, termed alveoli, adjacent to the plasma membrane (Cavalier-Smith 1991). Within this group, dinoflagellates are sister to the apicomplexans, which include several well-known human endoparasites such as *Toxoplasma gondii*, *Cryptosporidium* spp., and *Plasmodium falciparum*, a causative agent of malaria (Fast et al. 2002).

Dinoflagellates are united by possessing two heterodynamic flagellae, which beat with different rhythms and patterns, and a unique nucleus, termed a dinokaryon, which contains permanently condensed chromosomes that lack nucleosomes (Fensome et al. 1999). *Symbiodinium* is further classified as part of the order Suessiales, which is supported by recent phylogenetic studies (Bachvaroff et al. 2014; Gottschling and McLean 2013). This order contains a small number of extant free-living taxa described to date including four *Biecheleria* (=*Woloszynskia*) spp., *Gymnodinium* (=*Protodinium*) *simplex*, and *Polarella glacialis* (Fig. 10.2, Moestrup et al. 2009).

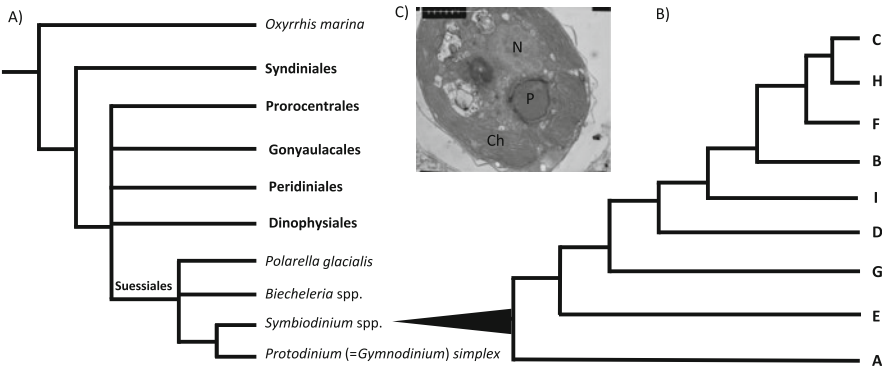

Fig. 10.2 Cladograms depicting the evolutionary relationships of the (**a**) dinoflagellates and (**b**) *Symbiodinium*. Monophyletic clades within *Symbiodinium* are differentiated by small subunit (18S) ribosomal DNA sequences. (**c**) Transmission electron micrograph of clade A *Symbiodinium in hospite* (i.e., living inside the host cell). The chloroplast (Ch) and attached pyrenoid (P) are denoted along with the nucleus (N) with permanently condensed chromosomes

10.3.2 Diversity

Symbiodinium was once described as a single pandemic species, *S. microadriaticum*, that associated with numerous cnidarian species (Freudenthal 1962). Molecular evidence has since revealed tremendous diversity within the genus. *Symbiodinium* divides into nine monophyletic clades, A-I, based on small subunit ribosomal (18S rDNA) sequence variation, and includes two clades, H and I (Fig. 10.2), which are found exclusively in foraminifera (Pochon and Gates 2010). Using 18S rDNA sequences, there is higher sequence divergence between *Symbiodinium* clades (~3 % nucleotide substitution) than between separate orders of free-living dinoflagellates (Rowan and Powers 1992) indicating high diversity within the genus. Several additional molecular markers from all three genetic compartments have been developed to address both evolutionary and ecological questions (Table 10.2). Genomic and cellular differences between *Symbiodinium* clades accompany these genetic differences. *Symbiodinium* genomes range in size from 1.5 to 4.8 pg/cell (LaJeunesse et al. 2005a) and contain varying numbers of chromosomes (Blank and Trench 1985). In addition they have some of the smallest dinoflagellate genomes (e.g., some dinoflagellate genomes top 240 pg/cell). Differences in chromosome number observed in light and electron micrographs have been suggested as means for differentiating species (Blank and Trench 1985). However, karyotyping data should be interpreted with caution due to the chromosome structure of *Symbiodinium* and dinoflagellates in general. Condensed chromosomal regions are connected by difficult-to-visualize "thin fibrils" of DNA that can lead to an overestimation in counts (Udy et al. 1993).

Symbiodinium diversity is also evident in differences in morphology, biochemistry, physiology, and ecology at the clade and subclade level. Cell sizes range between 5 and 19 μm, and different coral hosts harbor different-sized

Table 10.2 List of molecular markers used to determine phylogenetic relationships or genotype *Symbiodinium* spp. organized by genomic compartment

Genomic markers	References
Nucleus	
18S small subunit ribosomal DNA	Rowan and Powers (1992)
28S large subunit ribosomal DNA	Sampayo et al. (2009)
Internal transcribed spacer (ITS) region of rDNA	Sampayo et al. (2009)
Elongation factor (*elf*) 2	Pochon et al. (2012)
Calcium-modulated protein (*calmodulin*)	Pochon et al. (2012)
Damage checkpoint protein (*rad*24)	Pochon et al. (2012)
Actin	Pochon et al. (2012)
Microsatellite flanking regions	Santos et al. (2004)
Mitochondrion	
Cytochrome oxidase I (*cox*I)	Takabayashi et al. (2012)
Cytochrome oxidase III (*cox*III)	Pochon et al. (2012)
Cytochrome *b* (*cob*1)	Sampayo et al. (2009)
Chloroplast	
23S large subunit ribosomal DNA	Sampayo et al. (2009)
Photosystem II protein D1 (*psb*A)	Barbrook et al. (2006)
Noncoding *psb*A minicircle regions	Barbrook et al. (2006)

Symbiodinium, which correlate well with genetic similarity as assessed by isozyme profile (Schoenberg and Trench 1980a; Wilkerson et al. 1988). Cell size, in combination with phylogenetic, ecological, and physiological data, was used to differentiate between recently described species of *Symbiodinium*, indicating the presence of many undescribed species (Jeong et al. 2014; LaJeunesse et al. 2012). Although cells appear similar *in hospite* (i.e., living inside the host cell), there are differences in the ultrastructure and cellular morphology observed when *Symbiodinium* is cultured from different hosts (Blank and Trench 1985; Jeong et al. 2014; Schoenberg and Trench 1980a; Trench and Blank 1987). There are also biochemical differences in sterol composition, mycosporine-like amino acid (MAA) synthesis, photosynthetic pigments and associated proteins, and antioxidants (Banaszak et al. 2000; Blank and Trench 1985; Iglesias-Prieto et al. 1991; Steinke et al. 2011; Withers et al. 1982). Furthermore there are ecological differences among *Symbiodinium* types including motility and infectivity (Fitt and Pardy 1981; Schoenberg and Trench 1980b). Infectivity is a useful phenotype to assess, as it is the first step in the establishment of the symbiosis.

Given the molecular differences observed between *Symbiodinium* types, it is not surprising that significant physiological diversity exists within the genus at both the clade and subclade level. This includes differential photosystem function and repair, cellular growth and division, and heat tolerance, the ramifications of which will be discussed below (Iglesias-Prieto et al. 1992; Ragni et al. 2010; Robison and Warner 2006; Rowan 2004; Tchernov et al. 2004; Wang et al. 2012). The cellular causes of these phenotypic differences are only beginning

to be understood, but significant progress has been made in the last 10 years. For example, lipid composition of plastid thylakoid membranes can differentiate membrane stability and corresponding heat tolerance within clades of *Symbiodinium* (Tchernov et al. 2004). Because *Symbiodinium* is able to modify membrane lipid content to increase thermal stability in the warmer summer months and during acclimatization periods, lipid composition is of limited diagnostic applicability as a biomarker for determining thermotolerance (Díaz-Almeyda et al. 2011; Hill et al. 2009). Photophysiological differences have also been attributed to the differential damage and subsequent repair of the D1 protein, a protein component of photosystem II (Warner et al. 1999). In a final example, there are strain-specific differences in photoprotection afforded by UV light absorbing MAAs and cyclic electron transport (CET) which can dissipate excess energy to prevent photodamage (Banaszak et al. 2000; Reynolds et al. 2008). The combination of CET and MAAs in some clade A *Symbiodinium* has been hypothesized to explain its association with corals in shallow-water, high-light environments (Reynolds et al. 2008).

One of the current challenges in understanding the diversity of *Symbiodinium* is their categorization into meaningful functional groups, including determining species boundaries. Currently there are ~20 named species of *Symbiodinium*, but half are *nomina nuda* in need of proper species descriptions (for comprehensive list see LaJeunesse et al. 2012). Progress has lagged due to few morphological differences *in hospite* and the inability to grow many *Symbiodinium* types in culture (reviewed in Stat et al. 2012). Cultured isolates often differ genetically from the numerically dominant symbiont in vivo (LaJeunesse 2001; Santos et al. 2001). To avoid this culturing bias, researchers have successfully cultured *Symbiodinium* that was representative of the numerically dominant type by variously adding whole host tissue homogenates and other additives to each tested medium (Ishikura et al. 2004; Krueger and Gates 2012). These additives included amino acids, which were able to sustain C15 *Symbiodinium* isolated from the coral *Porites compressa* in culture for up to 2 weeks albeit with limited growth (Krueger and Gates 2012). These studies are necessary as isolating *Symbiodinium* strains representative of the dominant strain in the host is the first step for characterization.

10.3.3 Genome Structure

Despite the immense size of dinoflagellate genomes in general, a draft version of one of the smallest genomes, *Symbiodinium minutum* type B1 (LaJeunesse et al. 2012), has recently been published, yielding insights into its unusual genomic architecture (Shoguchi et al. 2013). Its 1.5 Gb genome contains approximately 42,000 predicted genes clustered in gene-rich regions that make up less than half of the genome. This leaves large regions of noncoding DNA, also described in other dinoflagellates (McEwan et al. 2008). In addition, genes are usually arranged unidirectionally, a phenomenon that is unusual in eukaryotes. As with other dinoflagellates, gene duplication is a dominant feature of the genome. An estimated

42 % of the encoded genes are predicted to have arisen by duplication. Some of these genes are present in tandem arrays with multiple copies in parallel orientation. This may be important for regulating transcription levels as several expressed genes are polycistronic (Boldt et al. 2012), a feature seen in other dinoflagellates that correlates to gene expression (Bachvaroff and Place 2008). An additional *Symbiodinium* genome has been published since writing this review. See Lin et al., 2015, *Science* 350:691-694.

To package, transcribe, and replicate these large genomes, dinoflagellates have evolved unique nuclear organization. Dinoflagellate DNA is permanently condensed in a cholesteric liquid crystalline state, unique among eukaryotes and similar to bacterial packaging (Teif and Bohinc 2011). This condensation arrangement decreases the amount of protein interacting with the DNA by tenfold (Chow et al. 2010; Rizzo and Noodén 1972). This led to a canonical view that dinoflagellates lack histones (Fensome et al. 1999) instead relying on histone-like proteins (HLP) that may be prokaryotic and viral in origin (Chan et al. 2006; Wong et al. 2003). However the sequenced *Symbiodinium* genome, as well as several dinoflagellate transcriptomes, confirms the presence of a full set of eukaryotic histone genes (Roy and Morse 2012; Shoguchi et al. 2013). Interestingly, expressed histone proteins were found to be below detection thresholds within the cells, and therefore it is unlikely that histones contribute significantly to the condensation of chromosomes (Roy and Morse 2012).

In contrast to the large nuclear genome, dinoflagellate plastids and mitochondria contain some of the smallest known functional genomes, coding for relatively few proteins (reviewed in Wisecaver and Hackett 2011). For example, the plastid genome of peridinin dinoflagellates consists of approximately 15 small plasmids or minicircles, 1–3 kbp in size. Recently the plastid genome of *Symbiodinium* type C3 was sequenced from a Caribbean *Agaricia* sp. (Barbrook et al. 2014). This plastid genome contains single genes, compared to multiple genes per minicircle observed in other dinoflagellates. This study also uncovered accelerated rates of plastid protein evolution, especially those involved in the light-harvesting complex of photosystem I. The authors hypothesize that this may explain photophysiological differences between *Symbiodinium* types. In addition, the noncoding regions of the minicircles have been used to differentiate *Symbiodinium* at the subclade level (Fig. 10.2 and Table 10.2) and, in combination with ecological data and other markers, to distinguish and describe new species of *Symbiodinium* (see below, LaJeunesse and Thornhill 2011).

10.4 Life History and Population Genetics

Symbiodinium spp. are found within numerous hosts on a reef, and to avoid confusion, for this chapter, we have adopted the nomenclature of Bush et al. (1997) to distinguish between different levels of populations. All *Symbiodinium* present within a coral colony represent the "infrapopulation," whereas all present on a given reef are encompassed by the "suprapopulation." When describing the

presence of individual *Symbiodinium* or populations in space, "location" refers to different regions within a colony (e.g., top, bottom, etc.), while "locality" will refer to the geographic locale or reef.

Symbiodinium alternates between an in vivo vegetative state and a motile zoospore (dinomastigote) stage, which occurs in culture and likely outside the host (reviewed in Stat et al. 2006). The vegetative stage is haploid (Santos and Coffroth 2003) and capable of undergoing binary fission with karyokinesis (division of nuclei) occurring prior to cytokinesis, as evidenced by the presence of binucleate cells (Kevin et al. 1969). Sexual isogametes, first described in the original species description (Freudenthal 1962), were observed in culture a few years later, but not since (Kevin et al. 1969). These observations are corroborated by molecular studies indicating that sexual reproduction is occurring (Andras et al. 2011; Chi et al. 2014; LaJeunesse 2001; Santos and Coffroth 2003; Wirshing et al. 2013). Using microsatellite markers, there are detectable differences between *Symbiodinium* suprapopulations (Santos et al. 2003; Thornhill et al. 2009; Wirshing et al. 2013) and even infrapopulations at a given locality (Andras et al. 2011; Howells et al. 2009; Kirk et al. 2009; Wirshing et al. 2013). This widespread diversity, coupled with mixing of alleles in linkage equilibrium, is consistent with the presence of sexual reproduction. In another approach to investigating the possibility of sexual reproduction in *Symbiodinium*, six meiosis-specific and 25 - meiosis-related genes were recently mined from the *S. minutum* genome and two additional *Symbiodinium* transcriptomes (Chi et al. 2014). Although these genes could serve other cellular purposes, these data certainly support the occurrence of sexual recombination.

Asexual reproduction of *Symbiodinium* occurs in the host, although at a slower rate than in culture (Davy et al. 2012) with the end result of one or a few symbiont genotypes populating a host (Goulet and Coffroth 1997). Symbiont population regulation *in hospite* has been of interest to researchers for years; however it remains only partially understood (Davy et al. 2012; Hill and Hill 2012). Strategies for symbiont regulation by the host include nitrogen, phosphorus, and potentially calcium limitation, digestion and expulsion of symbionts, and even selective expulsion of dividing cells.

Symbiodinium has been found in the environment. It has been identified in sediment and macroalgal samples taken from reef environments in the Pacific and Caribbean with densities reaching 4000 cells/mL (Coffroth et al. 2006; Littman et al. 2008; Takabayashi et al. 2012). *Symbiodinium* has also been detected in the water column above coral reefs (Manning and Gates 2008; Takabayashi et al. 2012; Yamashita et al. 2013); however densities are lower (80 cells/mL) than in sediments. This difference in cell density between benthic and water column locations is consistent with the high genetic differentiation of *Symbiodinium* populations across and even along reef tracks observed in microsatellite analyses (Andras et al. 2011; Howells et al. 2009; Kirk et al. 2009; Santos et al. 2003; Thornhill et al. 2009). This indicates low population connectivity, which could be explained by factors such as low migration and recruitment success.

Cnidarian hosts that acquire their symbionts via horizontal transmission must acquire them during one or more life-history stages. Both larvae and juvenile

polyps (coral recruits) have been observed to acquire symbionts in the laboratory but it is not clear at which stage symbiosis onset occurs in nature (Baird et al. 2009b). Species that initiate symbiosis as recruits may be exposed to relatively high densities of *Symbiodinium* in the benthos. Indeed, providing recruits and larvae with sediment during experimental colonization experiments in the laboratory helps speed colonization compared to seawater alone (Adams et al. 2009; Cumbo et al. 2013).

Although new aposymbiotic recruits are likely exposed to a benthic population derived from the adult population in a given locality, in some cases, the symbiont types acquired by juvenile corals are not the same as those of adults nor are they the most abundant types sampled from the environment (Poland et al. 2013; Yamashita et al. 2013). This process of obtaining suitable hosts from the environment does not appear to be specific to transmission process as even recruits harboring vertically transmitted symbionts are capable of acquiring symbionts from the environment (Byler et al. 2013). Host colonization is complex, dynamic, and potentially successional, resembling sequential habitation of a host by different symbiont types throughout the ontogeny of the host (Poland et al. 2013).

Not all *Symbiodinium* populations are genetically differentiated, and symbionts that are vertically transmitted between parent and offspring can be connected across large geographic distances. For example, symbiont populations associated with the brooding coral *Pocillopora damicornis*, which has vertically transmitted symbionts, are not structured across thousands of km^2 of area in the Eastern Pacific (Pettay and LaJeunesse 2013). *Symbiodinium* is also likely transmitted within a reef by paratenic (transport) hosts as evidenced by viable *Symbiodinium* detected in the feces of corallivorous fish and nudibranchs (Castro-Sanguino and Sánchez 2012; Muller-Parker 1984). These coral predators are capable of movement around reefs and contribute to the environmental populations discussed above. Finally, humans have likely contributed to the movement of *Symbiodinium* around the globe. *Symbiodinium* have been detected in the ballast water of ships arriving in Hawaii that had originated in multiple ports in the Pacific Ocean (Stat and Gates 2008). Vectored transport is also a possible explanation for the low genetic diversity associated with the sea anemone *Aiptasia* spp. and their *S. minutum* symbionts across several ocean basins (Thornhill et al. 2013).

10.5 Mechanisms of Inter-partner Recognition

The processes surrounding the initial onset of symbiosis and those that lead to persistence of *Symbiodinium* in host cnidarian cells have been subjects of interest to researchers for decades. Significant advances in genomic resources and the cell biology of some model systems have dramatically improved our understanding of the processes of inter-partner recognition. This topic has been recently extensively reviewed (Davy et al. 2012) and therefore we will only briefly summarize this area here.

As mentioned above, the majority of cnidarian–dinoflagellate partnerships are established anew with each host generation. The partners must therefore have a set of inter-partner recognition processes that result in establishment of the diverse sets of host–symbiont combinations observed in the natural world. This cascade of events, termed "the winnowing," involves a diverse array of biological processes, ranging from cell–cell interactions to inter-symbiont competition for the host intracellular niche, all of which are necessary but none of which alone are sufficient to result in specific host–symbiont combinations (Nyholm and McFall-Ngai 2004).

The host innate immune response to an invading microbe and the ability of *Symbiodinium* to modulate this response to survive and thrive within animals hosts have been the focus of a recent genomic and cell biology research. Based on genomic studies, invertebrates are now known to possess innate immune repertoires that are as complex and sophisticated as the well-understood mammalian innate immune system (Miller et al. 2005; Shinzato et al. 2011). In the context of cnidarian–dinoflagellate mutualisms, initial contact between host and symbiont has the trappings of an immune response. The very first interactions are likely classical microbe–host and cell–cell interactions so well described in other microbe–animal associations as *MAMP–PRR* (*m*icrobe-*a*ssociated *m*olecular *p*atterns–*p*attern *r*ecognition *r*eceptor) interactions (Janeway and Medzhitov 2002). All microbes display *MAMPs*, which are taxon-specific molecular patterns on their cell surfaces and include glycans, lipopolysaccharide, and peptidoglycan. Host cells recognize *MAMPs* using a diverse array of *PRRs* (McGuinness et al. 2003). *MAMP–PRR* interactions act as lock-and-key mechanisms that can launch downstream innate immune responses in the host cell. Beneficial microbes and some parasites and pathogens have evolved mechanisms to evade or co-opt this immune response for their benefit. There is now ample functional and genomic evidence that *Symbiodinium* glycan–host lectin interactions play a role in recognition. *Symbiodinium* spp. have a variety of glycans on their cell surfaces, depending on phylotype and strain (Bay et al. 2011; Logan et al. 2010; Wood-Charlson et al. 2006). Cnidarians have a huge variety of lectins, including C-type lectins typically involved in immune responses, in their genomes (Kvennefors et al. 2008; Vidal-Dupiol et al. 2009; Wood-Charlson and Weis 2009). Manipulation of glycan–lectin interactions can interfere with onset of symbiosis, and lectins have been localized to the host-derived membrane (symbiosome) surrounding symbionts in corals (Kvennefors et al. 2008; Wood-Charlson et al. 2006). Despite these advances, we still have only a glimpse of the role of glycan–lectin interactions in symbiosis recognition. Future studies in glycomics that compare the glycome of different symbiont types and align glycome profiles with host–symbiont specificity will result in a deeper understanding of the role of these interactions in the winnowing process. In addition, other *MAMP–PRR* interactions in cnidarian–dinoflagellate symbioses await discovery.

Cnidarian host gastrodermal cells are nutritive phagocytes and acquire invading *Symbiodinium* by phagocytosis. As part of a typical immune response to an invader, engulfed particles in phagosomes are destined for destruction, via an elaborate, highly conserved endosomal trafficking pathway, leading to the fusion of

phagosomes with lysosomes, which bathe the particle in degradative enzymes and a highly acidic environment (Davy et al. 2012). However, in the case of *Symbiodinium*, rather than being destroyed, it persists and ultimately thrives and divides within host cells (Colley and Trench 1983; Schwarz et al. 1999), a behavior that various other intracellular parasites including the prokaryote *Mycobacterium* spp. and the apicomplexan *Toxoplasma* spp. also exhibit (Davy et al. 2012). The signaling mechanisms that modulate the cnidarian host immune response are at present only partially understood but are thought to include the Rab GTPases, signaling gatekeepers in the trafficking process that guide phagosomal maturation (Chen et al. 2003, 2004). Work in the model anemone *Aiptasia* has shown that the presence of symbionts promotes tolerogenic signaling via the TGFβ pathway and sphingosine rheostat in the host (Detournay and Weis 2011; Detournay et al. 2012). This signaling is hypothesized to stabilize the partnership and discourage host immune attack of the symbiont. The specific *Symbiodinium* effectors, or molecules that promote colonization, remain completely undescribed. The recent publication of the first *Symbiodinium* genome is likely to generate candidate effectors for empirical testing in model systems (Shoguchi et al. 2013).

Once the partnership is established, the mechanisms driving the dynamic homeostasis of the relationship remain unknown. Although there is strong evidence that there is coordination of cell cycles and control of symbiont population size and host growth (Davy et al. 2012; Hill and Hill 2012), the regulatory mechanisms driving the process remain undescribed. The currencies of organic carbon moving from symbiont to host and inorganic nitrogen moving from host to symbiont could be signalers; however these hypotheses await future investigation.

The cellular and molecular mechanisms underlying coral bleaching, or the breakdown of the symbiosis due to environmental stress, have been extensively investigated and are beyond the scope of this review (see Table 10.1 for summary of reviews). To briefly summarize the growing extensive literature, bleaching is a result of symbiosis dysfunction involving (1) high amounts of reactive oxygen species, generated from both partners, that both damage the partners and act as immune response signalers and (2) the awakening of a host innate immune response against a compromised symbiont and resulting symbiont expulsion, by one or more means. The differential bleaching response displayed by different host–symbiont combinations and whether partnerships can adapt to or acclimatize to a changing environment are a major focus in the field of coral biology and are discussed further ahead (Palumbi et al. 2014).

10.6 Specificity

Specificity, defined as the taxonomic range of partners with which an organism associates (Douglas 2010), is an area of animal–*Symbiodinium* symbiosis that has been completely transformed by the advent of modern molecular genetic techniques. This area of study remains dynamic, fast moving, and controversial, due in part to the rapidly changing nature of molecular technologies. However despite the

contentions in the field, we will summarize here the resulting common themes and patterns that have emerged.

Many corals harbor one or a very few dominant *Symbiodinium* types (Coffroth and Santos 2005), but also host background populations detectable only with sensitive genetic monitoring such as next-generation amplicon sequencing, sequencing of cloned ITS (internal transcribed spacer) regions, and qPCR (e.g., Mieog et al. 2007; Silverstein et al. 2012). However, some coral species can form symbioses with multiple dominant *Symbiodinium* types that can vary depending on depth, thermal history of the reef, and locality (reviewed in Baker 2003). Host–symbiont partnership combinations can also be very stable. For example, corals can retain their symbionts in aquaculture and new environments. Corals kept in aquarium systems retain the field-associated symbiont types even if additional types are present in the tanks (Hartle-Mougiou et al. 2012). Likewise, *Fungia scutaria* colonies originally transplanted to a Caribbean reef in the 1960s still harbored the same symbiont type C1b found in conspecifics in their native range after 35 years, indicating remarkable longevity and specificity (LaJeunesse et al. 2005b).

Symbiodinium types can be categorized as generalists, those that associate with a variety of host species, and specialists, those that are found in one or a very few host species. For example, some of the basal lineages in clades B and C, such as types B1, C1, and C3, can associate with multiple coral hosts, whereas types B7 and C57a have only been found associated with *Madracis* spp. and *Millepora* spp., respectively (LaJeunesse 2005; LaJeunesse et al. 2010a). A complete list of host generalists and specialists, resolved by ITS sequence data, is available in the new GeoSymbio database (Franklin et al. 2012). Despite the apparent flexible, generalist nature of many types, there is emerging evidence of host specificity within ITS-delineated types as well. For example, *Symbiodinium* type B1 is found associated with numerous Caribbean octocorals (LaJeunesse 2002), but there is observed host specificity on a given reef if *Symbiodinium* microsatellites or their flanking regions are examined as well (Santos et al. 2004). More studies examining the fine-scale molecular markers are needed to determine the generality of this pattern and to help define host–symbiont interactions (Stat et al. 2009b; Thornhill et al. 2009).

While it is clear that specificity can range from corals harboring multiple dominant *Symbiodinium* types to those harboring a single dominant type with background strains (Fay and Weber 2012), the impact of this variability in specificity on holobiont physiology is less clear. Background symbionts may (1) represent surface contaminants or ingested algae that contribute nothing to host physiology; (2) contribute in some unknown capacity, for example, by priming the immune system or producing as yet undescribed products; or (3) represent "disaster taxa," which would allow background symbionts to rapidly replace dominant symbionts during bleaching events (*sensu* Correa and Baker 2011). A recent simulation study demonstrated that holobionts were less likely to go extinct if background types are capable of becoming dominant symbionts (Fabina et al. 2013). This was hypothesized in the adaptive bleaching hypothesis, which posits that internal symbiont shuffling among already present types (shuffling) or switching to newly acquired environmental types (switching) (Baker 2003) is

beneficial in changing environments (Buddemeier and Fautin 1993). Although there is evidence that the Caribbean octocoral *Briareum asbestinum* and scleractinian *Porites divaricata* can acquire novel symbionts from the environment (Coffroth et al. 2010; Lewis and Coffroth 2004), the relative contribution of switching vs. shuffling in symbiont change and the number of coral taxa capable of changing dominant types remain unclear and deserve further attention.

Finally, the topic of the underlying mechanisms conferring specificity brings us back to the winnowing process discussed above. What are the cellular, physiological, and ecological processes that result in the host–symbiont partner combinations observed in nature? At present these are only beginning to be understood in corals, but likely involve immune responses by the host and modulation of this response by symbionts. The lack of dramatic changes in expression profiles of hosts when challenged with appropriate symbionts suggests that these symbionts are cloaked in the host, successfully avoiding an immune response (Schnitzler and Weis 2010; Voolstra et al. 2009). In contrast, challenge by inappropriate symbionts in two coral species elicited significant upregulation of immune genes (Voolstra et al. 2009). Likewise, in studies of larvae of the coral *Fungia scutaria*, larvae challenged with inappropriate symbionts mounted an immune response (as evidenced by caspase activity) that eliminated these algae by 24 h post challenge (Dunn and Weis 2009).

10.7 The Role of *Symbiodinium* in the Response of Corals to Climate Change

10.7.1 Global Warming

Global climate change scenarios call for a warming of oceans by as much as 3 °C during the next 100 years, depending on CO_2 emission scenarios (Hoegh-Guldberg et al. 2007). Higher temperatures, as well as many other stressors, can cause a breakdown of the symbiosis (see Table 10.1 for a list of reviews). Loss of the pigmented *Symbiodinium* from coral tissue is responsible for the white color observed, aptly named coral bleaching.

This thermal increase will affect corals in a taxon- and symbiont-specific manner, and the ability of corals to persist will depend on adaptation, acclimatization, and colonization of the holobiont (Barshis et al. 2010; Marshall and Baird 2000; Sampayo et al. 2008). This section will focus on the potential role of the symbiont in affecting host tolerance to the impacts of climate change. Other studies and reviews focus on the role of the host (Baird et al. 2009a; Edmunds and Gates 2008).

Although many corals inhabit waters that are within ~1 °C of their thermal maximum where they bleach and suffer mortality (Hoegh-Guldberg et al. 2007), corals can withstand warmer temperatures with proper acclimation or acclimatization. Examination of corals before, during, and after warming events, as well as in

localities of natural high temperatures, provides insight into their capacity for thermal tolerance. For example, the Persian Gulf, north of the Arabian Sea, can reach summer temperatures of 36 °C, and *Porites lobata* colonies collected from these reefs were less susceptible to bleaching than conspecifics collected from Fiji in a common garden experiment (Hume et al. 2013). This may be due in part to a different symbiotic complement in the two populations: *P. lobata* from the Persian Gulf harbors type C3, while those from Fiji (Hume et al. 2013) and elsewhere (Barshis et al. 2010; LaJeunesse et al. 2004) harbor type C15. Likewise, the presence of clade D *Symbiodinium*, some of which have been shown to be heat tolerant and provide thermal protection to the host (Berkelmans and van Oppen 2006; Rowan 2004; Wang et al. 2012), associates with several species in the Persian Gulf (Baker et al. 2004).

Gaining thermotolerance from symbiont shuffling, switching, or both is likely to occur in a host- and *Symbiodinium* type-specific manner. Symbiont complement can remain stable (McGinley et al. 2012; Stat et al. 2009a), temporarily change only to return to the original symbiont type later during recovery (Coffroth et al. 2010; LaJeunesse et al. 2010b; Thornhill et al. 2006), or change and persist through the duration of the monitoring (Baker 2001; Berkelmans and van Oppen 2006; Jones et al. 2008). In one example of the latter, Australian *Acropora millepora* colonies that switched from type C2 to a more thermotolerant D *Symbiodinium* gained only a single-degree increase in thermal tolerance (Berkelmans and van Oppen 2006). Modeling of a widespread switch in Caribbean corals to *Symbiodinium* type D1a showed little gain in reef resiliency with increased temperatures (Ortiz et al. 2013). With an initial prevalence of type D1a set at 5 % and a 40 % chance of switching to this thermally tolerant symbiont, reef coral cover decreased compared to scenarios where corals maintained less heat-tolerant symbionts. This was caused by the modeled increased respiration of corals in higher temperatures combined with a decrease in growth of almost 40 % when corals harbor type D1a (Jones and Berkelmans 2010), but this model remains to be tested with empirical studies.

If corals are not able to switch symbionts, it is still possible that a combination of acclimatization and adaptation of their *Symbiodinium* complement may provide thermotolerance. For example, there are physiological differences between different suprapopulations of generalist symbionts type C1. *Symbiodinium* isolated from two populations of *A. millepora* with different thermal histories were used to infect aposymbiotic juveniles from a third population (Howells et al. 2011). When exposed to 32 °C, juveniles associated with *Symbiodinium* from the warmer population had significantly higher survivorship, indicating potential adaptation or acclimatization even within ITS type.

Rising oceanic temperatures may also have a negative effect on coral larvae depending on the symbiotic state. High seawater temperatures decrease planula survivorship and larval duration in the water column (reviewed in Gleason and Hofmann 2011). In addition, *Symbiodinium* negatively affects the ability of the larval corals to cope with temperature stress. In Pacific *Acropora* species, larvae exposed to freshly isolated *Symbiodinium* have significantly higher antioxidant activity, DNA damage, and mortality in elevated temperature or UV light treatments compared to aposymbiotic conspecifics (Nesa et al. 2012; Yakovleva

et al. 2009). High temperature also decreased the prevalence of *Symbiodinium* in the Pacific corals *Acropora intermedia* and *Fungia scutaria* over time despite high initial infection rates across treatments (Baird et al. 2008; Schnitzler et al. 2012). Temperature can also affect the symbiotic complement during initial onset of the symbiosis as the more thermal-tolerant clade D *Symbiodinium* has a higher prevalence in warmer conditions (Abrego et al. 2012). However, *Acropora* recruits harboring these clade D symbionts have a threefold decrease in growth compared to type C1 *Symbiodinium* indicating a major trade-off between survival and growth (Cantin et al. 2009; Little et al. 2004).

10.7.2 Ocean Acidification

Ocean acidification is concomitant with global climate change and is a direct result of increasing atmospheric CO_2 levels that in turn cause an acidic shift in the carbonate pH balance in seawater. This pH drop causes a decrease in aragonite saturation values, potentially compromising the ability of corals and other calcifying organisms to deposit skeletons and therefore threatening reef formation (Hoegh-Guldberg et al. 2007). The study of the impacts of ocean acidification on corals is in its infancy and is a very active area of research. Thus the impact of decreased pH on corals is unclear. While some studies have shown coral calcification to be negatively impacted (Anthony et al. 2008), others are finding evidence that corals resist decreasing pH by tightly controlling pH in the animal and at the site of calcium deposition (Edmunds et al. 2013; Venn et al. 2013). Impacts on corals are variable and depend in part on host taxon and environmental conditions (Chan and Connolly 2013). In contrast, sea anemones excel in higher CO_2 environments, due to increased availability of inorganic carbon for photosynthesis which in turn leads to increased growth rates (Suggett et al. 2012).

The effect of elevated pCO_2 on coral bleaching and *Symbiodinium* photophysiology is not clear, and understanding the correlation is made more difficult by other environmental conditions (i.e., temperature and light). For example, there was a bleaching response in the Australian corals *Acropora intermedia* and *Porites lobata* when exposed to low-pH waters in both high and control temperature treatments (Anthony et al. 2008). Conversely, high CO_2 concentrations alone did not elicit a bleaching response in several other coral hosts, although temperature and light affected photophysiology in some experiments (Gabay et al. 2013; Godinot et al. 2011; Wall et al. 2014). Differing host–symbiont partnerships may have different physiological responses to higher CO_2 concentrations. In cultured *Symbiodinium*, doubling pCO_2 increased the growth rate and photosynthetic capacity of ITS types A13 and A2, respectively (Brading et al. 2011). However, neither phenotype (i.e., growth and photosynthetic rates) was affected in types A1 and B1. It is also uncertain if corals will benefit from switching or shuffling *Symbiodinium* type to adapt to ocean acidification. There are natural locations where pCO_2 in seawater mimics predicted increased oceanic conditions

within the next 100 years (500–900 ppm) including natural CO_2 seeps. Six coral species in elevated CO_2 seeps near Papua New Guinea harbor similar symbionts types compared to nearby control populations at ambient conditions (390 ppm) (Noonan et al. 2013). The effects of increased pCO_2 in combination with other aspects of climate change need to be further examined to understand the role *Symbiodinium* may play in the ability of corals to cope with these changes.

10.8 Conclusions

Animal–*Symbiodinium* symbioses play a fundamental role in globally important coral reef ecosystems. Recent advances in genetic, genomic, and cell biological aspects of the symbiosis are strengthening our understanding of these interactions. Insights into the high diversity of the *Symbiodinium* genus, the complexity of its large genome, and the detail of inter-partner recognition and specificity are helping to inform us on the ability of these partnerships to adapt and acclimatize to a rapidly changing climate.

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Chapter 11
Fiat Lux: The Squid–Vibrio Association as a Model for Understanding Host–Microbe Associations

Spencer V. Nyholm

Abstract The symbiosis between the Hawaiian bobtail squid *Euprymna scolopes* and the bioluminescent bacterium *Vibrio fischeri* offers an experimentally tractable model for understanding the role of beneficial bacteria on animal development and the mechanisms by which host and symbionts establish and maintain highly specific associations. The symbiont is transmitted from the environment each generation, and mechanisms must be in place to ensure specificity. Research over the years has revealed some of the "molecular dialogue" that occurs between the partners during and after colonization. Many of these interactions involve microbe-associated molecular patterns (MAMPs) and host pattern recognition receptors (PRRs) as well as components of the host's innate immune system. The role of light production by the symbiont and light detection by the host is also critical to the association and has likely served as a driving force during the evolution of this symbiosis. Finally, the host harbors a second symbiosis, housing a consortium of bacteria in the female reproductive system. *Euprymna scolopes* therefore offers the unique opportunity to study both a binary and consortial symbiosis in the same host.

11.1 Importance of Model Associations in Symbiosis Research

All animals and plants form beneficial associations with microorganisms, and such associations have had a profound effect on the evolution of these groups (McFall-Ngai et al. 2013; Oldroyd 2013). In recent years it has become evident that symbionts play a critical role in the development and health of not only individual hosts, but entire ecosystems [e.g., coral reefs; see Chap. 10 by V. Weis and hydrothermal vent and other chemoautotrophic ecosystems (Dubilier et al.

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2008)]. Understanding the molecular mechanisms by which these associations are established and maintained can be difficult because often the symbionts occur as complex consortia where delineating the role of any one member is challenging. Therefore, employing the use of model systems with fewer partners is often advantageous. The binary association between the Hawaiian bobtail squid *Euprymna scolopes* and the bioluminescent bacterium *Vibrio fischeri* is one such system that has been used to understand how beneficial host–microbe associations are formed (McFall-Ngai 2014). This chapter will review how this highly specific association is established and maintained, highlighting the molecular mechanisms by which the partners communicate to achieve this goal and how the host's innate immune system contributes to the specificity of the symbiosis.

11.2 Bioluminescent Symbioses

Bioluminescence (the production of light by living organisms) is a common biological phenomenon in many environments but is especially common in marine ecosystems (Widder 2010). The light produced by organisms is used for a number of behaviors including finding prey, camouflage to avoid predation, and attracting mates. The vast majority of fauna use autogenic bioluminescence, meaning they produce the chemicals (substrate luciferin and enzyme luciferase) necessary for light generation. A few groups, mainly found among fishes and squid, rely on a symbiotic relationship with bacteria for light production.

The majority of bioluminescent bacteria in the marine environment belong to members of the *Gammaproteobacteria* group *Vibrionaceae* and primarily within the genera *Vibrio* and *Photobacterium* (Guerrero-Ferreira et al. 2013; Urbanczyk et al. 2011). Members of these groups have formed associations with a number of different species of fish and squid (Guerrero-Ferreira and Nishiguchi 2007; Urbanczyk et al. 2011). Perhaps the best studied of this group is *Vibrio fischeri* (also referred to as *Aliivibrio fischeri*) which produces light through a process known as quorum sensing. This phenomenon, which was first discovered in *V. fischeri*, regulates light production based on density-dependent cell–cell communication [reviewed in Verma and Miyashiro (2013) and Miyashiro and Ruby (2012)]. *Vibrio fischeri* produces a freely diffusible chemical autoinducer known as an N-acyl homoserine lactone (3-oxo-C6-HSL) that initiates gene expression when a quorum or critical density of bacterial cells is present (e.g., as found in culture or contained within the light organ of a host). The chemistry of bacterial bioluminescence in *V. fischeri* is based on production of an enzyme (luciferase) that oxidizes substrates [bacterial luciferin; reduced flavin mononucleotide (FMNH2)] and a long-chain fatty acid (RCHO) into FMN and aliphatic acid (RCOOH). The genes for all of these factors are encoded by the *lux* operon (Lux ICDABEG) (Gray and Greenberg 1992) and are transcriptionally activated when 3-oxo-C6-HSL binds the LuxR activator. A positive feedback loop allows for the production of more autoinducer (LuxI) and thus increases luminescence output. Luminescence in *V. fischeri* is also regulated by