

From bacterial bleaching to the hologenome theory of evolution

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Abstract. Extensive bleaching of the coral *Oculina patagonica* in the eastern Mediterranean Sea occurs every summer when the seawater temperature exceeds 25°C. The infection by *Vibrio shiloi* and subsequent bleaching occurred only above 25°C because several of the bacterial virulence factors, superoxide dismutase, adhesion and toxin P, are only expressed above 25°C. Starting in 2002, the corals developed resistance to *V. shiloi*. Attempts to explain the resistance led to the Coral Probiotic Hypothesis, which posits that corals can adapt to their environment, including resistance to pathogens, by changing their symbiotic bacteria. The hologenome theory considers the holobiont a unit of natural selection. The hologenome is defined as the sum of the genetic information of the host and its microbiota. The theory is based on four well documented generalizations: (1) All animals and plants establish symbiotic relationships with microorganisms. (2) Symbiotic microorganisms are transmitted between generations. (3) The association between host and symbiont affects the fitness of the holobiont. (4) Under environmental stress, the symbiotic microbial community can change rapidly. These points taken together suggest that the genetic wealth of diverse microbial symbionts can play an important role both in adaptation and in evolution of higher organisms.

Keywords: Coral bleaching; *Vibrio*; holobiont, hologenome, evolution

Introduction

Extensive bleaching of the coral *Oculina patagonica* in the eastern Mediterranean Sea occurs every summer. Kushmaro et al. (1996, 1997) reported that the bleaching of *O. patagonica* was the result of an infection by *Vibrio shiloi*. The demonstration that *V. shiloi* was the causative agent of the disease was established by rigorously satisfying all of Koch's postulates, including the fact that bleached corals in the sea contained the bacterium (Kushmaro et al. 1996, 1997) whereas it was absent from healthy corals. Furthermore, Kushmaro et al. (1998) showed that the infection and subsequent bleaching only occurred at temperatures above 25°C. Thus, for bleaching to occur, both elevated temperature and the causative agent must be present.

The specific steps in the infection of *O. patagonica* by *V. shiloi* have been studied extensively (Rosenberg and Falkovitz 2004). The bacteria are chemotactic to the coral mucus, adhere to a β -galactoside-containing receptor on the coral surface, penetrate into the epidermal layer and multiply intracellularly, reaching 10^8 - 10^9 cells per cm^3 . The intracellular *V. shiloi* produces an extracellular peptide toxin (PYPVYPPVVP) that inhibits algal photosynthesis. Another important factor for the virulence of *V. shiloi* is the expression of superoxide dismutase (SOD). Adhesion, production of the toxin and expression of SOD are all temperature-dependent reactions, occurring at summer (25-30°C) but not winter (16-20°C) temperatures. Thus, *V. shiloi* can not infect, multiply

or survive in the coral during the winter. Sussman et al. (2003) demonstrated that the marine fireworm *Hermodice carunculata* is a winter reservoir and spring-summer vector for *V. shiloi*.

Development of resistance of *O. patagonica* to *V. shiloi*

We have been studying the *V. shiloi/O. patagonica* model system of coral bleaching in the sea and in the laboratory for over ten years. Sometime between 2002 and 2004 we found that the corals became resistant to the pathogen. The evidence for the development of resistance is based on the following (Reshef et al. 2006):

1. From 1995-2002, the pathogen *V. shiloi* was readily isolated from 46/50 bleached and bleaching corals collected from the wild; from 2004 to the present, we have been unable to isolate *V. shiloi* from bleached or bleaching corals.
2. From 1995-2002, all laboratory strains of *V. shiloi* caused bleaching in controlled aquaria experiments; from 2004 to the present, none of the same strains bleach *O. patagonica* in the laboratory.
3. From 1995-2002, *V. shiloi* adhered to the corals, penetrated into the ectoderm and multiplied intracellularly to 10^8 - 10^9 cells per cm^3 ; now, *V. shiloi* adheres, penetrates the ectoderm and is rapidly killed.

Before the corals became resistant, the isolation and infection was so reproducible that for several years we used the system to demonstrate Koch's postulates in the teaching laboratory. Not only can we now **not**

isolate *V. shiloi* from bleached corals, but molecular techniques failed to recover the 16S rRNA gene from ca. 1000 clones that were sequenced (Koren and Rosenberg 2006). Recently, Ainsworth et al. (2008) confirmed that *V. shiloi* is not currently present in bleached *O. patagonica*, using FISH technology.

The coral probiotic hypothesis

The studies summarized above indicate that corals can indeed adapt rapidly to changing environmental conditions by altering their population of symbiotic bacteria. These studies led us to propose the Coral Probiotic Hypothesis (Reshef et al. 2006). This hypothesis posits that a dynamic relationship exists between symbiotic microorganisms and environmental conditions which brings about the selection of the most advantageous coral holobiont. Changing their microbial partners would allow the corals to adapt to changing environmental conditions more rapidly (days to weeks) than via mutation and selection (many years). An important outcome of the Probiotic Hypothesis would be development of resistance of the coral holobiont to diseases. The following evidence supports this hypothesis: (i) Corals contain a large and diverse bacterial population associated with their mucus and tissues. (ii) The coral-associated bacterial population undergoes a rapid change when environmental conditions are altered. (iii) Although lacking an adaptive immune system (no antibodies), corals can develop resistance to pathogens. One potentially contributing factor is the production of antibacterials by coral mucus bacteria (Ritchie 2006). The Coral Probiotic Hypothesis may help explain the evolutionary success of corals and moderate the predictions of their demise.

The hologenome theory of evolution: role of microorganisms in the evolution of animals and plants

The hologenome theory of evolution (Rosenberg et al. 2007; Zilber-Rosenberg and Rosenberg 2008) is a generalization of the coral probiotic hypothesis. More than a hundred years of biological research has demonstrated the importance of microorganisms in the health and disease of higher organisms. As a result of the recent development of culture-free molecular techniques, it is now accepted that in many cases the number of symbiotic microorganisms and their combined genetic information far exceed that of their hosts. The hologenome is defined as the sum of the genetic information of the host and its microbiota. In the hologenome theory of evolution, we suggest that the holobiont (Margulis 1993; Rohwer et al. 2002) (the host and its symbiotic microbiota) with its hologenome, should be considered a unit of selection in evolution, and that relatively rapid variation in the diverse microbial symbionts can

have an important role in the adaptation and evolution of the holobiont. In essence, the hologenome theory of evolution focuses on the holobiont as a single dynamic entity in which a vast amount of the genetic information and variability is contributed by the microorganisms. Evolution of the holobiont can occur by changes in the host genome and/or in any of the associated microbial genomes, and relies on cooperation between the genomes within the holobiont, as much as on competition with other holobionts. A large body of empirical data provides the foundation for the hologenome theory of evolution. We choose to discuss this information within the following framework: (1) all animals and plants establish symbiotic relationships with diverse microorganisms. (2) Symbiotic microorganisms can be transmitted between generations with fidelity. (3) The association between host organism and its microbial community affects the fitness of the holobiont within its environment. (4) Genetic variation in holobionts can be enhanced by incorporating different symbiont populations and can change under environmental demand more rapidly and by more processes than the genetic information encoded by the host organism alone.

All animals and plants establish symbiotic relationships with microorganisms

Eukaryotes presumably arose from prokaryotes (Margulis 1993) and have remained in close relationship with them ever since. It is therefore not surprising that the surfaces of animals and plants contain a great abundance and variety of microorganisms. In addition, some microorganisms are able to grow inside animal or plant cells, i.e., endosymbionts. Because the vast majority of microorganisms that have been observed on or in animal and plant tissues cannot be cultured, current research on the diversity of microorganisms associated with a particular species relies primarily on culture-free DNA-based technology (Hugenholtz et al., 1998). Although censuses of microorganisms associated with different animal and plant species are only in an early stage, certain interesting generalizations have emerged: (1) The diversity of microbial species associated with a particular animal or plant species is high. (2) The host associated microbial community is very different from the community in the surrounding environment (Frias-Lopez et al. 2002; Rohwer et al. 2002; Sharp et al. 2007). (3) In some cases it has been shown that similar, but not identical, microbial populations are found on the same species that are geographically separated, while different populations are found on different species at the same location (Rohwer et al. 2002; Lambais et al. 2006; Fraune and Bosch 2007). (4) Different microbial communities often dominate different tissues of the same organism (Tannock 1995; Koren and Rosenberg 2006; Dethlefsen et al.

2007). (5) In several cases where a large diversity of associated bacterial species exists, certain bacterial groups dominate.

Transmission of symbionts between holobiont generations

The hologenome theory of evolution relies on ensuring the continuity of partnerships between holobiont generations. Accordingly, both host and symbiont genomes must be transmitted with accuracy from one generation to the next. The precise modes of vertical transmission of host genomes are well understood and need not be discussed here. However, in recent years, it has become clear that microbial symbionts can also be transmitted from parent to offspring hosts by a variety of methods. McFall-Ngai (2002), in an insightful review on the influence of bacteria on animal development, divided the modes for maintaining symbionts faithfully between generations into two categories, transovarian and environmental transmissions, while correctly acknowledging that there are numerous intermediate cases. We would like to take this approach one step further by suggesting that the numerous intermediate cases, in fact, best describe the large variety in modes of transmission, which are known at present to reconstitute plant and animal holobionts. It is this continuum of modes of transmission from direct to indirect that makes it impractical to place them in any specific categories.

Direct transmission from parent to offspring occurs with some symbionts where the microorganisms are in or on the reproductive cells. For example, in the aphid–Buchnera symbiosis, bacteria are intracellularly situated in bacteriocytes and are transferred to and transmitted via the eggs (Baumann et al., 1995). Direct contact is another slightly less direct mode of transmission demonstrated in mammals in which many of the symbionts are derived during passage through the birth canal or subsequently by close physical contact with parent or family and community members.

Another slightly less direct mode of transmission is used in the termite hindgut–microbiota symbiosis where feces of adult termites (containing abundant microorganisms) are fed to newly hatched juveniles by workers in the colony (Abe et al. 2000).

Cooperation between the host and the microbiota contributes to the fitness of the holobiont

Natural selection is the central concept of Darwinian theory – the fittest survive and spread their advantageous traits through populations, but ‘fittest’ is not an absolute property because it varies with environmental influences. Considering the holobiont as a unit of selection in evolution, we argue that the cooperation between the normal microbiota and the host generally leads to improved fitness. In addition, the genetic diversity of the

microbiota can extend the range of environments in which the holobiont can compete successfully.

In several well-studied cases, neither the host nor the primary symbiont can survive without the other (absolute mutualism). For example, in the aphid–Buchnera symbiosis, the primary endosymbiotic bacterium has lost many genes required for independent growth during evolution, whereas the aphid partner depends on essential amino acids lacking in its diet that are synthesized and furnished by the symbiont (Baumann et al., 1995). While the aphid–Buchnera primary endosymbiosis is an example of absolute dependency, most of the symbiosis systems are not based on life or death interactions, but rather the microbial partners contribute in different degrees to the holobiont’s well-being. One such example, also found in the aphids, is the secondary endosymbioses with bacteria belonging to a number of distinct lineages within the Gamma- and Alphaproteobacteria. These microorganisms are intracellular endosymbionts, which are not essential for growth and reproduction, but that contribute to the fitness of the holobiont (Russell et al. 2003). Regardless of the extent of dependency, a large body of data has been accumulated in recent years demonstrating that both endosymbionts and exosymbionts play numerous roles in metabolism, regulation, disease resistance and in sex and fertility determination (which may lead to species determination) of their hosts.

In the human gut–microbiota relationship a substantial amount of recent information has been gathered regarding many facets of this interaction (Xu and Gordon 2003; O’Hara and Shanahan 2006; Xu et al. 2007). The diverse interactions reported between the human gut and its microbiota include not only the cooperation in food breakdown [e.g. fiber into short-chain fatty acids (Hooper et al. 2002)] and biotransformation of certain molecules [e.g. bile acids (Hylemon and Harder, 1998)] but also participation in the development and normal function of the innate and adaptive immune systems in the gut (Hooper and Gordon 2001; O’Hara and Shanahan 2006), participation in the structural buildup of blood vessels [angiogenesis (Stappenbeck et al. 2002)] and participation in the regulation of fat accumulation (Bäckhed et al. 2004).

Genetic variation in holobionts

Variation is the raw material for evolution. According to the hologenome theory of evolution, genetic variation can arise from changes in either the host or the symbiotic microbiota genomes. Variation in host genomes occurs during sexual reproduction, chromosome rearrangements and ultimately by mutation. These same processes occur in microorganisms with the noteworthy difference that in haploid bacteria recombination occurs, within the same species, by conjugation, transduction and DNA transformation. In addition to recombination and

mutation, changes in the genome of the microbiota of holobionts can occur by three other processes: microbial amplification, acquisition of novel strains and horizontal gene transfer between different species. These three processes can occur rapidly under environmental demand and may be important elements in the evolution of animals and plants (Dinsdale et al. 2008).

Microbial amplification is the most rapid and easy to understand mode of variation in holobionts. It involves changes in the relative numbers of the diverse types of associated microorganisms that can occur as a result of changing temperatures (for plants and invertebrates), nutrient availability, exposure to antibiotics or other environmental factors. The holobiont is a dynamic entity with certain microorganisms multiplying and others decreasing in number as a function of local conditions. An increase in the number of a particular microbe is equivalent to gene amplification. Considering the large amount of genetic information encoded in the diverse microbial population of holobionts, microbial amplification is a powerful mechanism for adapting to changing conditions.

Another mechanism for introducing variation into holobionts is acquiring new symbionts from the environment. Animals and plants come in contact with billions of microorganisms during their lifetime. It is reasonable to assume that occasionally, as a random event, one of these microorganisms will find a niche and become established in the host. Under the appropriate conditions, the novel symbiont may become more abundant and affect the phenotype of the holobiont. Unlike microbial amplification, acquiring new symbionts can introduce entirely new genes into the holobiont.

Based solely on the host genome, animals and plants would evolve slowly because of (1) their relatively long generation times, (2) the fact that only changes in the DNA of the germ line are transmitted to the next generation and (3) often a whole set of new genes is required to introduce a beneficial phenotypic change. If the environment changes relatively rapidly, the host genome alone may not evolve quickly enough and the organism may lose competitiveness and become extinct. We argue that rapid changes in the symbiotic microbiota could allow the holobiont to adapt and survive under changing environmental conditions, thus providing the time necessary for the host genome to evolve. In some cases, the required new function remains within the symbiotic microbiota. For example, the animals that first evolved to feed on cellulose never developed the genes to degrade cellulose, but rather adapted to provide better conditions for microbial degradation of the polymer.

Discussion

The distinguishing feature of the hologenome theory is that it considers all of the diverse microbiota

associated with the animal or the plant as part of the evolving holobiont and that changing the microbial community by amplification, novel acquisition from the environment and horizontal gene transfer provide additional mechanisms for rapid evolution. The consequences of considering all of the diverse symbiotic microbiota of the holobiont (endo and exocellular) are far reaching. Most importantly, they can increase the genetic information of the holobiont severalfold. Microbially directed processes can regulate and extend the metabolic capability of holobionts, including degradative and biosynthetic reactions in addition to regulatory mechanisms.

In terms of modern Darwinian theory, the unit of selection in evolution must contain two properties (Lloyd 1994): the replicator or genome that is subject to variation, and the interactor or phenotype, that interacts with the environment in a way that creates differential reproduction. The data reviewed in this article clearly demonstrate that both properties are expressed by the holobiont. The usefulness of considering the holobiont, with its hologenome, as a unit of selection is that it makes adaptability to changing environments in a relatively short time frame more comprehensible and amenable to experimental tests.

Interestingly, the hologenome theory incorporates aspects of both Darwinism and Lamarckism. Individual organisms evolve by selection of random variants, whereas the holobiont can evolve by adaptive processes. Consider, for example, marine invertebrates living in an ocean of increasing temperatures. The hologenome will change as a result of the shift-up in temperature by amplification of those microbiota symbionts that grow better at the higher temperature and possibly by gaining more thermophilic microorganisms from the environment. The holobiont will not only be better adapted to its environment, but it will also have an increased probability of passing on the acquired genetic traits to the next generation. Thus, when considering a holobiont, rather than the individual organisms that comprise it, it is possible to have an inheritance of acquired characteristics. How important this is in long-term evolution is debatable. However, in the short term, inheritance of acquired characteristics by holobionts may help them survive, multiply and buy the time necessary for the host genome to evolve.

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