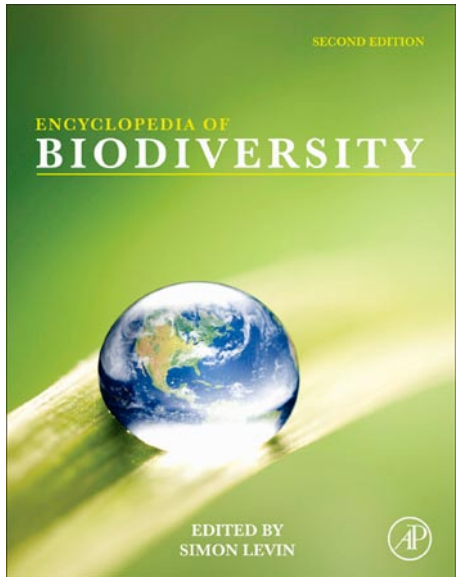

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Origins, Evolution, and Breakdown of Bacterial Symbiosis

Joel L Sachs, University of California, Riverside, CA, USA

Glossary

Bacterial mutualist A bacterium that interacts closely with a host and significantly enhances the host's fitness.

Bacterial parasite A microbe that interacts closely with a host and significantly reduces the host's fitness.

Endosymbiotic bacteria Bacterial species that live within the cells of hosts, are transmitted by hosts to their offspring, and often cannot survive outside of the host.

Infection Colonization of a host organism by bacteria that reproduce within the host and are ultimately transmitted to new hosts.

Introduction

The primitive earth was dominated by single-celled bacteria and archaea; the two major lineages of unicellular organisms that lack nuclei and the membrane-bound organelles of the eukaryotes. Beginning with the first fossil evidence of life, approximately 3.5 billion years ago and continuing for 2 billion years thereafter, most evidence of life appeared bacterial and archaeal. Not until a period that began about 1.5 billion years ago did eukaryotic lineages emerge and only in the last 1 billion years did multicellular plants and animals begin to diversify and ultimately to dominate. Thus, we can imagine a period of at least 2 billion years in which bacteria and archaea persisted mostly as free-living cells in the terrestrial and aquatic habitats. The last 500 million years of earth's history has witnessed an explosion in eukaryotic diversity including major radiation events of animal and plant lineages. This diversification of eukaryotic lineages has also driven a massive and parallel radiation of symbiotic bacteria (Sachs *et al.*, 2011b). Although archaea can also form symbioses with eukaryotic hosts, these interactions do not appear as common or diverse.

In the last billion years bacterial lineages have evolved a diverse array of mechanisms to gain entry into the tissues and cells of eukaryotes and to proliferate within these hosts (Medina and Sachs, 2010; Toft and Andersson, 2010). Here, the author refers to both harmful and beneficial bacterial infections as symbioses, a term used to describe any persistent and intimate association between microbes (the symbionts) and host species (animals, plants, or other eukaryotes; Medina and Sachs, 2010). Much of the research uncovering the mechanisms of symbiotic infection has focused on microbial parasitism (Sachs *et al.*, 2011a). In parasitic interactions, the microbes infect and actively exploit hosts resulting in reduced host survival and reproduction (evolutionary fitness). Bacterial parasites are a major challenge to human health and also represent a huge cost in terms of lost crops and animal stocks. However beneficial bacterial infections are known to be just as ubiquitous as parasites and can be found in virtually any eukaryotic species and in any ecosystem (Sachs *et al.*, 2011b). Such mutualistic infections occur when microbes infect and offer resources and services to hosts that enhance host fitness. Here the author's goal is to trace the deep evolutionary history of bacterial symbiosis and particularly to examine events leading to the origins and macroevolution of the bacterial mutualism.

Bacterial mutualists exhibit a great deal of lifestyle variation in their interactions with the eukaryotic hosts. Three key sources of variation are thought to bear particular importance to the evolution of mutualist bacterial lineages (Sachs *et al.*, 2011b). One source of variation is the degree to which the bacteria reproduce inside their host versus free in the environment. Many (and perhaps most) mutualist bacteria infect hosts but also spend a large portion of their lifecycle free in soils or aquatic environments (Sachs *et al.*, 2011a). The animal and plant hosts of these bacteria are born symbiont-free and thus must acquire their infections from the environmental pools (Szathmary and Maynard-Smith, 1995; Nyholm and Mcfall-Ngai, 2004; Sachs *et al.*, 2011a). At the opposite end of the spectrum are endosymbiotic bacteria and bacterially-derived organelles (Sagan, 1967; Keeling, 2010), which are the bacteria that cannot live independently without the hosts, only divide within host tissue and can only form new infections via transmission from host parent to offspring. A second source of variation is the array of animal and plant host tissues that bacterial mutualists can inhabit, including cell surfaces ranging from skin, mucosa, leaves and roots, and spaces between and within cells. In some cases the hosts have evolved specialized structures to house beneficial symbionts (e.g., Douglas, 1989; Bright and Sorgo, 2003; Nyholm and Mcfall-Ngai, 2004; Currie *et al.*, 2006; Goettler *et al.*, 2007; Vaishnava *et al.*, 2008) whereas in other cases beneficial bacterial symbionts can range widely within unstructured host tissues (Hirose *et al.*, 2009; Kaltenpoth *et al.*, 2009; Sachs *et al.*, 2011b). A final source of variation is represented by the array of mechanisms by which bacterial mutualists can provide fitness benefits to hosts, including the transfer of key nutrients, protection via toxicity as well as bioluminescence (Sachs *et al.*, 2011a, b). As described below each of these variables can have implications for the long-term evolution of symbiotic bacterial lineages.

The author describes how the study can be done on the diversification of such mutualist bacterial traits over the millions of years that they have developed and moreover how the study can be done on the origins of bacterial mutualist lineages and their persistence over evolutionary time. One approach to characterize such evolutionary history is to distill the key events and investigate the forces that shaped them (Szathmary and Smith, 1995). Bacteria can be understood to have undergone several major transitions in their evolutionary history and route to become mutualists with the eukaryotic

hosts. Some of these transitions represent key evolutionary milestones that have occurred many times in the history of bacteria. Five of these evolutionary transitions will be explored in depth over the course of this article (Sachs *et al.*, 2011b).

Evolutionary Origins of Bacterial Associations with Hosts

In the past history of bacteria, evolutionary origins of host association have occurred when environmentally existing bacteria initially evolved to form persistent and intimate interactions with the eukaryotic hosts (Sachs *et al.*, 2011b). Since the planet was first dominated only by the bacterial and archaeal cells, none of these organisms had large bodied hosts to live in or infect. Once eukaryotic lineages evolved, in particular the diversification of animals and plants, there were many evolutionary origins in which bacteria that once lived independently in the environment evolved to form intimate and persistent associations with these hosts (Sachs *et al.*, 2011b). For bacteria to undergo the transition to host association they must have had to overcome major hurdles, including evasion of host defenses, competition with other microbes to inhabit host surfaces, uptake of novel compounds in hosts and the ability to gain transmission from one host to the next (Sachs *et al.*, 2011b). To examine the difficulty of these potential hurdles, evolutionary methods can be used to assess the degree to which origins of host association are rare in bacterial lineages (Sachs *et al.*, 2011b).

One method to quantitatively investigate past evolutionary events is to build an evolutionary tree of genetically related species (a phylogeny) and then use the tree as a tool to test evolutionary hypotheses. The first step in this case is to reconstruct a species phylogeny of bacteria. This is becoming increasingly easier with the great availability of whole genome sequences (e.g., Wu *et al.*, 2009). The recent analysis by Wu and colleagues (2009) used genetic data from fully sequenced bacterial genomes to reconstruct an evolutionary tree of over 350 bacterial species. Because of the large amount of data that is available from the whole genome sequence, a very robust phylogeny can result (Wu *et al.*, 2009; Sachs *et al.*, 2011b; see **Figure 1**). The next step is to map species traits that can be added to the tree to study past events. In this case the traits of importance characterize each bacterial species in terms of their interactions with eukaryotic hosts (Boussau *et al.*, 2004; Merhej *et al.*, 2009; Toft and Andersson, 2010; Philippot *et al.*, 2010). Many bacterial species in this analysis exhibit no symbiotic interactions and are thus characterized as environmental whereas other species interact intimately with eukaryotic hosts and are categorized as parasites and mutualists depending on their fitness effects on hosts during infection (Sachs *et al.*, 2011b). Once a phylogenetic tree has been reconstructed for a lineage and phenotypes (traits) became known for each of the species, algorithms can be used to infer ancestral character states onto the tree. Such inference methods (known as ancestral state reconstruction) can be used to retrace the order of evolutionary events as well as the number of times the particular traits have independently evolved within a lineage.

Analyzing the evolution of host association origins across bacteria (Wu *et al.*, 2009; Toft and Andersson, 2010) a couple of conclusions can be made. First of all, simply from viewing the tree (**Figure 1**) it becomes clear that many independent origins of bacterial symbiosis have evolved. These origins can be found in almost every well-known lineage. For instance, on the 350 species tree a minimum of 42 origins of host-association are inferred from the environmental (free living) ancestral condition (Sachs *et al.*, 2011b). This pattern is interesting because it suggests that the hurdle in evolving from an environmental bacterium into a symbiont might not be as difficult as first imagined. Secondly, it appears that these origins of host association are more-or-less randomly distributed among bacteria (Sachs *et al.*, 2011b). Whereas some well-studied lineages appear to have many origins (Proteobacteria, Actinobacteria, and Firmicutes; **Figure 1**) and a few lineages (such as Chlorobi, Chloroflexi, and Planctomycetes) have never evolved the host association (Madigan *et al.*, 2009; Wu *et al.*, 2009; Toft and Andersson, 2010) there does not seem to be any significant bias where the origins occur on the tree (Sachs *et al.*, 2011).

To explore the mechanisms of symbiosis origins, researchers have begun to investigate the changes within the bacterial genomes that are correlated with these evolutionary transitions. One newly available approach is to study bacteria for which whole genome datasets are published and to compare the genomes of bacteria with different lifestyles. The goal here is to specifically retrace the particular sets of genes or genetic loci that are gained or lost concurrently with the origins of host association. Two interesting results have come from these studies so far. One pattern is that there are often few consistent genetic differences between host associated and environmental bacterial species (Carvalho *et al.*, 2010). Similarly, few genetic differences are often found between related bacteria that are beneficial versus those that are harmful to the hosts during infection (Sachs *et al.*, 2011a). Among the small set of genes that do differ in host associated and environmental species, many of them exhibit a strong evidence of horizontal gene transfer (movement of genes from one organism to an unrelated organism; Dale *et al.*, 2001; Horn *et al.*, 2004; Frank *et al.*, 2005; Ruby *et al.*, 2005; Ma *et al.*, 2006). Whereas only a few studies have rigorously compared the genetics of environmental versus beneficial or harmful host-associated bacteria (Dale *et al.*, 2001; Horn *et al.*, 2004; Frank *et al.*, 2005; Ruby *et al.*, 2005; Ma *et al.*, 2006; Carvalho *et al.*, 2010), one initial conclusion that can be made from these studies is that horizontal gene transfer is likely an important mechanism that promotes shifts in the lifestyles of bacteria. This pattern suggests that transitions to host association might be caused by a relatively smaller set of genes that are mobilizable among the bacterial lineages. At a broader level, these data suggest that very simple ecological factors might consistently promote or constrain a bacterium to gain the ability to infect eukaryotic hosts. These ecological factors might be as simple as access to habitats with compatible hosts and access to compatible genes (gained from other bacterial lineages) that allow an environmental bacterium to infect the hosts (Sachs *et al.*, 2011b).

The Origins of Bacterial Mutualism

Among the host-associated lineages, bacterial mutualists are incredibly diverse. Beneficial bacteria are diverse in the sense

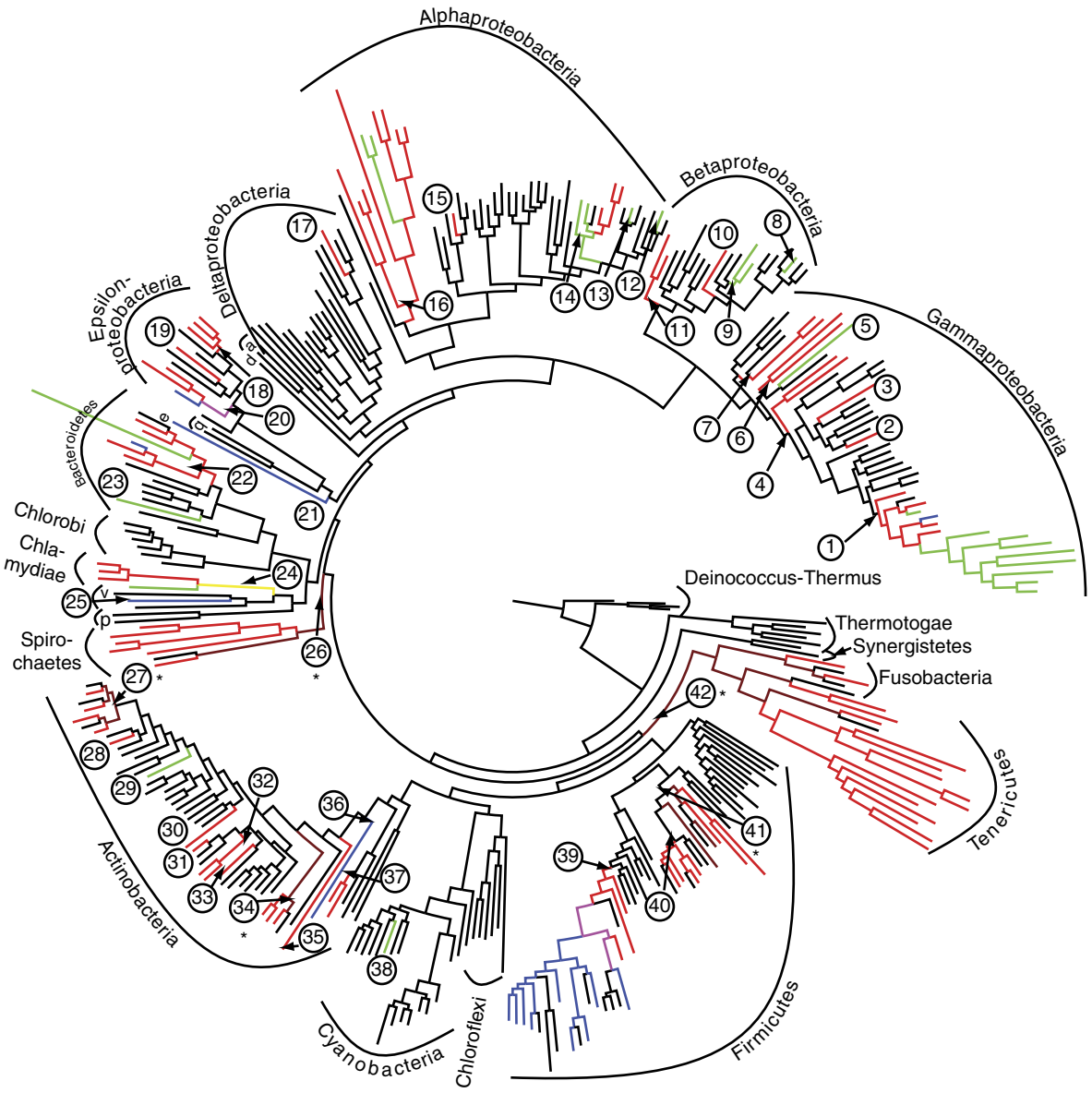

Figure 1 Inferred evolutionary history of bacterial host-association. Ancestral states of symbiosis are inferred on a bacterial evolutionary tree (modified from a previously published figure; Sachs JL, Skophammer RG, and Regus JU (2011b) Evolutionary transitions in bacterial symbiosis. *Proceedings of the National Academy of Sciences USA* 108:10800–10807). The tree is reconstructed from 31 single copy genes taken from 350 bacterial species for which whole genome data are available (Wu *et al.*, 2009). Phyla and proteobacterial classes are labeled with their full names (e.g. Gammaproteobacteria; Firmicutes) or single-letter abbreviations (a = Acidobacteria; d = Defferribacteres; q = Aquificae; e = Elusimicrobia; v = Verrucomicrobia; p = Planctomycetes). Branch colors represent host-associated traits on the tips of the tree and inferred states on ancestral nodes (black = environmental; blue = commensal; green = mutualist; red = parasite). Host association traits were obtained from a prior review (Toft and Andersson, 2010). Sachs and colleagues (2011b) inferred a minimum of 42 origins of host-association (labeled 1–42).

that this strategy has not only evolved independently in many anciently diverged lineages but also because so many types of hosts are infected in many different ways (Sachs *et al.*, 2011a). Other questions risen by the author include: what evolutionary process brought about these interactions in the first place? In other words, from the perspective of the bacterial evolutionary tree, what are the ancestral conditions that predate the origins of bacterial mutualists? Furthermore can evolutionary predictions be made about scenarios that favor the evolution of bacterial mutualists from other lifestyles?

Most of these questions remain unresolved. However, just as the author has done above for the origins of host association, information from bacterial phylogenies can be used to begin retracing the origins of bacterial mutualism. Perhaps the most perplexing question about bacterial mutualists is where they came from (Ewald, 1987; Szathmary and Smith, 1995; Corsaro *et al.*, 1999; Moran and Wernegreen, 2000; Medina and Sachs,

2010). Two competing hypotheses describe evolutionary scenarios for the origins of bacterial mutualism. One hypothesis proposes that bacterial mutualists have come about from parasitic ancestors that have evolved to be less harmful over time to the point that beneficial interactions could evolve with the hosts (Ewald, 1987). This attenuation of virulence is thought to be linked with the type of transmission that the bacteria exhibit (Ewald, 1987). Many bacteria are horizontally transmitted, meaning that individual bacteria travel from one host to a new and unrelated host to initiate a new infection. Horizontal transmission can actually promote parasitism of hosts because the ability to escape and infect a new host allows the bacterium to exploit the current host without harming itself (Fine, 1975). In contrast, vertical transmission occurs when bacteria are transmitted from a host to its offspring and in this case the bacteria can benefit themselves by being mutualistic (Fine, 1975). In a seminal paper Paul Ewald hypothesized that microbial mutualism can evolve from parasitism when there are evolutionary shifts from horizontal to vertical transmission among the hosts (Ewald, 1987). The author reviews data for and against this hypothesis below. The competing hypothesis states that bacterial mutualists must evolve directly from ancestors that lived free in the environment (Ewald, 1987). This hypothesis is based on the idea that it is difficult or impossible for bacteria switching from parasitism to mutualism because of constraints of genetic architecture (Moran and Wernegreen, 2000).

To examine empirical support for these scenarios we can again use the evolutionary reconstruction of bacterial species to infer past evolutionary transitions. However, this kind of analysis (that investigates transitions between different bacterial lifestyles) must be used with caution. The goal of hypothesis testing here is to examine general patterns of evolutionary transitions, however the dataset that is being used is relatively small because there are very few bacterial species on the tree (350 compared to the likely millions of actual bacterial species on earth), and because only a fraction of them ever associate with hosts. This sparse sampling means that many transitions are missing and that some inferred transitions might be spurious because intermediate steps have not been sampled. With these caveats in mind it can seen in the dataset that both the above hypotheses are supported. Three independent transitions from parasitism to mutualism can be observed on the tree and also mutualist lineages are inferred to have originated directly from the environmental ancestors (**Figure 1**). Unfortunately, there is not enough data on the tree to sufficiently test Ewald's hypothesis (1987) that the evolution of vertical transmission is responsible for the transitions from parasitism to mutualism. Phylogenies that examine specific bacterial lineages that include different host-associated lifestyles have also been used to examine and test these hypotheses and are consistent with the conclusion that mutualist bacteria can evolve from both environmental and parasitic ancestors (Sachs *et al.*, 2011b).

The Evolutionary Maintenance of Bacterial Mutualism

Mutualism is inherently unstable over evolutionary time (Sachs and Simms, 2006). For any cooperative interaction among species to be maintained, a mechanism must be in place to prevent the invasion of individuals that gain benefits from the interaction but do not pay any costs (cheaters). Since cheaters only take but do not give in an interaction they can be strongly favored by natural selection. The challenge then, is to explain how natural selection can promote cooperative individuals in a mutualism over cheaters. Three types of models for the maintenance of mutualism have been proposed (Axelrod and Hamilton, 1981; Bull and Rice, 1991; Sachs *et al.*, 2004; Foster and Wenseleers, 2006). The simplest of these models is called byproduct cooperation, which occurs when the bacterial partner cooperates with hosts as an automatic consequence of an unrelated and selfish trait (Sachs *et al.*, 2004). For instance, many bacteria produce toxins and antibiotics to protect themselves in the environment and these bacterial traits can automatically offer protection to hosts. One interesting outcome of such 'selfish cooperation' is that there is no opportunity for the bacterium to cheat since the cooperative trait usually bears no net cost of the bacterium. The next type of model, called partner fidelity feedback, works under the idea that there is a feedback in benefits between the bacterial and host partners. Feedbacks can work whenever the host and symbiont interact repeatedly or over a long enough period that their fitness interests become linked. The best example of this linkage has already been described in the example of vertical transmission. When hosts transmit bacteria from parent to offspring, both the bacterium and host can share a common interest in maximizing each other's survival and reproduction. The last type of model, called partner choice, occurs when the host can exert some level of control over the bacteria and has the ability to act as a selective force in the bacterial population. Specifically, with partner's choice the host can selectively favor cooperative over uncooperative infections and thus select for mutualists (and select against cheater mutants). To some degree, each of these models was developed without any particular biological interaction in mind and none of these models was initially created to study microbial symbioses. Hence, an interesting question to address is the degree to which different bacterial–host mutualisms are stabilized by byproducts, partner fidelity, or partner choice (Sachs *et al.*, 2011b).

Byproducts cooperation has not had a great deal of attention from empirical biologists. In part, this might be because it can be difficult to resolve byproducts cooperation into clear mechanisms. Yet, a feature that differentiates byproducts from partner fidelity feedback and partner choice is that the cooperative act bears no net cost for the bacterium; the trait is beneficial to the bacterium for some purpose unrelated to the host and exists irrespective of the host interaction. As described above, bacteria that exhibit toxicity or antibiotics might fulfill this description well. For instance, bacteria in the lineage Actinomycetes produce antibiotics as a benefit for their ant hosts. In this case the ants are fungus farmers; they grow fungi from the leaf litter collected in the forest and then eat the fungus for sustenance. The farming ants use the Actinomycete bacteria that grow on their exoskeleton to keep their fungal gardens pathogen-free (Currie *et al.*, 1999). As described above the trait of antibiotic production is a function that also benefits bacteria directly when it is in the environment, and this trait is expressed whether the bacteria are in the host or

not (Currie *et al.*, 1999; Sachs *et al.*, 2011b). Another likely example comes from bacteria that inhabit the lower gastrointestinal tracts of humans, *Bacteroides thetaiotaomicron*. One of the benefits that these bacterial mutualists are thought to provide for humans is to breakdown macromolecules that we cannot otherwise digest because we lack the enzymes to do it (Sonnenburg *et al.*, 2005). This is likely true for many functions that occur within the human body as the bacteria that inhabit us and carry our beneficial functions within the human body contain about 100 times as many genes as are found in our genome. Specifically in the case of *B. thetaiotaomicron*, the action of breaking down complex energy-filled food molecules into simpler compounds also must benefit the bacteria directly (Sachs *et al.*, 2011b). As there has been so little research on this topic there are likely many more examples such as these, but more work needs to be done to understand the breadth and importance of the byproducts cooperation in bacterial mutualists.

Partner-fidelity between bacteria and their hosts occurs when the bacteria are vertically transmitted from hosts to their offspring (Fine, 1975) and more generally in any scenario in which the symbiont interacts intimately with the host for a prolonged period (Sachs *et al.*, 2004; Sachs and Wilcox, 2006). Whereas vertical transmission is only one mechanism to maintain partner fidelity between bacteria and hosts, it remains the only driver that is well characterized or understood. In most cases vertically transmitted bacteria are very cooperative with hosts, but some exceptions exist. Some vertically transmitted bacteria harmfully manipulate the host's reproduction to maximize their own spread in the host population. For instance, many vertically transmitted symbionts are only passed on through females to offspring (via the ovum) and thus male hosts represent an evolutionary dead-end to these symbionts. In the case of *Wolbachia*, a common bacterial symbiont of most insects, have sometimes evolved mechanisms to bias host sex ratio toward females in order to maximize their own transmission (Stouthamer *et al.*, 1999).

Partner choice occurs when hosts exhibit mechanisms to selectively benefit beneficial bacterial partners and or punish harmful strains (Sachs *et al.*, 2004). Yet, how can a large eukaryotic host such as a plant or animal individually recognize individual bacterial genotypes and species and react toward them differentially in an adaptive manner? Despite a great deal of research, this question remains one of the major unsolved mysteries of symbiosis. One recent idea of how hosts can manage a complex group of genetically diverse symbionts is that hosts spatially separate their symbionts so that each genotype can be dealt with on an individualized basis (Denison, 2000; Sachs *et al.*, 2004, 2011b; West *et al.*, 2002a, b). Consistent with this hypothesis, many hosts of beneficial bacteria have evolved specialized structures that only exist in the context of a bacterial interaction and that have the potential to order symbionts into a fine level of spatial structure (e.g., Douglas, 1989; Bright and Sorgo, 2003; Nyholm and Mcfall-Ngai, 2004; Currie *et al.*, 2006; Goettler *et al.*, 2007; Vaishnava *et al.*, 2008). In many of these hosts, little is known about how or whether the structure promotes partner choice. Perhaps the best understood system is in legumes that are infected with root-nodule forming bacteria (rhizobia) that fix nitrogen for the host in exchange for plant sugars (Sprent *et al.*, 1987). In this case each nodule is most often infected by a single rhizobial genotype and thus nodules serve as a structural means for the plant to reward or punish rhizobial strains depending on how cooperative they are (Kiers *et al.*, 2003; Simms *et al.*, 2006; Sachs *et al.*, 2011a). The question is whether other hosts can wield such power over their bacterial symbionts. Some animals that host gastrointestinal bacteria have evolved complex crypt structures within their guts that appear specially to compartmentalize bacterial symbionts. But whether these crypt structures actively enforce partner choice is poorly understood. Humans exhibit crypt structures in their gastrointestinal tracts that bear importance to interaction with gut flora (Vaishnava *et al.*, 2008) but it is not clear whether these crypts are necessary for the maintenance of the mutualism or not. In some lineages of stinkbugs, the hosts have bacterial symbionts in the genus *Burkholderia* (Kikuchi *et al.*, 2011) that proliferate in the specialized gut crypts. Interestingly, only the stinkbug species that exhibit these crypts form beneficial symbioses with *Burkholderia*. This pattern suggests that the structural separation of the symbionts is a prerequisite to the evolutionary maintenance of the interaction (Sachs *et al.*, 2011b), but more data are needed.

The Evolutionary Capture of Bacterial Symbionts within Host Lineages

Most beneficial bacteria exhibit a rich existence both inside and outside their hosts. Since symbiotic bacteria are often environmentally acquired by hosts, the bacteria experience long phases in which they persist in soils or water separated by infection and growth within the hosts. To maintain this dual lifestyle the host-associated bacteria must be able to replicate in diverse environmental settings and must also be able to successfully localize and infect new hosts (Bright and Bulgheresi, 2010). As discussed above in the paragraph on partner–fidelity feedback, a subset of bacteria have evolved the ability to be vertically transmitted within host lineages (parent to offspring) and thus have lost their independence. The evolution of strict vertical transmission allows the symbionts to completely lose any environmental phase and thus persist only within the tissues or cells of hosts. How does this process of symbiont capture evolve and is it driven by the hosts or bacteria themselves (Sachs *et al.*, 2011b)?

A conflict of interests over symbiont capture appears to exist. Whereas vertically transmitted symbionts appear to suffer reduced fitness over time, their hosts experience clear benefits (Frank, 1996; Sachs *et al.*, 2011b). A great deal of data has accumulated regarding the genomic consequences of evolving vertical transmission for bacteria. When bacteria make the transition from free-living to strict vertical transmission their population size drops as a consequence of losing environmental phases. This simple change is thought to have enormous consequences. One of the fundamental rules of population biology is that natural selection only works efficiently on large populations: once populations drop to a small enough size they are only effected by random genetic change and tend to evolve reduced fitness over time (Nilsson *et al.*, 2005). Consistent with this theory, vertically transmitted

bacteria are known to accumulate harmful mutations over time, lose genetic pathways (Moran, 2003; Toh *et al.*, 2006), and ultimately lose so much function that they are obliged to rely on the host for basic nutrient synthesis (Shigenobu *et al.*, 2000). Because of this evolutionary pattern of genome degradation, once vertical transmission evolves it can lead to an irreversible evolutionary endpoint for bacteria that ends in reliance on hosts (Sachs *et al.*, 2011b). Whereas all of these changes appear to be harmful for vertically transmitted symbionts, the hosts mostly experience benefits. Hosts that transmit their beneficial bacteria vertically guarantee that their offspring will also receive a beneficial infection and by virtue of partner fidelity promote selection for beneficial traits in their bacterial symbiont population (Sachs *et al.*, 2004; Sachs and Bull, 2005; Sachs, 2006).

Hosts appear to have evolved many types of structures and mechanisms that promote symbiont capture (Bright and Bulgheresi, 2010). In many cases hosts have specific structures that appear to have no purpose at all except to efficiently transfer bacteria to offspring (Sachs *et al.*, 2011b). It is fascinating to observe the diversity of specialized structures that animal hosts have evolved to engage in vertical transmission of bacteria (Bright and Bulgheresi, 2010). Moreover, animals have also evolved complex behaviors to pass on symbionts to offspring, for instance by smearing symbionts onto eggs, onto egg cases, into cocoons, or directly on growing offspring (Douglas, 1989; Kaltenpoth *et al.*, 2005; Hirose *et al.*, 2009; Kikuchi *et al.*, 2009; Kojima and Hirose, 2010; Kaltenpoth *et al.*, 2010). In contrast to these well-studied mechanisms, little is known about the ability of bacteria to promote or counteract symbiont capture. In some interesting cases bacteria can be seen to actively migrate within their host to get to the ovaries (Douglas, 1989) or even move outside the parent to infect offspring (Ran *et al.*, 2010). Nonetheless, these examples are not necessarily evident against hosts being responsible for symbiont capture. This is because once capture has evolved the bacteria have no other option to infect new hosts other than vertical transmission (Sachs *et al.*, 2011a) Whereas hosts clearly benefit from the structures and behaviors that promote symbiont capture, little is known about the evolution of capture from the symbiont's perspective.

Evolutionary Breakdown of Bacterial-eukaryotic Symbioses

A great deal of theoretical work has modeled the ecology and evolution of mutualistic interactions. Although bacterial mutualists are extremely common, much of this theory predicts that evolutionary instability which leads to the breakdown of mutualism over time (Sachs and Simms, 2006). One major prediction that has come from this work is that mutualisms are prone to shift into parasitism as cheater mutants invade mutualist populations (Axelrod and Hamilton, 1981; Bull and Rice, 1991). Another prediction is that mutualists can evolve to abandon their partners if costs of the interaction come to outweigh potential benefits (Keeler, 1985; Holland *et al.*, 2004). To what degree does evidence support these hypotheses of 'mutualisms-breakdown' (e.g., Sachs and Simms, 2006)? As described above, phylogenetic analyses can be used to retrace the steps of evolution and infer how often mutualist bacterial lineages shift into other lifestyles. Interestingly, on the phylogenetic tree described above that samples lineages across bacteria (Wu *et al.*, 2009; Toft and Andersson, 2010; **Figure 1**) there are only two observed switches in which bacterial mutualists shift into other lifestyles. One of these shifts is a transition from a mutualist to a parasite and the other is a reversion back to an environmental (nonhost-associated) lifestyle (Sachs *et al.*, 2011b). Compared to the total number of lifestyle switches on the tree (72; Sachs *et al.*, 2011b) this dataset makes mutualism-breakdown seem surprisingly rare among bacterial lineages. Yet, it is difficult to tell whether this pattern represents an accurate reflection of bacterial evolution, or if it is an artifact of the dataset. As the author described above, the one big challenge to this kind of analysis is that so much of the dataset is missing: the tree represents a very sparse sampling of the actual bacterial diversity that exists.

For filling the potential gaps on the bacterial tree (Wu *et al.*, 2009; Toft and Andersson, 2010; **Figure 1**) one option is to 'zoom in' and investigate the potential for mutualism among much more closely related species or even strains of bacteria. Only in the last several years has there been a pulse of phylogenetic studies that have sampled bacterial lineages deeply enough to investigate the losses or gains of mutualism with hosts. Although several recent studies show repeated losses (abandonment) of bacterial mutualism (Nishiguchi and Nair, 2003; O'Brien *et al.*, 2005; Mueller *et al.*, 2010; Sachs *et al.*, 2010; Kikuchi *et al.*, 2011) no phylogenetic study that the author is aware of has uncovered a transition from mutualism to parasitism within a well-studied lineage. Hence, mutualism-breakdown appears to occur when bacterial mutualists evolve to abandon hosts (and revert to an environmental lifestyle) but there is extremely little evidence to support the idea that cheaters evolve within bacterial mutualist populations and drive transitions from mutualism to parasitism (Sachs *et al.*, 2011b).

Discussion

The history of bacterial interactions with animal, fungal, and plant hosts likely stretches back to the very origins of these lineages. It is virtually impossible to imagine organisms in any of these lineages existing without many intimate interactions with bacterial species (both harmful and beneficial). In the article the author has reviewed what is known about the evolution of bacterial interactions with eukaryotic hosts with a special focus on bacteria that cause beneficial infections (mutualists). Particularly, the author has explored the origins, maintenance, and ultimate breakdown of the bacterial interactions with hosts.

From the available data it can concluded that the evolutionary milestone of bacteria first achieving symbioses with hosts has evolved many times in almost every bacterial lineage that is known to science. The repeated and convergent evolution of persistent and intimate interactions between bacteria and hosts suggests that these interactions are highly advantageous to bacteria, relatively easier to evolve and mostly independent of the specific habitat requirements (Sachs *et al.*, 2011b). Among the many intimate interactions that bacteria

exhibit with eukaryotes a large subset are beneficial to hosts. These bacterial mutualists have evolved from the diverse ancestors that include parasitic species (that evolved over time to offer benefits to hosts) as well as environmental bacteria (that evolved from unassociation with hosts directly to a beneficial interaction; Ewald, 1987; Sachs *et al.*, 2011b). Among these types of origins it appears that bacterial mutualism most often evolves directly from the ancestors that live free in the environment.

Bacteria that have evolved mutualist interactions with hosts are incredibly diverse and provide almost any kind of nutrient or biological service to hosts that can be imagined (Sachs *et al.*, 2011a). In some cases hosts have evolved mechanisms to actively select bacterial cooperation, for instance by selectively rewarding beneficial strains or by punishing cheaters (Sachs *et al.*, 2004). In other cases bacterial cooperation is maintained in a more passive manner because host and symbiont exhibit a feedback in terms of fitness benefits (Fine, 1975). Most bacteria that provide beneficial infections to hosts are acquired from environmental sources and exhibit horizontal transmission among hosts. In some cases, bacterial symbionts have switched to vertical transmission and thus can lose any environmental phase and evolve to live only within the hosts. Although transitions from horizontal to strict vertical transmission (symbiont capture) involve both host and symbiont evolution, both theory and evidence suggest that hosts are likely to dominate control of these transitions. Finally, the evolutionary breakdown of bacterial mutualism appears to be relatively rare compared to other transitions in bacterial symbiosis. This lack of transitions from mutualism to parasitism or other bacterial lifestyles suggests either that bacterial mutualisms are evolutionarily robust or that transitions from mutualism to other lifestyles are themselves unstable (and lead to extinctions or other stable states; Sachs and Simms, 2006).

See also: Adaptive Radiation. Geologic Time, History of Biodiversity in. Phylogeny

References

Axelrod R and Hamilton WD (1981) The evolution of cooperation. *Science* 211: 1390–1396.

Boussau B, Karlberg EO, Frank AC, Legault BA, and Andersson SGE (2004) Computational inference of scenarios for alpha-proteobacterial genome evolution. *Proceedings of the National Academy of Sciences of the United States of America* 101: 9722–9727.

Bright M and Bulgheresi S (2010) A complex journey: Transmission of microbial symbionts. *Nature Reviews Microbiology.* 8: 218–230.

Bright M and Sorgo A (2003) Ultrastructural reinvestigation of the trophosome in adults of *Riftia pachyptila* (Annelida, Siboglinidae). *Invertebrate Biology* 122: 347–368.

Bull JJ and Rice WR (1991) Distinguishing mechanisms for the evolution of cooperation. *Journal of Theoretical Biology* 149: 63–74.

Carvalho SFM, Souza RC, Barcellos FG, Hungria M, and Vasconcelos ATR (2010) Genomic and evolutionary comparisons of diazotrophic and pathogenic bacteria of the order Rhizobiales. *Bmc Microbiology* 10: 37.

Corsaro D, Venditti D, Padula M, and Valassina M (1999) Intracellular life. *Critical Reviews in Microbiology* 25: 39–79.

Currie CR, Poulsen M, Mendenhall J, Boomsma JJ, and Billen J (2006) Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. *Science* 311: 81–83.

Currie CR, Scott JA, Summerbell RC, and Malloch D (1999) Fungus-growing ants use antibiotic-producing bacteria to control garden Parasites. *Nature* 398: 701–704.

Dale C, Young SA, Haydon DT, and Welburn SC (2001) The insect endosymbiont *Sodalis glossinidius* utilizes a type III secretion system for cell invasion. *Proceedings of the National Academy of Sciences of the United States of America* 98: 1883–1888.

Denison RF (2000) Legume sanctions and the evolution of symbiotic cooperation by rhizobia. *American Naturalist* 6: 567–576.

Douglas AE (1989) Mycetocyte symbiosis in insects. *Biological Reviews of the Cambridge Philosophical Society* 64: 409–434.

Ewald PW (1987) Transmission modes and evolution of the parasitism–mutualism continuum. *Annals of the New York Academy of Sciences* 503: 295–306.

Fine PEM (1975) Vectors and vertical transmission: An epidemiological perspective. *Annals of the New York Academy of Sciences* 266: 173–194.

Foster KR and Wenseleers T (2006) A general model for the evolution of mutualisms. *Journal of Evolutionary Biology* 19: 1283–1293.

Frank AC, Alsmark CM, Thollesson M, and Andersson SGE (2005) Functional divergence and horizontal transfer of type IV secretion systems. *Molecular Biology and Evolution.* 22: 1325–1336.

Frank SA (1996) Host control of symbiont transmission: The separation of symbionts into germ and soma. *American Naturalist* 148: 1113–1124.

Goettler W, Kaltenpoth M, Herzner G, and Strohm E (2007) Morphology and ultrastructure of a bacteria cultivation organ: The antennal glands of female European beewolves, *Philanthus triangulum* (Hymenoptera, Crabronidae). *Arthropod Structure and Development* 36: 1–9.

Hirose E, Neilan BA, Schmidt EW, and Murakami A (2009) Enigmatic life and evolution of Prochloron and related Cyanobacteria inhabiting colonial Ascidians. In: Gault PM and Marler HJ Hauppauge (eds.) *Handbook on Cyanobacteria: Biochemistry, Biotechnology and Applications.* New York: Nova Sciences Publishers Inc. pp. 161–189.

Holland JN, DeAngelis DL, and Schultz ST (2004) Evolutionary stability of mutualism: Interspecific population regulation as an evolutionarily stable strategy. *Proceedings of the Royal Society of London Series B – Biological Sciences* 271: 1807–1814.

Horn M, Collingro A, and Schmitz-Esser S (2004) Illuminating the evolutionary history of chlamydiae. *Science* 304: 728–730.

Kaltenpoth M, Gottler W, Herzner G, and Strohm E (2005) Symbiotic bacteria protect wasp larvae from fungal infestation. *Current Biology* 15: 475–479.

Kaltenpoth M, Goettler W, Koehler S, and Strohm E (2010) Life cycle and population dynamics of a protective insect symbiont reveal severe bottlenecks during vertical transmission. *Evolutionary Ecology* 24: 463–477.

Kaltenpoth M, Winter SA, and Kleinhammer A (2009) Localization and transmission route of *Coriobacterium glomerans*, the endosymbiont of pyrrhocorid bugs. *Fems Microbiology Ecology* 69: 373–383.

Keeler KH (1985) Cost: Benefitmodels of mutualism. In: Boucher DH (ed.) *The Biology of Mutualism, Ecology and Evolution*, pp. 100–127. Oxford: Oxford University Press.

Keeling PJ (2010) The endosymbiotic origin, diversification and fate of plastids. *Philosophical Transactions of the Royal Society B – Biological Sciences* 365: 729–748.

Kiers ET, Rousseau RA, West SA, and Denison RF (2003) Host sanctions and the legume–rhizobium mutualism. *Nature* 425: 78–81.

Kikuchi Y, Hosokawa T, and Fukatsu T (2011) An ancient but promiscuous host–symbiont association between Burkholderia gut symbionts and their heteropteran hosts. *ISME Journal* 5: 446–460.

Kikuchi Y, Hosokawa T, Nikoh N, Meng XY, Kamagata Y, and Fukatsu T (2009) Host–symbiont co-speciation and reductive genome evolution in gut symbiotic bacteria of acanthosomatid stinkbugs. *Bmc Biology* 7.

Kojima A and Hirose E (2010) Transfer of prokaryotic algal symbionts from a tropical Ascidian (*Lissoclinum punctatum*) colony to its larvae. *Zoological Science* 27: 124–127.

Ma WB, Dong FFT, Stavrinides J, and Guttman DS (2006) Type III effector diversification via both pathoadaptation and horizontal transfer in response to a coevolutionary arms race. *Plos Genetics* 2: 2131–2142.

Madigan MT, Martinko JM, Dunlap PV, and Clark DP (2009) *Brock Biology of Microorganisms.* San Francisco: Pearson Benjamin-Cummings.

Medina M and Sachs JL (2010) Symbiont genomics, our new tangled bank. *Genomics* 95: 129–137.

Merhej V, Royer-Carenzi M, Pontarotti P, and Raoult D (2009) Massive comparative genomic analysis reveals convergent evolution of specialized bacteria. *Biology Direct* 4.

Moran NA (2003) Tracing the evolution of gene loss in obligate bacterial symbionts. *Current Opinion in Microbiology* 6: 512–518.

Moran NA and Wernegreen JJ (2000) Lifestyle evolution in symbiotic bacteria: Insights from genomics. *Trends in Ecology and Evolution* 15: 321–326.

Mueller UG, Ishak H, Lee JC, Sen R, and Gutell RR (2010) Placement of attine ant-associated Pseudonocardia in a global Pseudonocardia phylogeny (Pseudonocardiaceae, Actinomycetales): A test of two symbiont-association models. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* 98: 195–212.

Nilsson A, Koskiniemi S, Eriksson S, Kugelberg E, Hinton JCD, and Andersson DI (2005) Bacterial genome size reduction by experimental evolution. *Proceedings of the National Academy of Sciences of the United States of America* 102: 12112–12116.

Nishiguchi MK and Nair VS (2003) Evolution of symbiosis in the Vibroonaceae: A combined approach using molecules and physiology. *International Journal of Systematic and Evolutionary Microbiology* 53: 2019–2026.

Nyholm SV and McFall-Ngai MJ (2004) The winnowing: Establishing the squid-Vibrio symbiosis. *Nature Reviews Microbiology* 2: 632–642.

O'Brien H, Miadlikowska J, and Lutzoni F (2005) Assessing host specialization in symbiotic cyanobacteria associated with four closely related species of the lichen Peltigera. *European Journal of Phycology* 40: 363–378.

Philippot L, Andersson SGE, and Battin TJ (2010) The ecological coherence of high bacterial taxonomic ranks. *Nature Reviews Microbiology* 8: 523–529.

Ran LA, Larsson J, and Vigil-Stenman T (2010) Genome erosion in a nitrogen-fixing vertically transmitted endosymbiotic multicellular cyanobacterium. *Plos One* 57: e11486.

Ruby EG, Urbanowski M, and Campbell J (2005) Complete genome sequence of *Vibrio fischeri*: A symbiotic bacterium with pathogenic congeners. *Proceedings of the National Academy of Sciences of the United States of America* 102: 3004–3009.

Sachs JL (2006) Cooperation within and among species. *Journal of Evolutionary Biology* 19: 1415–1418.

Sachs JL and Bull JJ (2005) Experimental evolution of conflict mediation between genomes. *Proceedings of the National Academy of Sciences of the United States of America* 102: 390–395.

Sachs JL, Ehinger MO, and Simms EL (2010) Origins of cheating and loss of symbiosis in wild Bradyrhizobium. *Journal of Evolutionary Biology* 23: 1075–1089.

Sachs JL, Essenberg CE, and Turcotte MM (2011a) New Paradigms for the Evolution of Beneficial Infections. *Trends in Ecology Evolution* 26: 202–209.

Sachs JL, Mueller UG, Wilcox TP, and Bull JJ (2004) The evolution of cooperation. *Quarterly Review of Biology* 79: 135–160.

Sachs JL, Russell JE, Lii YE, Black KC, Lopez G, and Patil SA (2010) Host control over infection and proliferation of a cheater symbiont. *Journal of Evolutionary Biology* 23: 1919–1927.

Sachs JL and Simms EL (2006) Pathways to mutualism breakdown. *Trends in Ecology and Evolution* 21: 585–592.

Sachs JL, Skophammer RG, and Regus JU (2011b) Evolutionary transitions in bacterial symbiosis. *Proceedings of the National Academy of Sciences USA* 108: 10800–10807.

Sachs JL and Wilcox TP (2006) A shift to parasitism in the jellyfish symbiont *Symbiodinium microadriaticum*. *Proceedings of the Royal Society B – Biological Sciences* 273: 425–429.

Sagan L (1967) On Origin of Mitosing Cells. *Journal of Theoretical Biology* 14: 225–274.

Shigenobu S, Watanabe H, Hattori M, Sakaki Y, and Ishikawa H (2000) Genome sequence of the endocellular bacterial symbiont of aphids Buchnera sp APS. *Nature* 407: 81–86.

Simms EL, Taylor DL, and Povich J (2006) An empirical test of partner choice mechanisms in a wild legume–rhizobium interaction. *Proceedings of the Royal Society B – Biological Sciences* 273: 77–81.

Sonnenburg JL, Xu J, and Leip DD (2005) Glycan foraging *in vivo* by an intestine-adapted bacterial symbiont. *Science* 307: 1955–1959.

Sprent JI, Sutherland JM, and Faria SM (1987) Some aspects of the biology of nitrogen-fixing organisms. *Philosophical Transactions of the Royal Society of London, B. Biological Sciences* 317: 111–129.

Stouthamer R, Breeuwer JAJ, and Hurst GDD (1999) *Wolbachia pipientis*: Microbial manipulator of arthropod reproduction. *Annual Review of Microbiology* 53: 71–102.

Szathmary E and Smith JM (1995) The major evolutionary transitions. *Nature* 374: 227–232.

Toft C and Andersson SGE (2010) Evolutionary microbial genomics: Insights into bacterial host adaptation. *Nature Reviews Genetics* 11: 465–475.

Toh H, Weiss BL, and Perkin SAH (2006) Massive genome erosion and functional adaptations provide insights into the symbiotic lifestyle of *Sodalis glossinidius* in the tsetse host. *Genome Research* 16: 149–156.

Vaishnava S, Behrendt CL, Ismail AS, Eckmann L, and Hooper LV (2008) Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host–microbial interface. *Proceedings of the National Academy of Sciences of the United States of America* 105: 20858–20863.

West SA, Kiers ET, Pen I, and Denison RF (2002a) Sanctions and mutualism stability: When should less beneficial mutualists be tolerated. *Journal of Evolutionary Biology* 15: 830–837.

West SA, Kiers ET, Simms EL, and Denison RF (2002b) Sanctions and mutualism stability: Why do rhizobia fix nitrogen. *Proceedings of the Royal Society of London Series B – Biological Sciences* 269: 685–694.

Wu DY, Hugenholtz P, Mavromatis K, *et al.* (2009) A phylogeny-driven genomic encyclopaedia of Bacteria and Archaea. *Nature* 462: 1056–1060.