Self-Extending Symbiosis: A Mechanism for Increasing Robustness Through Evolution

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Abstract

Robustness is a fundamental property of biological systems, observed ubiquitously across species and at different levels of organization from gene regulation to ecosystem. The theory of biological robustness argues that robustness fosters evolvability and that together they entail various tradeoffs as well as characteristic architectures and mechanisms. We argue that classes of biological systems have evolved to enhance their robustness by extending their system boundary through a series of symbioses with foreign biological entities ("self-extending symbiosis"). A series of major biological innovations has been achieved by events consistent with this framework: horizontal gene transfer, serial endosymbiosis, oocytes-mediated vertical infection, and host-symbiont mutualism for bacterial flora. Self-extending symbiosis contributes to robustness because symbiotic foreign biological entities can enhance the adaptative capacity of the system against environmental perturbations as well as contribute novel functions. In addition, evolutionary history indicates that the degree of symbiosis achieved has substantially changed from tight integration into the genome (which is less adaptive) to loose integration as bacterial flora (which can be highly adaptive). The most dramatic example can be seen in the symbiosis of host immune system and bacterial flora in which substantial function of host defense depends on the proper maintenance of bacterial flora and its adaptive capability. Biological systems following this type of evolutionary path might have attained high levels of functionality, robustness, and evolvability. Thus, robustness, evolution, and self-extending symbiosis may form essential system principles for biology.

Keywords

bacterial flora, evolvability, immune system, robustness, symbiosis

September 30, 2005; accepted October 25, 2005

Robust Evolvable Systems

Robustness is one of the fundamental properties of biological systems that is ubiquitously observed. The theory of biological robustness argues that it fosters evolvability and that selection tends to favor individuals with robust traits, and thus evolvable robust systems, such as living organisms, progressively adapt to become more robust against the environment in which they are embedded (Kitano 2004). At the same time an inherent tradeoff exists in which systems that are robust against certain perturbations are often extremely fragile against unexpected perturbations. In addition, being a more robust system is often accompanied by additional tradeoffs in resource demands and therefore in the performance of specific functions of the system. This suggests an interesting principle whereby the potentially conflicting demands of robustness and its associated tradeoffs play an important role in defining the properties of evolvable complex systems. The theory of biological robustness also argues for the existence of a characteristic global architecture that is universal for robust evolvable systems, an architecture that is a nested modularized bow-tie structure with a number of system controls imposed (Kitano 2004). The essence of the bow-tie structure is that there is a highly conserved core network connected to diverse input and output networks (Csete and Doyle 2004; Kitano 2004). The system can be robust against external perturbations because diverse inputs and outputs enable the system to detect these perturbations and take adequate countermeasures. Analysis of the number of genes in each functional category in a number of fully sequenced genomes of various sizes reveals that the proportion of genes that constitute input networks, such as signal transduction pathways, grows much faster than the entire genome, whereas the number of genes for the conserved core network, such as DNA repair and cell cycle control, increases only moderately (van Nimwegen 2003). This suggests that over evolutionary time robustness against external perturbations is enhanced by adding diverse new functions to the input and output parts of the bow-tie structure (Figure 1). We argue that this is a general strategy of biological systems.

So far, robustness and its evolutionary history have been addressed within the framework of Mendelian genetics in which mutation and sexual recombination have been considered as mechanisms for evolutionary innovations. The emergence of specific mechanisms for increasing robustness and the enrichment of the bow-tie structure have been discussed within this paradigm. In this article, we argue that this conception has to be expanded to include what we termed "self-extending symbiosis" as a mechanism to further enhance robustness. Self-extending symbiosis refers to phenomena where evolvable robust systems continue to extend their system boundary by incorporating foreign biological forms (genes, microorganisms, etc.) to enhance their adaptive capability against

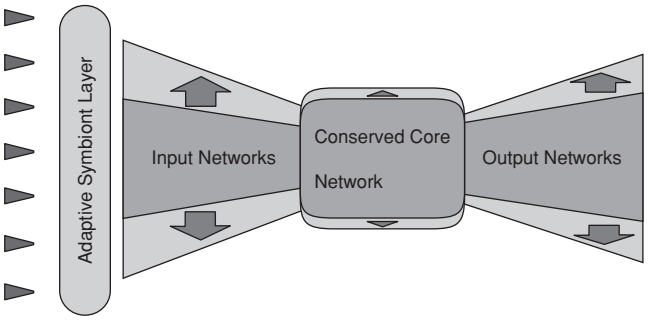

Figure 1.
Evolution of bow-tie structure and augmentation by an adaptive symbiont layer. Comparison of the numbers of genes in each functional category over various genomes of different sizes revealed a major increase in the number of genes involved in signal transduction, two-component system, and cell-cell communication, while the number of genes involved in core functions such as DNA repair and cell cycle has increased only moderately. Enhancement of input networks may provide greater detection capacity and increased flexibility in coping with environmental perturbations. The addition of an adaptive symbiont layer, such as commensal bacterial flora, further enhances the robustness of the system by allowing the whole system to adapt to greater levels of perturbations.

environmental perturbations and hence improve their survivability and reproduction potential. In other words, robust evolvable systems have consistently extended themselves by incorporating "non-self" features into tightly coupled symbiotic states.

Evolutionary History of Self-Extending Symbiosis

Looking at the history of evolutionary innovations, it is clear that some of the major innovations are the result of the acquisition of "non-self" into "self" at various levels (Figure 2). Horizontal gene transfer (HGT) facilitates evolution by exchanging genes between different species that have evolved within different contexts of optimization. HGT was shown to be a frequent phenomenon in prokaryotes, archea, and unicellular eukaryotes (Brown 2003; Gogarten and Townsend 2005). Microorganisms acquire novel functions, mostly those that enhance their robustness against environmental challenges, through horizontal exchange of genes. For example, it has been argued that the global emergence of antibiotic-resistant bacteria may be the result of horizontal transfer of antibiotic-resistant genes (Monroe and Polk 2000; Levy and Marshall 2004; Smets and Barkay 2005). In metazoan species, HGT has not been reported (at best, reported highly controversially) except in some rare instances of insect-bacteria symbiosis between the adzuki bean beetle *Callosobruchus chinensis* and *Wolbachia* (Kondo et al. 2002).

The serial endosymbiosis theory proposed by Lynn Margulis (Margulis 1971; Margulis and Bermudes 1985) argues that eukaryotic cells have initially been created through the acquisition of bacteria as their organelles. This process resulted in greater functionalities of eukaryotic cells, which in

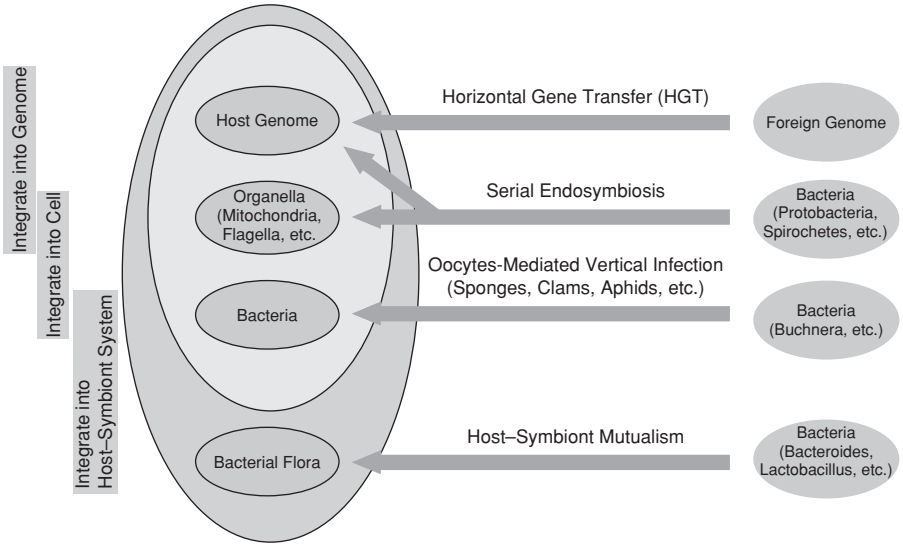

Figure 2.
Extending symbiotic systems by acquisition of nonhost systems. In addition to evolution by Mendelian genetics, living systems have increased their robustness against environmental perturbations by acquiring a variety of nonhost systems. Horizontal gene transfer (HGT) has been observed between different kingdoms of archea, prokaryotes, and eukaryotes. Through serial endosymbiosis, various species have merged into host cellular systems and formed organelles such as mitochondria. During this process, genes in the acquired species have been integrated into the host genome. In some metazoan species, infected commensal bacteria are vertically infected through oocytes. Although this mutualism has resulted in highly similar phylogenic trees between host and symbiont genes, genes are not transferred into the host. Bacterial flora are indispensable in the host system, although they are not integrated into genomes or intracellular systems in general. The clear pattern observed here is that acquisition of nonhost systems ranges from gene and intracellular organelles to bacterial flora reflecting diverse levels of flexibility and contributes to robustness of the symbiotic system.

turn made them more robust against environmental challenges. Here symbiosis led to the incorporation of foreign biological entities into cytoplasm of a cell as well as eventually into its own genome.

While HGT and endosymbiosis resulted in the incorporation of foreign biological entities into the genome and cellular structure of host cells, there are other forms of symbiosis that do not directly alter the genome but are nevertheless essential to the survival of a species. There are species that allow certain bacteria to be vertically inherited through the host's oocytes as observed in sponges, clams (Cary and Giovannoni 1993), and aphids (Baumann et al. 1995). Aphids, for example, are infected with the genus *Buchnera*, resulting in an endosymbiotic relationship that dramatically improves energy utilization and terrain exploration capability of the host. It was shown that aphids and *Buchnera* undergo parallel evolution. As a result the phylogenetic trees of the host (aphids) and symbionts (genus *Buchnera*) are consistent with each other (Baumann et al. 1995). Parallel evolution has also been observed in a case of endosymbiosis between *Psyllid* and *Candidatus* (Thao et al. 2001).

Aside from such tight coupling of host and symbiont, horizontal (environmental) acquisition of symbionts (Nyholm and McFall-Ngai 2004) is yet another approach to extend the self by incorporating a broader range of microbes, thereby allowing the host to be able to adapt to a wide range of environments and nutrients. Commensal bacteria are ubiquitously observed in the gut of various metazoan species including termites (Schmitt-Wagner et al. 2003), cockroaches (Bracke et al. 1979), prawns (Oxley et al. 2002), and mammals. In all these cases bacteria have established inseparable relationships with the host organisms, and are even considered to have coevolved with their hosts (Backhed et al. 2005). In human beings, the commensal bacterial flora in the gut consists of a diverse set of microorganisms (up to 500–1,000 species), amounting to about 10^{14} bacteria and weighing a total of 1.5 kg (Xu and Gordon 2003). The human being as a symbiotic system consists of approximately 90% prokaryotes and 10% eukaryotes (Savage 1977), and a random shotgun sequencing of the whole human symbiotic system would result in predominantly bacterial genome readouts of about two million genes with sporadically interspersed mammalian genes (Hooper et al. 2002). Such commensal intestinal bacteria play a critical role in various aspects of host physiology. Mammalian bacterial flora has also been considered to be an integral part of host protection by mutually beneficial symbiosis with the host immune system.

These lines of observations point to a characteristic property of biological systems, namely that a greater level of robustness and functionalities is gained by incorporating foreign biologic entities into their own system in the form of different degrees of symbiosis. HGT and endosymbiosis incorporate foreign entities into genome and cellular structures, while vertical inheritance based endosymbiosis does not directly alter genome. The bacterial flora simply adds another layer that is

symbiotically interacting with the mucosal immune system of the host to its adaptive system. The general tendency observed here is the continuous addition of external layers to the host system by means of symbiotic incorporation of foreign entities, and it results in an increased level of robustness against environmental perturbation.

Symbiosis of the Host Immune System and Bacterial Flora

The relationship between the bacterial flora and the host immune system provides an interesting example of how symbiosis adds robustness to the host organism by offering flexibility against environmental changes. This is a significant example because the role of the host immune system is to reject pathogens including bacteria. The symbiotic relationship between bacteria and host is thus a consequence of a complex dynamic interaction between the host immune system and the bacteria. Some species of bacteria not only successfully colonize our body but also provide us with essential functions without which we could not survive, such as stimulation of the gut immune system development, rejection of pathogenic bacteria, and contributions to our nutrient supply.

It was demonstrated that germ-free mice that have no commensal bacterial flora have an undeveloped mucosal immune system that has hypoplastic Peyer's patches, as well as significantly reduced numbers of IgA-producing plasma cells and lamina propria $CD4^+$ T cells (Macpherson et al. 2001, 2002).

Commensal bacteria even protect against pathogenic bacteria, a phenomenon known as colonization resistance. Continuous flow experiments using a smaller subset of commensal bacteria revealed that this effect is due to the antipathogen function of commensal bacteria including *Escherichia coli* and competition for nutrients and spaces that are sustained by the dynamics of an intermicrobial metabolic network (Ushijima and Ozaki 1986, 1988). Such a phenomenon is medically relevant, as perturbation of commensal bacteria due to the use of antibiotics causes antibiotic-associated diarrhea (AAD) (Bergogne-Berezin 2000) by allowing pathogenic bacteria such as *Clostridium difficile* to proliferate. In addition, the intestinal bacterial flora contributes to the host's nutrient supply by starch digestion, amino acid homeostasis, vitamin synthesis, etc. (Hooper et al. 2002). Some metabolic interactions are so tightly coupled that metabolites are produced which neither the host nor bacteria alone can produce (Nicholson and Wilson 2003; Nicholson et al. 2005). Such Sym-xenobiotic metabolism signifies the level of symbiosis achieved between the host and commensal bacteria. The composition of bacterial flora also changes dynamically with changes in dietary and nutrient contents (Harmsen et al. 2000; Mai and Morris 2004); thus it is essential for the host to be able to harbor a broad range of bacteria to enable its flora to cope with environmental perturbations. A genomic study revealed that the bacterial flora even manipulates host gene expression in order to establish mutually advantageous partnerships (Xu et al. 2003), and that host gene expression changes according to the composition of microbes in the flora (Hooper et al. 2001). Not only does the bacterial flora affect the host, but the host also affects the activity and composition of the flora through its genotype (Zoetendal et al. 2001) and immunological responses (Hornef et al. 2002). Because of the intricate relationship between bacterial flora and the host, some believe that the flora should be considered as an additional "organ" rather than as an unwanted guest (Hooper et al. 2002).

It should be noted that this "organ" provides enhanced robustness against environmental perturbations by stimulating immune system development, nutrient processing and biosynthesis, and resistance to pathogenic microbes, and it has been argued that this relationship has coevolved (Backhed et al. 2005). It has also been found that loss of biodiversity of the bacterial flora of the intestine is associated with inflammatory bowel diseases (Ott et al. 2004). These findings suggest that the bacterial flora is an essential part of the system, and that the immune system might have coevolved to allow diverse symbionts to reside within the host without compromising the defense of the host against other pathogens.

Has Bacterial Flora Been Enriched by the Emergence of Adaptive Immunity?

Given the line of reasoning so far, we hypothesize that the immune system evolved to provide robustness to the host-symbiont system against a variety of pathogenic threats from the environment, instead of providing defense to the host organism alone. This means that the immune system enhances the robustness of the organisms by enabling them to harbor a broader range of symbionts, yet rejecting pathogenic microorganisms and toxins. The premise of this hypothesis is that harboring a broader range of symbionts contributes more to the robustness of the host organism by enabling a spectrum of adaptive responses against changes in dietary and nutrient contents and the exposure to pathogenic microorganisms.

If this hypothesis is correct, the diversity of the commensal bacterial flora may correlate with a variety of perturbations that the species encountered. This means that a species that has been exposed to a broader range of foods, microorganisms, and threats from the environment possibly caused by an expanding range of habitat and food intake behavior will harbor a more diverse bacterial flora. Another interesting hypothesis may provide additional support for this speculation. Studies of evolution of the immune system show that adaptive immunity emerged primarily after the origin of the jawed vertebrates, possibly when the variable (V) type of an Ig

superfamily (*Igsf*) gene was invaded by transposable elements that contain *RAG1* and *RAG2* genes (Bernstein et al. 1996; Agrawal et al. 1998), as well as after rounds of genome-wide chromosomal duplications (Kasahara 1998) that might foster a dramatic increase in the complexity of the system. Proponents of the "jaw hypothesis" argue that the acquisition of a jaw imposed selective pressure for the emergence of an adaptive immune system, because the development of a jaw conferred the ability to bite and swallow foods and increased the incidence of injury in the wall of digestive organs because of hard substances and bones (Andersson and Matsunaga 1996; Matsunaga 1998; Matsunaga and Rahman 1998). Although the jaw hypothesis does not explain the pivotal role of the bacterial flora in mucosal immune development, it is possible to assume that a dramatic increase in the exposure of the gut surface to bacteria imposed evolutionary pressure for the emergence of adaptive immunity to back up innate immunity, thus enabling a stronger defense against invading pathogens to be established. We speculate that adaptive immunity emerged not only because of increased incidence of injury, but also because of a drastic increase in exposure to pathogens, in both amount and variety, owing to changes in food and behavioral patterns. During this process, some bacteria species might have coevolved with the host system to create mutually beneficial relationships involving nutrient supply, digestive assistance, and pathogen rejection. Because of this symbiotic state, the bacterial flora may have been significantly enriched to cope with the increased exposure and diversity of microbes, viruses, nutrients, and toxins. Reinforcement of the immune system with the emergence of the adaptive immune system may have been essential in keeping diverse microorganisms without allowing them to penetrate the host. If this hypothesis is correct, species with adaptive immunity will have a more diverse and variable commensal bacterial flora than species without adaptive immunity.

Conclusion

In this article, we proposed a self-extending symbiosis hypothesis that argues that extending the "self" through varying degrees of symbiosis with foreign biological entities enhances robustness of the system and has been selected in the evolutionary path of eukaryotic metazoans. Such augmentations of the organism's architecture enrich the bow-tie architecture of complex systems by adding layers of adaptation and novel functions. Evolutionary history indicates that the level of symbiosis tends to be more loosely coupled as the complexity of organism increases. Early during evolution, HGT and endosymbiosis were the major vehicles of evolutionary innovation whereas various forms of exosymbiosis with bacteria are dominant in metazoans. However, this shift of the level of symbiosis provides greater levels of adaptability against environmental change, and hence greater degrees of robustness. The hypothesis predicts that tumors, which are evolvable robust systems by themselves, may undergo self-extending symbiosis to acquire a higher degree of robustness, perhaps by acquiring genomes from local cells, microenvironment remodeling, and controlling tumor-associated macrophages.

The most recent innovation due to self-extending symbiosis is the symbiosis between the bacterial flora and the host immune system. Symbiont biodiversity within individuals may provide a selective advantage, so species might have evolved to embrace a greater degree of symbionts while simultaneously preventing the harmful side effects. This may be what the immune system has been optimized for, and may represent a recent evolutionary innovation that is consistent with the idea of enhanced robustness by extending the self.

Self-extending symbiosis may be a fundamental process of significant evolutionary innovation that adds greater levels of robustness and functionalities to the species. Evolution in the context of Mendelian genetics may shape architecture at the genomic level, whereas self-extending symbiosis augments the system's robustness by adding new adaptive layers. Evolution and symbiosis are both linked to robustness. This could lead to a fundamental principle of biological systems that may explain architectural and system-level principles and their evolution. Deeper understanding of this principle may provide us with a novel concept and connected countermeasures for medically significant diseases such as cancer and immunological disorders.

Acknowledgments

This research was supported in part by the Exploratory Research for Advanced Technology (ERATO) and the Solution-Oriented Research for Science and Technology (SORST) programs (Japan Science and Technology Organization), a NEDO Grant (New Energy and Industrial Technology Development Organization) of the Japanese Ministry of Economy, Trade and Industry (METI), the Special Coordination Funds for Promoting Science and Technology and the Center of Excellence Program for Keio University (Ministry of Education, Culture, Sports, Science, and Technology), and the Genome Network Project by the Ministry of Education, Culture, Sports, Science, and Technology.

References

Agrawal A, Eastman QM, Schatz DG (1998) Transposition mediated by *RAG1* and *RAG2* and its implications for the evolution of the immune system. Nature 394: 744–751.

Andersson E, Matsunaga T (1996) Jaw, adaptive immunity and phylogeny of vertebrate antibody VH gene family. Research in Immunology 147: 233–240.

Backhed F, Ley R, Sonnenburg J, Peterson D, Gordon JI (2005) Host-bacterial mutualism in the human intestine. Science 307: 1915–1920.

Baumann P, Baumann L, Laui CY, Rouhbakhsh D, Moran NA, Clark MA (1995) Genetics, physiology, and evolutionary relationships of the genus *Buchnera*: Intracellular symbionts of aphids. Annual Review of Microbiology 49: 55–94.

Bergogne-Berezin E (2000) Treatment and prevention of antibiotic associated diarrhea. International Journal of Antimicrobial Agents 16: 521–526.

Bernstein RM, Schluter SF, Bernstein H, Marchalonis JJ (1996) Primordial emergence of the recombination activating gene 1 (*RAG1*): Sequence of the complete shark gene indicates homology to microbial integrases. Proceedings of the National Academy of Sciences USA 93: 9454–9459.

Bracke JW, Cruden DL, Markovetz AJ (1979) Intestinal microbial flora of the American cockroach, *Periplaneta americana L*. Applied and Environmental Microbiology 38: 945–955.

Brown JR (2003) Ancient horizontal gene transfer. Nature Reviews Genetics 4: 121–132.

Cary SC, Giovannoni SJ (1993) Transovarial inheritance of endosymbiotic bacteria in clams inhabiting deep-sea hydrothermal vents and cold seeps. Proceedings of the National Academy of Sciences USA 90: 5695–5699.

Csete M, Doyle J (2004) Bow ties, metabolism and disease. Trends in Biotechnology 22: 446–450.

Gogarten JP, Townsend JP (2005) Horizontal gene transfer, genome innovation and evolution. Nature Reviews Microbiology 3: 679–687.

Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, Bindels JG, Welling GW (2000) Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. Journal of Pediatrics, Gastroenterology and Nutrition 30: 61–67.

Hooper LV, Midtvedt T, Gordon JI (2002) How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annual Review of Nutrition 22: 283–307.

Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI (2001) Molecular analysis of commensal host-microbial relationships in the intestine. Science 291: 881–884.

Hornef MW, Wick MJ, Rhen M, Normark S (2002) Bacterial strategies for overcoming host innate and adaptive immune responses. Nature Immunology 3: 1033–1040.

Kasahara M (1998) What do the paralogous regions in the genome tell us about the origin of the adaptive immune system? Immunological Reviews 166: 159–175.

Kitano H (2004) Biological robustness. Nature Reviews Genetics 5: 826–837.

Kondo N, Nikoh N, Ijichi N, Shimada M, Fukatsu T (2002) Genome fragment of *Wolbachia* endosymbiont transferred to X chromosome of host insect. Proceedings of the National Academy of Sciences USA 99: 14280–14285.

Levy SB, Marshall B (2004) Antibacterial resistance worldwide: Causes, challenges and responses. Nature Medicine 10: S122–S129.

Macpherson AJ, Hunziker L, McCoy K, Lamarre A (2001) *IgA* responses in the intestinal mucosa against pathogenic and non-pathogenic microorganisms. Microbes and Infection 3: 1021–1035.

Macpherson AJ, Martinic MM, Harris N (2002) The functions of mucosal T cells in containing the indigenous commensal flora of the intestine. Cellular and Molecular Life Science 59: 2088–2096.

Mai V, Morris JG Jr (2004) Colonic bacterial flora: Changing understandings in the molecular age. Journal of Nutrition 134: 459–464.

Margulis L (1971) Symbiosis and evolution. Scientific American 225(2): 48–57.

Margulis L, Bermudes D (1985) Symbiosis as a mechanism of evolution: Status of cell symbiosis theory. Symbiosis 1: 101–124.

Matsunaga T (1998) Did the first adaptive immunity evolve in the gut of ancient jawed fish? Cytogenetics and Cell Genetics 80: 138–141.

Matsunaga T, Rahman A (1998) What brought the adaptive immune system to vertebrates?—The jaw hypothesis and the seahorse. Immunological Reviews 166: 177–186.

Monroe S, Polk R (2000) Antimicrobial use and bacterial resistance. Current Opinion in Microbiology 3: 496–501.

Nicholson JK, Holmes E, Wilson ID (2005) Gut microorganisms, mammalian metabolism and personalized health care. Nature Reviews Microbiology 3: 431–438.

Nicholson JK, Wilson ID (2003) Opinion: Understanding "global" systems biology: Metabonomics and the continuum of metabolism. Nature Reviews Drug Discovery 2: 668–676.

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

Ott SJ, Musfeldt M, Wenderoth DF, Hampe J, Brant O, Fölsch UR, Timmis KN, Schreiber S (2004) Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. Gut 53: 685–693.

Oxley AP, Shipton W, Owens L, McKay D (2002) Bacterial flora from the gut of the wild and cultured banana prawn, *Penaeus merguiensis*. Journal of Applied Microbiology 93: 214–223.

Savage DC (1977) Microbial ecology of the gastrointestinal tract. Annual Review of Microbiology 31: 107–133.

Schmitt-Wagner D, Friedrich MW, Wagner B, Brune A (2003) Phylogenetic diversity, abundance, and axial distribution of bacteria in the intestinal tract of two soil-feeding termites (*Cubitermes* spp.). Applied and Environmental Microbiology 69: 6007–6017.

Smets BF, Barkay T (2005) Horizontal gene transfer: Perspectives at a crossroads of scientific disciplines. Nature Reviews Microbiology 3: 675–678.

Thao ML, Clark MA, Burckhardt DH, Moran NA, Baumann P (2001) Phylogenetic analysis of vertically transmitted psyllid endosymbionts (*Candidatus Carsonella ruddii*) based on atpAGD and rpoC: Comparisons with 16S-23S rDNA-derived phylogeny. Current Microbiology 42: 419–421.

Ushijima T, Ozaki Y (1986) Potent antagonism of *Escherichia coli*, *Bacteroides ovatus*, *Fusobacterium varium*, and *Enterococcus faecalis*, alone or in combination, for enteropathogens in anaerobic continuous flow cultures. Journal of Medical Microbiology 22: 157–163.

Ushijima T, Ozaki Y (1988) Factors influencing potent antagonistic effects of *Escherichia coli* and *Bacteroides ovatus* on *Staphylococcus aureus* in anaerobic continuous flow cultures. Canadian Journal of Microbiology 34: 645–650.

van Nimwegen E (2003) Scaling laws in the functional content of genomes. Trends in Genetics 19: 479–484.

Xu J, Bjursell MK, Himrod J, Deng S, Carmichael LK, Chiang HC, Hooper LV, Gordon JI (2003) A genomic view of the human-bacteroides thetaiotaomicron symbiosis. Science 299: 2074–2076.

Xu J, Gordon JI (2003) Inaugural article: Honor thy symbionts. Proceedings of the National Academy of Sciences USA 100: 10452–10459.

Zoetendal EG, Akkermans ADL, Akkermans van-Vliet WM, de Visser JAGM, de Vos WM (2001) The host genotype affects the bacterial community in the human gastrointestinal trace. Microbial Ecology in Health and Disease 13: 129–134.