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A SYMBIOTIC VIEW OF LIFE: WE HAVE NEVER BEEN INDIVIDUALS

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ABSTRACT

The notion of the "biological individual" is crucial to studies of genetics, immunology, evolution, development, anatomy, and physiology. Each of these biological subdisciplines has a specific conception of individuality, which has historically provided conceptual contexts for integrating newly acquired data. During the past decade, nucleic acid analysis, especially genomic sequencing and high-throughput RNA techniques, has challenged each of these disciplinary definitions by finding significant interactions of animals and plants with symbiotic microorganisms that disrupt the boundaries that heretofore had characterized the biological individual. Animals cannot be considered individuals by anatomical or physiological criteria because a diversity of symbionts are both present and functional in completing metabolic pathways and serving other physiological functions. Similarly, these new studies have shown that animal development is incomplete without symbionts. Symbionts also constitute a second mode of genetic inheritance, providing selectable genetic variation for natural selection. The immune system also develops, in part, in dialogue with symbionts and

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thereby functions as a mechanism for integrating microbes into the animal-cell community. Recognizing the "holobiont"—the multicellular eukaryote plus its colonies of persistent symbionts—as a critically important unit of anatomy, development, physiology, immunology, and evolution opens up new investigative avenues and conceptually challenges the ways in which the biological subdisciplines have heretofore characterized living entities.

IN THE EARLY modern period, mirroring the appearance of the independent citizen, the notion of the autonomous individual agent framed a biology that was organized around the study of particulate, interacting, living entities (Taylor 1989). Anatomical, physiological, and developmental criteria were conceived solely in terms of individuals, and the Darwinian view of life regarded aggregates of individuals of common ancestry as identifiable units in competition with one another. With the understanding that plants and animals are comprised of living "cells," a new orientation quickly developed concerning the integration of physiological processes and anatomic units, but still these cells were understood as agents in constructing and sustaining a singular organism that would in turn maintain its autonomy and integrity. Only with the emergence of ecology in the second half of the 19th century did organic *systems*—comprised of individuals in cooperative and competitive relationships—complement the individual-based conceptions of the life sciences.

The development of such complex formulations of individuals and systems depends on myriad factors, of which technology constitutes a major component in the characterization process. We perceive only that part of nature that our technologies permit and, so too, our theories about nature are highly constrained by what our technologies enable us to observe. But theory and technology act on each other reciprocally: we construct those technologies that we think are important for examining a particular perspective of nature. The development of the microscope, for example, revealed the hitherto invisible microbial world of bacteria, protists, and fungi; and the descendants of that instrument further allowed the discovery of subcellular organelles, viruses, and macromolecules. New technologies such as polymerase chain reaction, high-throughput RNA analysis, and next generation sequencing continue to dramatically transform our conceptions of the planet's biosphere. They have not only revealed a microbial world of much deeper diversity than previously imagined, but also a world of complex and intermingled *relationships*—not only among microbes, but also between microscopic and macroscopic life (Gordon 2012). These discoveries have profoundly challenged the generally accepted view of "individuals." Symbiosis is becoming a core principle of contemporary biology, and it is replacing an essentialist conception of "individuality" with a conception congruent with the larger systems approach now pushing the life sciences in diverse directions. These findings lead us into directions that transcend the self/nonself, subject/object dichotomies that have characterized Western thought (Tauber 2008a,b).

This reorientation is not new for the microbial or botanical sciences. In the world of protists, hereditary symbiosis, the inheritance of acquired symbionts is legion. In the microbial world, "you are what you eat" can be taken literally. In botanical science, the concept of the autonomous individual has also been challenged by discoveries concerning rhizobia, mycorrhizae, and endocytic fungae. Nonetheless, zoologists long subscribed to a more individualist conception of the organism, since the role of microbial symbionts had been more difficult to document in animal evolution (Sapp 1994, 2002, 2009). We report here that the zoological sciences are also finding that animals are composites of many species living, developing, and evolving together. The discovery of symbiosis throughout the animal kingdom is fundamentally transforming the classical conception of an insular individuality into one in which interactive relationships among species blurs the boundaries of the organism and obscures the notion of essential identity.

Our aims in this overview are to: outline the data demonstrating that animals are symbiotic complexes of many species living

together; demonstrate how a thoroughly symbiotic perspective opens important areas of research and offers fundamentally new conceptions of the organism; and explore what this new evidence means for biology, medicine, and for the conservation of biodiversity.

Criteria for Individuality

What would biological science be if symbiosis were seen as the rule, not the exception? What scientific questions would become paramount and how might this change our view of life if intimate cooperation between species were a fundamental feature of evolution? What could "individual selection" mean if all organisms were chimeric, and there were no real monogenetic individuals?

There are many ways in which the term "individual" is used in biology. Individuals can be defined anatomically, embryologically, physiologically, immunologically, genetically, or evolutionarily (see Geddes and Mitchell 1911; Clarke 2010; Nyhart and Lidgard 2011). These conceptions, though, are not wholly independent of one another. Nor have these definitions of individuality often been explicitly articulated as such. Indeed, even in biology today there is a dearth of definition in what constitutes the individual organism. Still, definitions are implied, and each stems from the common tenet of genomic individuality: one genome/one organism. As such, all classical conceptions of individuality are called into question by evidence of all-pervading symbiosis.

Anatomical Individuality

Anatomically, the individual animal is regarded as a structured whole. Yet, data from PCR show that the cells and bodies of animals are shared with numerous "species" of bacteria and other microbes. In some sponges, nearly 40% of the volume of the organism is comprised of bacteria, which contribute significantly to host metabolism (Taylor et al. 2007). The algal symbiont, *Symbiodinium*, provides up to 60% of the nutrients needed by its host coral (the term "host" is used here in the classical sense to denote the larger, eukaryotic, multicellular organism in which the "symbiont" resides). When this symbiosis is broken by a prolonged increase in sea-surface temperatures, corals "bleach." They lose their algal symbionts and die. Similarly, the entity we call a cow is an organism whose complex ecosystem of gut symbionts—a diverse community of cellulose-digesting bacteria, ciliated protists, and anaerobic fungi—informs its specialized anatomy, defines its plant-digesting physiology, regulates its behaviors, and ultimately determines its evolution (Kamra 2005).

In addition to the mitochondrial vestiges of ancient symbiosis, thousands of bacterial "species" (themselves genetic composites) live in intimate association with our own eukaryotic cells. Estimates that 90% of the cells that comprise our bodies are bacterial (Bäckhed et al. 2005; Ley et al. 2006) belie any simple anatomical understanding of individual identity. Metagenomic sequencing (Qin et al. 2010) has shown that each human gut has entered into a persistent partnership with over 150 species of bacteria, and that the human species maintains about 1000 major bacteria groups in our gut microbiome. The gene set contained by this symbiotic metagenome is about 150 times larger than that of the human eukaryotic genome. And this does not include the symbionts of human airways, skin, mouth, or reproductive orifices.

Mastotermes darwiniensis, a termite of northern Australia, may claim the title of "poster organism" for the chimeric individual. The worker termites eat trees and entire homes, digesting the cellulose in their guts and constructing elaborate subterranean nests. But as Lewis Thomas (1974) and Lynn Margulis and Dorion Sagan (2001) have asked: What constitutes the individual organism? How can a worker termite be considered an individual when it is the hive that is the reproductive unit of the species, and the worker cannot even digest cellulose without its gut symbiont, *Mixotricha paradoxa*, which is itself a genetic composite of at least five other species? Neither humans, nor any other organism, can be regarded as individuals by anatomical criteria. To capture this complexity, the term "holobiont" has been introduced as the anatomical term that describes the integrated organism comprised of both host elements and persistent

populations of symbionts (Rosenberg et al. 2007).

Developmental Individuality

The *developmental* view of animal individuality was originally proposed by Thomas Huxley in his published lecture, "Upon Animal Individuality" (Huxley 1852). A variant of the anatomical version of biological individuality, the individual animal proposed here is understood to be that which proceeds from ovum to ovum. Yet, this view of life is belied by evidence that what we understand to be the "individual" develops as consortia of animal cells and microbes (McFall-Ngai 2002; Gilbert and Epel 2009; Fraune and Bosch 2010; Pradeu 2011). Indeed, the development of both vertebrates and invertebrates (especially larval and postembryonic development) is predicated on intimate relations with microbes.

In some instances, the symbiosis may be parasitic, one organism benefiting at the expense of another. For example, the development of the European blue butterfly *Maculinea arion* requires that the female lays her eggs on thyme plants. The larvae, however, do not eat thyme, but drop to the ground, where they produce a mixture of volatile chemicals mimicking the smell of the larvae of the ant species *Myrmica sabuleti*. Patrolling Myrmicae mistake the butterfly larva as one of their own, and carry it into the ant nest. Once in the nest with the ant larvae, the caterpillar is fed by the workers, eventually eating young ants until it is ready to pupate. It undergoes metamorphosis in the ant colony and emerges as an adult (Thomas 1995; Nash et al. 2008). This type of life-cycle symbiosis occurs throughout marine invertebrates, where larvae require cues, often from their food sources, in regard to where and when to settle and undergo metamorphosis.

The importance of symbiotic organisms for the completion of host life cycles is also evident in parasitic worms, where bacteria are crucial for embryogenesis and molting (Hoerauf et al. 2003; Coulibaly et al. 2009) and in salamander development, where symbiotic algae on the egg jelly produce the oxygen necessary for the survival of the spotted salamander embryos (Olivier and Moon 2010; Kerney et al. 2011).

In numerous organisms, the development of particular organs is predicated on chemical signals from symbionts (Douglas 1988, 2010). For example, the ovaries of the parasitoid wasp, *Asobara*, undergo apoptosis if signals from their *Wolbachia* symbionts are lacking (Pannebakker et al. 2007). And the newborn of the squid *Euprymna scolopes* lacks a light organ, which is developed in cooperation between the squid and the luminescent bacteria (*Vibrio fisheri)* absorbed by its ventral epithelium (McFall-Ngai et al. 2012). Without the bacteria, the organ does not develop.

In "germ-free" asymbiotic mice, the development of the immune system and the digestive system cannot be completed without gut bacteria (Ley et al. 2006, 2008; Lee and Mazmanian 2010). Rather, these mice have insufficient intestinal capillaries, poorly developed or absent gut-associated lymphoid tissue, and a diminished T-cell repertoire that gives them an immunodeficiency syndrome (Stappenbeck et al. 2002; Rhee et al. 2004; Niess et al. 2008; Duan et al. 2010). In zebrafish, microbes regulate (through the canonical Wnt pathway) the normal proliferation of the intestinal stem cells. Without these microbes, the intestinal epithelium has fewer cells, and it lacks goblet cells, entroendocrine cells, and the characteristic intestinal brush border enzymes (Rawls et al. 2004; Bates et al. 2006).

Microbial symbionts appear to be a normal and necessary part of the life cycle of all mammals, which acquire the microbes as soon as the amnion breaks or when infants suckle or hug. The microbes colonize the guts and induce appropriate gene expression in the intestine of the newborn (Hooper et al. 2001). In the developing guts of mice and zebrafish, hundreds of genes are activated by bacterial symbionts (Hooper et al. 2001; Rawls et al. 2004). The coevolution of mammals and their gut bacteria has in effect resulted in the "outsourcing" of developmental signals from animal cells to microbial symbionts. Thus, the symbionts are integrated into the normal networks of animal development, interacting with the eukaryotic cells of their "host" (Gilbert 2001, 2003; McFall-Ngai 2002). Development then becomes a matter of interspecies communication. We are

not individuals from the viewpoint of developmental biology.

Physiological Individuality

Since the classical writings of Henri Milne-Edwards (1827) and Rudolf Leuckart (1851), the *physiological* view of animal individuality regards the organism as comprised of parts that cooperate for the good of the whole. Complexity of animal organization is accompanied by the increasing division of labor among organs, a concept derived from Adam Smith's conception that socioeconomic progress in complex societies results from the division of labor (Limoges 1994). In the post-Darwinian era, this individualistic view of the organism extended to the organization of the cell, as well as projected onto the organism formed by intercellular relations. Accordingly, all complex organization resulted from the struggle for existence, providing an ever-increasing integration through a division of labor (Sapp 1994, 2003). A common assumption underlays this classical conception, namely, that each organism is derived from one germplasm, the zygote.

Yet, far removed from this classical conception, a small but growing body of evidence accumulated, which reveals that this physiological division of labor could also be accomplished by different species living together, as exemplified by the discoveries, in the latter 19th century, of the duality of lichens, of fungi living in the roots of orchids and forest trees, of nitrogen-fixing bacteria in root nodules of legumes, and of algae living inside the cells of translucent cnidarians. Later, in the early 20th century, findings that microbes inherited through the eggs of insects caused morphological changes with no apparent pathogenic effects on their host further suggested how organisms living in close proximity shared their respective physiologies (Buchner 1965; Sapp 1994).

Still, evidence of such intimate microbial interactions, especially with animals, was relatively scarce, and the evidence for the life-giving properties of microbial infections could not compete with the great success and importance of the germ theory of disease. Indeed, the view of microbial infections as disease-causing "germs" defined the antagonistic view that microbes were "the enemy of man."

Current molecular biological research has underscored how symbionts can become part of an obligatorily integrated commonality (MacDonald et al. 2011; Vogel and Moran 2011). For example, the "genome" of the mealy bug *Planococcus* is the product of a nested symbiosis: animal cells harbor the betaproteobacterium *Tremblaya princeps*, which in turn harbor a gammaproteobacterium, provisionally named *Moranella endobia*. The synthesis of amino acids appears to be coordinated between these two microbes and the host. Three of the enzymes needed for phenylalanine biosynthesis are encoded by the *Moranella* bacterium, five other enzymes are encoded by the *Tremblya* bacterium, and a final enzyme in this pathway is encoded by the insect (McCutcheon and von Dihlen 2011). Note, the genomes of *all three* organisms have been altered through this symbiosis. Such metagenomic sequencing has demonstrated the importance of microbes in insect physiological systems (Vásquez et al. 2012; Weiss et al. 2012).

Microbial symbiosis also has been demonstrated in vertebrate physiology. Lipid metabolism, the detoxification of xenobiotics, regulation of colonic pH, vitamin synthesis, and intestinal permeability are each biological functions provided to the holobiont by bacteria (Nicholson et al. 2012). Specific bacteria also induce the formation of regulatory T-lymphocytes that suppress potentially dangerous immune responses that can cause inflammatory bowel disease (Mazmanian et al. 2008; Chow et al. 2010). The role of symbiotic microbes in mammalian disease prevention is well recognized today (Mazmanian et al. 2008; Lee and Mazmanian 2010; Ballal et al. 2011), and new metagenomic sequencing continues to provide new insights into the relationships between human physiological states and the microbial populations found in humans (Turnbaugh and Gordon 2009; Greenblum et al. 2012). And there is reciprocity. The common gut symbiont *Bacteroides thetaiotaomicron* induces angiogenin-4 gene expression in the intestinal Paneth cell. This protein functions to produce new blood vessels. But it is also a bacteriocidal factor against *Listeria*, the major competitor of *Bacteroides* and a gut pathogen (Cash

et al. 2006). From these examples, we may conclude that on classical physiological grounds, animals are not individuals.

Genetic Individuality

The classical *genetic* conception of the individual is rooted in sex and based on the inheritance of the chromosomal complement acquired at fertilization. That conception of the genetic individual, at the basis of the Weismannian biology of the 19th and 20th centuries (Weismann 1893), was fortified by classical Mendelian genetics and later came to include the mitochondrial chromosome as well (Chapman et al. 1982; Avise 1991). In population genetics, this genetic identity supersedes all others, as it is postulated to contain the allelic variations that are the bases of phenotypic variation.

The one-genome/one-organism doctrine of classical genetics has been eclipsed by studies of hereditary symbiosis. Microbial symbionts form a second type of genetic inheritance (Moran 2007; Gilbert 2011). Arthropods often acquire their symbionts vertically though the maternal germline as well as horizontally from the environment. Mammals obtain them through the maternal reproductive tract and from the mutual licking and grooming following birth. The microbial symbionts represent diverse genomes; and those genomes can also be coselected together with the genome of their host. In aphids, symbiotic bacteria provide selectable allelic variation (thermotolerance, color, parasitoid resistance) that enable some hosts to persist better under different environmental conditions (Dunbar et al. 2007; Tsuchida et al. 2010). There is also allelic variation in the human microbiome. The genes of *Bacteroides plebeius* differ in different human populations. The Japanese strain contains at least two genes (horizontally transferred from a marine relative) that enable the bacteria to metabolize complex sugars, such as those found in seaweeds (Heheman et al. 2010). Indeed, the Human Microbiome Project (Turnbaugh et al. 2007) has applied ecological metagenomics to explore the microbial world within the human species.

The evolutionary importance of microbial symbionts goes well beyond increasing the fitness of hosts or providing hereditable variation that might stabilize a community. Recent studies in *Drosophila*, for instance, demonstrate that the symbionts (not alleles of nuclear genes) provide important pheromonal cues necessary for mating preference (Sharon et al. 2010). Symbionts can therefore provide selectable allelic variation such that the entire group—the holobiont—is the selectable entity rather than either host or symbiont alone (see Zilber-Rosenberg and Rosenberg 2008; Gilbert et al. 2010). Thus, microbes provide a second hereditary system that enables holobiont survival and selection. Indeed, as the mealy bug example mentioned earlier demonstrates, genomes evolve in such a manner that they need their partners to achieve complex genetic integration. None of the three species in that symbiosis has a "complete" genome. It is the holobiont that does. We are not individuals by genetic criteria.

Immune Individuality

The "*immune* self" model of individuality, first proposed by Sir McFarlane Burnet (Burnet and Fenner 1949), portrays the immune system as a defensive network against a hostile exterior world. The immune individual rejects anything that is not "self." Indeed, the discipline of immunology has been called "the science of self/non-self discrimination" (Klein 1982). In this view, the immune system is a defensive "weaponry," evolved to protect the body against threats from pathogenic agents: worms, protists, fungi, bacteria, and viruses. Accordingly, if it were not for the immune system, opportunistic infections would prevail (as they do in cases of immune deficiencies) and the organism would perish.

In a fascinating inversion of this view of life, however, recent studies have shown that an individual's immune system is in part created by the resident microbiome. In vertebrates, the gut-associated lymphoid tissue is specified and organized by bacterial symbionts (Rhee et al. 2004; Lanning et al. 2005). The immune system does not function properly and its repertoire is significantly reduced when symbiotic microbes are absent in the gut (see Lee and Mazmanian 2010; Round et al. 2010). Similarly, Hill et al. (2012) have shown that microbial symbionts provide developmental signals that limit the proliferation of basophil progen-

itor cells and thereby prevent basophil-induced allergic responses. Lee and Mazmanian conclude, "multiple populations of intestinal immune cells require the microbiota for their development and their function" (2010:1768).

This ability of symbionts to condition and promote the immune capacities of the holobiont is not exclusive to vertebrates. In several insect species, bacteria of the genus *Wolbachia* appear to play an important role in antiviral protection (Teixeira et al. 2008; Moreira et al. 2009; Hanson et al. 2011). In plants, endophytes, the diverse and widespread fungi that live out most of their life cycle in plant tissue, provide enhanced pathogen immunity to their host; they can also ward off herbivores, among other benefits (Herre et al. 2007). Thus, immune systems are created, in part, by microbial symbionts. We will return to these newer concepts of immunity below, in a discussion of how the holobiont community can be an evolutionarily viable "individual."

Evolutionary Individuality: The Revised Immune Individual

Biological individuality has also been defined evolutionarily, as that which can be selected (see Maynard Smith and Szathmáry 1995; Michod and Roze 1997; Okasha 2006). Usually, these individuals are genes or monogenomic organisms. But, from the above discussion, it is evident that organisms are anatomically, physiologically, developmentally, genetically, and immunologically multigenomic and multispecies complexes. Can it be that organisms are *selected* as multigenomic associations? Is the fittest in life's struggle the multispecies group, and not an individual of a single species in that group?

An instructive example comes from studies of the pea aphid, *Acyrthosiphon pisum* and the several species of bacteria that live in its cells: variants of *Buchnera* provide the aphid with thermotolerance (at the expense of fecundity at normal temperatures; Dunbar et al. 2007); *Rickettsiella* provides color change, turning genetically red aphids green through the synthesis of quinones (Tsuchida et al. 2010); and some variants of *Hamiltonella* provide immunity against parasitoid wasp infection (Oliver et al. 2009). But in the last case, the protective variants *Hamiltonella* result from the incorporation of a specific lysogenic bacteriophage within the bacterial genome. The aphid must be infected with *Hamiltonella*, and the *Hamiltonella* must be infected by phage APSE-3. As Oliver et al. (2009) write, "In our system, the evolutionary interests of phages, bacterial symbionts, and aphids are all aligned against the parasitoid wasp that threatens them all. The phage is implicated in conferring protection to the aphid and thus contributes to the spread and maintenance of *H. defensa* in natural *A. pisum* populations" (Oliver et al. 2009:994). But there is a cost to the host in having this beneficial protection, for in the absence of parasitoid infection, those aphids carrying the bacteria with lysogenic phage are not as fecund as those lacking them. Similarly, a tradeoff occurs in aphids that carry the thermotolerant genetic variants of *Buchnera*, i.e., while more heat resistant, they have less fecundity at milder temperatures than their sisters whose bacteria lack the functional allele for the heat-shock protein. However, the *population* as a whole can survive hot weather, which would otherwise prevent reproduction.

This symbiotic relationship appears to fulfill the criteria for group selection: alleles can spread throughout a population because of the benefits they bestow on groups, irrespective of the alleles' effect on the fitness of individuals within that group. Except, in this case, the beneficial alleles are genetic variations in bacterial *symbionts*, which provide their hosts with a second source of inherited selectable variation. We are not genetic or anatomical individuals; and if there is no "individual organism," what remains of classic notions of "individual selection"?

This moves the biological discussion of symbiotic associations into the venerable conception of "group selection," so abhorrent to neo-Darwinian sensibilities, and so denigrated by sociobiologists' conceptions based on game theory. Most discussions of group selection (see Williams 1966; Lewontin 1970; Hull 1980; Keller 1999) are not germane here, because they assume that the group in question is composed of a single species. However, one important concern is relevant: cheaters. The major problem for all group selection theo-

ries (and the groups, themselves) are potential "cheaters," those lower-level parts of the group that would proclaim their own autonomy and that would multiply at the expense of the others. As Stearns has pointed out, "conflicts within lower levels and between lower and higher levels must be suppressed or otherwise resolved" (2007:2275).

This problem of cheaters, it has been argued, has rendered many models of group selection mathematically untenable (see Keller 1999; Leigh 2010; Eldakar and Wilson 2011). The problem of "cheaters" then has to be solved in such a way that associates in a symbiotic relationship are under the social control of the whole, the holobiont. This strong socializing and unifying force is found in the immune system, and there we find a solution to the problem of cheaters in a symbiotic complex.

The immune system may be formulated as having two "limbs": an *outward*-looking limb that defines the organism as that which is to be protected from foreign pathogens, and an *inward*-looking arm that looks for potential dangers arising from within the organism itself (see Burnet and Fenner 1949; Tauber 2000, 2009; Ulvestad 2007; Eberl 2010; Pradeu 2010). This dualistic vision was the original conception of Metchnikoff at the end of the 19th century. He regarded immunity as a general physiology of inflammation, which included repair, surveillance for effete, dying, and cancer cells, as well as responsibility for the defense against invading pathogens (Tauber 1994). This larger, systemic understanding thus places defensive properties as only part of a continuous negotiation of numerous interactions between the organism and its biotic environment—both "internal" and "external" (Ulvestad 2007; Tauber 2008a,b).

If the immune system serves as the critical *gendarmerie* keeping the animal and microbial cells together, then to obey the immune system is to become a citizen of the holobiont. To escape immune control is to become a pathogen or a cancer. In cancer, such autonomously proliferating (lower-level) cells must escape the innate, acquired, and anoikis-mediated immune systems of the host in order to survive (Hanahan and Weinberg 2011; Buchheit et al. 2012). Infections are those microbes that that have similarly evaded the immune-enforced social modes of conformity (Hoshi and Medzhitov 2012). Most *Neisseria* species, for instance, can become symbionts. The two pathogenic *Neisseria* species that will not be part of the symbiotic community (*N. gonorrhoeae* and *N. meningitidis)* have escaped the social control of the holobiont by circumventing the immune system (Mulks and Plaut 1978; Welsch and Ram 2008).

In some cases, the internal immune surveillance of symbionts can actually be observed. In insects, symbionts are sequestered in bacteria-bearing host cells, called the bacteriocytes, which, in some species, cluster together to form a bacteriome (Buchner 1965). In weevils, antimicrobial peptide coleoptericin-A selectively targets endosymbionts within the bacteriocytes and inhibits their cell division (Login et al. 2011). If the synthesis of this peptide is blocked, the bacteria escape from the bacteriocytes and spread into the insect tissues. Here, it seems that the coevolution of host and symbiont has enabled the immune system to facilitate the endosymbiotic relationship. In squids (McFall-Ngai et al. 2010) and mammals (Hooper et al. 2012), elements of the host immune system have been co-opted to support the colonization, limitation, and persistence of symbiotic bacteria within the host.

Medzhitov et al. (2012) have discussed "disease tolerance" as a strategy whereby the defensive factors are minimalized to prevent damage to the infected organism. However, what we suggest is not merely "tolerance" toward microbes, but active recruitment of symbiotic bacteria by the immune system. Peterson et al. show that IgA, in addition to its well-known role in attacking polio virus and other pathogens, plays a "critical role in establishing a sustainable host-microbial relationship" (2007: 328). Similarly, these Peyer's Patch antibodies, which are essential in fighting opportunistic pathogens, appear to be involved in "the creation of an optimal symbiotic environment on the interior of the PPs" (Obata et al. 2010: 7419). Even the Toll-like receptors that mediate innate immunity are utilized by *Bacteroides* to establish a host-commensal relationship. The ability of symbiotic bacteria to use the innate and acquired immunity pathways to initiate symbioses has led Round et al. (2011)

to conclude that "the immune system can discriminate between pathogens and the microbiota through recognition of symbiotic bacterial molecules in a process that engenders commensal colonization" (Round et al. 2011:974). To use an anthropomorphic analogy, the immune system is not merely the body's "armed forces." It is also the "passport control" that has evolved to recognize and welcome those organisms that help the body.

Thus, the immune system looks inward, in surveillance, to monitor potential microbial cheaters. The "defensive" role of immunity, so prominent in the medical and agricultural contexts, must be balanced from evolutionary and ecological viewpoints. Immunity does not merely guard the body against other hostile organisms in the environment; it also mediates the body's participation in a community of "others" that contribute to its welfare (Tauber 2000; Agrawal 2001; Hooper et al. 2001; Dale and Moran 2006). The immune system has learned through evolution which organisms to exclude and kill, and which organisms to encourage, allow entry, and support. If accepted, the symbiont can mutually participate in development and physiological processes. Moreover, it can help mediate the holobiont's response to other organisms, effectively becoming "self." From this vantage, there is no circumscribed, autonomous entity that is *a priori* designated "the self." What counts as "self" is dynamic and context-dependent.

"*E pluribus unum*": The Negotiated Surveillance of Parts and Wholes

Negotiated surveillance is a general mechanism that has evolved to permit the incorporation of potentially self-replicating parts into coherent wholes (see Maynard Smith and Szathmáry 1995; Michod and Roze 1997; Okasha 2003, 2006). We see this, as Lynn Margulis (Sagan 1967) long predicted we would, in the main transitions in evolution; for in addition to providing variation needed for intraspecies selection (see above), symbiosis has been critically important in macroevolutionary innovation (see, for example, Margulis and Fester 1991).

First, and foremost, as mentioned above, eukaryotic cells are themselves the result of several symbioses. Suggestions that their nuclei, mitochondria, and chloroplasts originated from ancient symbioses had been repeatedly postulated throughout the 20th century, but they were dismissed and ridiculed in so much as they conflicted with the main tenets of classical biology (Sapp 1994). The tipping point occurred in the 1960s when mitochondria and chloroplasts were shown to possess their own genes and their own translation machinery. And with that discovery, symbiosis in the origin of the eukaryotic cell was brought to the fore of cell biology (Sagan 1967; Margulis 1970, 1981).

Still definitively demonstrating the symbiotic origin of eukaryotic organelles required the development of new molecular methods for showing evolutionary relationships in the microbial world. Methods based on comparisons of ribosomal RNA were developed by Carl Woese and colleagues, for exploring the hitherto unknown evolutionary relationships of microbes (see Sapp 2009). Those methods, when applied to mitochondria and chloroplast origins, revealed them to be relics of formerly free-living alphaproteobacteria and cyanobacteria, respectively. Today, molecular phylogeneticists generally agree that the nuclear genome of the mother cell, the engulfing host, was itself formed from the symbiotic fusion of an Archaean and one or perhaps two other lineages. The nature of those non-Archaean symbionts remains a subject of discussion among microbial phylogeneticists (Hartman and Federov 2002; Hall 2011; see also Sapp 2005, 2009).

Second, multicellularity may also have been initiated by interactions between bacteria and protists. Certain species of choanoflagellates, the unicellular clade thought to be the sister group of multicellular animals, can be transformed into multicellular organisms by interactions with specific bacteria (Dayel et al. 2011). In the presence of certain bacteria, cells remain together after cell division, and the cells form epithelial rosettes sharing a common extracellular matrix and intercellular bridges. Based on this finding, one mode of multicellularity may have arisen as a consequence of a multispecies association of bacteria and protists altering cellular development.

Third, the origin of placental mammals may have been predicated on genomic-level inte-

gration of exogenous DNA. Every genome is a historical product and, just like the cell, it is the result of ancient symbioses and horizontal gene transfers. We are genomic chimeras: nearly 50% of the human genome consists of transposable DNA sequences acquired exogenously (Lander et al. 2001; Cordaux and Batzer 2009), possibly by the horizontal gene transfer from microbial symbionts to animal cells (see Dunning Hotopp et al. 2007; Altincicek et al. 2012). Although much of this added DNA is thought to be "parasitic," some transposable elements may have been critical in creating new patterns of transcription (Sasaki et al. 2008; Oliver and Greene 2009; Kunarso et al. 2010). The emergence of the uterus, the defining character of eutherian mammals, appears to have been facilitated independently in several mammalian families by transposons integrating into the regions controlling the expression of the prolactin gene. These transposons contain transcription factor binding sites that enable the prolactin gene to become expressed in the uterine cells (Lynch et al. 2011; Emera et al. 2012). Moreover, this convergent evolution of gene expression via the insertion of transposable elements also suggests that such transposons can mediate adaptive evolution. The selective silencing of such transposons by DNA methylation or small interfering RNAs appears to be another policing mechanism that has facilitated evolution (Chung et al. 2008; Kaneko-Ishino and Ishino 2010; Castañeda et al. 2011).

Thus, animals can no longer be considered individuals in any sense of classical biology: anatomical, developmental, physiological, immunological, genetic, or evolutionary. Our bodies must be understood as holobionts whose anatomical, physiological, immunological, and developmental functions evolved in shared relationships of different species. Thus, the holobiont, with its integrated community of species, becomes a unit of natural selection whose evolutionary mechanisms suggest complexity hitherto largely unexplored. As Lewis Thomas (1974:142) commented when considering self and symbiosis: "This is, when you think about it, really amazing. The whole dear notion of one's own Self—marvelous, old free-willed, free-enterprising, autonomous, independent, isolated island of a Self—is a myth."

New Perspectives, New Questions

The understanding that symbionts are critical for animal development, health, and homeostasis brings with it "new" problems and opens up novel avenues of investigations. In regard to evolutionary biology, much needs to be investigated in terms of understanding the very diversity of microbes, trying to unravel their complex relations with each other and with their animal host. The evolution of bacterial symbionts and their animal hosts is still an untapped research domain of central importance for evolutionary biology, medicine, and agriculture.

The field of research on *Wolbachia* endosymbionts that has emerged over the past decade exemplifies the importance of understanding symbiotic associations in each of these fields. *Wolbachia* are transmitted sexually through the cytoplasm of the eggs of many species of insects and of nematodes. Their effects range from mutualism to parasitism. They cause cytoplasmic incompatibility and parthenogenesis, and they can change male offspring to females so as to enhance their own transmission and reproduction (Werren 2005). Molecular phylogenetic analysis has also shown that horizontal gene transfer from *Wolbachia* to host genomes is widespread (Dunning Hotopp et al. 2007). *Wolbachia* are held to be important in understanding rapid speciation and the rich species diversity of insects and nematodes of symbiosis, and also in controlling insects pests and disease (see, for example, Brelsfoard and Dobson 2009).

In regards to medicine, first and foremost stands the challenge of elucidating the complex relationship between health, disease, and changes in the human microbiome. The interactions of host genome, symbionts, and diet become critical. The genomes of certain mice, for example, have been shown to enable colonization of specific gut bacteria, which results in an obese or a lean phenotype, depending upon the bacteria's ability to utilize nutrients (Turnbaugh et al. 2006). In zebrafish, a particular cohort of gut bacteria is selected when given mouse intestinal microbes (Rawls et al. 2004, 2006). Although

the adage "no man is island" works for human interactions, each person is precisely an island to a bacterial cell. The island biogeographical perspectives of colonization, succession, resource allocation, and division of functional modules may be critical in symbiotic relationships (see Morowitz et al. 2011; Muegge et al. 2011; Costello et al. 2012).

This new symbiotic perspective makes sense of certain data and provides a fresh outlook on human anatomy and physiology. The milk oligosaccharides produced by human mothers cannot be utilized by newborn infants; however, they serve as an excellent food for strains of *Bifidobacillus* that enhance infant nutrition (Zivkovic et al. 2011). The vermiform appendix, long thought of as a vestigial organ, may actually serve as a reservoir for normal gut bacteria such that symbionts can be rapidly replaced after bouts of diarrhea (Smith et al. 2009). Diarrhea remains the leading cause of death in children of less-developed countries (CDC 2010), and antibiotic-induced colitis, caused by the spread of *Clostridium* after the normal symbionts have been killed, can be cured by the low-tech procedure of fecal transplants (usually from the spouse; Bakken 2011).

If we have evolved the ability to select microbial symbionts, perhaps genetically modifying these bacteria may enhance health. The curative effect of *Lactobacillus* on experimentally induced intestinal inflammation can be even more pronounced by genetically modifying the *Lactobacillus* to induce more IL-10 (Mohamadzadeh et al. 2011). Also, since microbes are in part responsible for detoxifying xenobiotic chemicals, our responses to drugs might depend on our microbial populations (Haiser and Turnbaugh 2012).

What we think is worth studying can be affected by our paradigms. One of the most important areas of developmental biology has been the study of mammalian brain formation. Although environmental stimuli were known to affect behaviors and learning, the possibility that microbes could regulate neural development had not been considered until recently. Now, however, a microbiota-gut-brain axis has recently been proposed (Cryan and O'Mahony 2011; McLean et al. 2012). Germ-free mice, for example, have lower levels of NGF-1A and BDNF (a transcription factor and a paracrine factor associated with neuronal plasticity) in relevant portions of their brains than do conventionally raised mice. Heijtz et al. (2011:3051) have concluded that "during evolution, the colonization of gut microbiota has become integrated into the programming of brain development, affecting motor control and anxiety-like behavior." In another investigation, a particular *Lactobacillus* strain has been reported to help regulate emotional behavior through a vagus nerve-dependent regulation of GABA receptors (Bravo et al. 2011). Investigations into the regulation of brain development by bacterial products were unthinkable before this challenge to the prevailing paradigm.

Conservation zoology is also greatly affected by acknowledging the diverse effects of symbiont relationships. For instance, knowledge of symbiosis is crucial in preventing the extinction of the spotted salamander in the central states of America; and knowledge of the parasitic symbiosis of *Maculinea* and the *Myrmica* ants has been critical for the return of *Maculinea* to Great Britain (Thomas 1995). In agriculture, "curing" insects of their vital symbionts may be an environmental friendly way of controlling pests such as aphids. This destroying of the host by killing the symbiont has been shown to work in the case of *Mansonella*, a worm that parasitizes humans (Coulibaly et al. 2009).

Last, this new appreciation of symbiosis, where even microevolution might involve interspecies interactions, opens up a range of new questions for evolutionary biology. The change of a localized, interacting, multispecies collective over time has been modeled by ecological succession, and in one of the first formulations of ecological succession, Clements (1916) likened succession to development, viewing the climax community as the adult phenotype. Each organism may have to become modeled in a web of ecosystem dynamics, where cells come from diverse genotypes.

In the 2009 "Homage to Darwinism" debate held at Oxford University, Richard Dawkins questioned the bringing of symbiosis into evolutionary theory:

> Take the standard story for ordinary animals, [where] you've got a distribution of animals [and] you've got a promontory, or

> an island or something and so you end up with two [geographical] distributions. And then on either side you get different selection pressures, and so one [group] starts to evolve this way, and [the other] one starts to evolve that way, and what's wrong with that? It's highly plausible, it's economical, it's parsimonious. Why on Earth would you want to drag in symbiogenesis when it's so unparsimonious and uneconomical?
>
> To which Lynn Margulis replied, Because it's there (Dawkins and Margulis 2009).

And it is significant. For animals, as well as plants, there have never been individuals. This new paradigm for biology asks new questions and seeks new relationships among the different living entities on Earth. We are all lichens.

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