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Towards A Developmental Biology Of Holobionts

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Abstract

We do not develop as monogenomic organisms, instructed solely from the DNA and cytoplasm of the zygote. Rather, we are *holobionts*, symbiotic consortia containing numerous microbial genomes, whose signals are critically important for our normal development. Microbes play crucial roles in forming and maturing animal guts, immune systems, nervous systems, and reproductive organs. In some species, they regulate such developmental phenomena as the proper orientation of the anterior-posterior axis and metamorphosis. One of the biggest challenges to developmental biology, then, is studying the developmental biology of holobionts, where co-development is the rule, and where the body is seen as a collection of interdependent ecosystems.

What a profession this is – this daily inhalation of wonder.
(Jean Rostand, 1962)

You complete me.
(Dorothy Boyd, in *Jerry Maguire*, by Cameron Crowe, 1996)

Becoming with others

In the past century, the biological world has gone from a Darwin-Wallace paradigm, through a Dawkins-Collins phase, and is now entering the Margulis-Lewontin era. To be sure, the worldviews of each of the earlier eras, like evolving species or religions, are still present while the newer ones arise; but the Margulis-Lewontin view of biology emphasizes cooperative interactions and interpenetrations between individuals, rather than the predominantly competitive

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interactions of the earlier paradigms. As Richard Lewontin (2002) documented, the boundaries of the organism are more porous, interpenetrating and interacting with the environment. The genotype gives us a repertoire of potential phenotypes, and the phenotype is often determined by environmental agents. And as Lynn Margulis (1998) showed, organisms can no longer seen as "monogenomic," bearing only the genes derived from the zygote. Rather, each organism is a *holobiont*, a symbiotic consortium with numerous microbes. Each organism is an ecosystem, and complex organisms, such as ourselves, are biomes, containing numerous ecosystems. Indeed, in this new view of the world, those animals most fit to survive are often those with the best systems of cooperation. As Richard Powers (2018, p. 142) concluded in his analysis of forests and their humans, "Competition is not separable from endless flavors of cooperation."

The Margulis-Lewontin perspective of biology highlights developmental plasticity and symbiosis (Levins and Lewontin, 1985; Margulis and Sagan, 2003; Gilbert and Tauber 2016). Developmental plasticity is most obviously seen in "individuals", where the environment has agency, along with the genome, such that environmental agents generate different phenotypes from the same genotype (West-Eberhardt 2003; Minelli and Fusco 2010; Sultan 2017). Temperature, for instance, can determine the pigment patterns of some butterflies and the sex of many reptiles. Plasticity can also be seen in the "environment". Here, the environment is not a given context. Rather, habitats are formed by interactions between the organisms developing in them and as part of them. This extension of plasticity into the environment is called *niche construction* (Laland *et al.*, 2008).

Symbiosis can be a source of both constraint and flexibility (Bennett and Moran 2016). In the latter mode, it provides the organism with flexibility derived from numerous other genetic systems. Indeed, while we receive some 22,000 pairs of genes from our parents, we get on the order of 8 million different genes from our symbionts (Funkhauser and Bordenstein 2013; McFall-Ngai et al., 2013). Although symbiosis can be parasitic or mutualistic, symbiosis is usually used to describe mutualistic, reciprocally beneficial, interactions between consenting adults. The cow, for instance, is a domesticated female bovid that digests grass. Only, it can't digest grass, as its genome contains no gene encoding cellulose-digesting enzymes. Ditto for wood-eating termites, whose genome contains no lignin-digesting genes. In both cases, their respective abilities to digest cellulose and wood come from the colonies of microbial symbionts located within their guts. The microbes get food and shelter; the animal gets a crucial source of nutrition.

Indeed, symbiosis is the signature of life on earth, whether we are speaking about the nitrogen-fixating symbioses of legumes and rhizobacteria, the mycor-

rhizal interactions with plant roots and seeds, the coral reef and tidal seagrass symbioses that sustain oceanic diversity, or the insect pollinators of plants. And within these grand symbioses are the smaller symbioses we call organisms, cells, and genomes. The "organism" is not an "individual," in the sense of being a solitary organism. Rather, it is a collection of interpenetrating ecosystems. The microbes on our skin and in our guts are essential for our normal physiological, mental, and immune relationships (Gilbert *et al.*, 2012, 2015; McFall-Ngai *et al.*, 2013).

What fascinates me is that symbionts are not only required for normal animal functioning; they are also necessary for normal animal development. This is revolutionary. Throughout the Twentieth Century, it had been assumed that the zygote contained all the genes and proteins needed for normal development under permissive conditions. Development was seen as a read-out of the genes acquired at fertilization (Keller, 1992, 2002). This was our origin story, following the standard Western origin narrative of unity, diversity, and restoration (Haraway, 1985, 2017). Developmental symbiosis – *sympoiesis* – has literally queered the story, adding an important layer of interactive non-heterosexual intercourse – the microbes.

The new story of developmental symbiosis has several points of origin, of which two groups framed much the discussion – Margaret McFall-Ngai and Ned Ruby's studies of the squid light organ and Jefferey Gordon's studies of mouse intestines. The squid isn't born with a light organ. Rather, it binds members of a particular marine bacteria species onto its abdomen (poisoning all others), and the light organ is formed by the interactions of the squid cells and the *Vibrio fischeri* bacteria. The light organ then houses the bacteria, brings them to a critical density, and controls its bioluminescence (McFall-Ngai, 2014; Aschtgen *et al.*, 2016). In Gordon's laboratory, the Paneth cells of the mouse intestine were seen to transcribe different amounts of mRNA depending on whether particular bacteria are present (Hooper *et al.*, 2001; Camp *et al.*, 2014). Certain species of *Bacteroides* are responsible for the "normal" amounts of mRNA that encode enzymes (such as colipase), paracrine factors (such as angiogenin-4), and structural proteins (such as Sprr2a). Germ-free mice (having no gut microbes) have about 10% the amount of angiogenin-4 mRNA as conventionally raised mice; and the normal amount of this message can be regained by adding *Bacteroides* to the gut. The Angiogenin-4 protein helps make gut capillaries, the blood vessels that bring food to the rest of the body. The gut capillary network of germ-free mice is very poor (Stappenbeck *et al.*, 2002). So we mammals get a lot of work from our *Bacteroides* symbionts. They help make us who we are. And *Bacteroides* gets help from the host, the zoon. Not only does the mammalian gut provide

Bacteroides with good food and housing; the host's Angiogenin-4 has a second use – it kills *Listeria*, the major competitor of *Bacteroides* (Hooper *et al.*, 2003; Cash *et al.*, 2006). Development involves some niche construction on the part of the microbes.

Developmental symbiosis has been found throughout the animal kingdom (McFall-Ngai, 2002; Douglas, 2010, 2018; Gilbert and Epel, 2015). In mammals, bacteria are critical for the development of the gut capillaries, the enteric neurons, and the gut-associated lymphoid tissue. In zebrafish, bacteria regulate the division of the gut stem cells as well as the normal proliferation of the insulin-producing beta-cells of the pancreas. Without these particular microbes, there is a paucity of differentiated gut epithelium (Rawls, 2004; Hill *et al.*, 2016). Moreover, some of these developmentally critical bacteria are rather rare members of the microbiome. In zebrafish, for instance, the *Aeromonas* bacteria that stimulates beta cell proliferation are such a very rare component of the gut microbiome that it has no signature in the genomic sequence data (Hill *et al.*, 2016). This leads to the concern that our desire for cleanliness might be wiping out bacteria that are essential for *our* normal development (Blaser, 2014).

We mammals inherit most of our microbes from our mother. Indeed, this is a third pattern of inheritance, following those of nuclear chromosomes and mitochondria (Funkhauser and Bordenstein, 2013; Chiu and Gilbert, 2015; Roughgarden *et al.*, 2017). After our amnion breaks and we pass through the birth canal, we become colonized by microbes. Moreover, the microbes we pick up are not the usual ones. Rather, the microbial populations of the vagina and distal gut are changed during the last trimester of human pregnancy (Koren *et al.*, 2012: Romero *et al.*, 2014). And when the mother feeds the new baby, not all of the food is for the baby. Another part, consisting of oligosaccharides unable to be digested by mammals, are specifically for *Bifidobacteria*, one of the microbes that is helpful for the colonization of the gut by other beneficial microbes (Garrido *et al.*, 2016). The bacteria in mothers' milk appear to be particularly important in inducing the formation of the helper T cells that prevent opportunistic infections (Ardeshir *et al.*, 2014). A specific set of microbes is passed from generation to generation to complete normal development. Birth is the passing from one set of symbiotic relationships to another.

In invertebrates, there are particularly strong associations between bacteria, immune defense, and metamorphosis (Douglas 2010, 2018). Here, the interactions of microbes and development are so strong that many insects develop special cells, *bacteriocytes*, to contain the symbionts. These interactions between invertebrates and microbes can start very early. In the nematode *Brugia malayi*, *Wolbachia* bacteria are responsible for the correct anterior-posterior pattern of

the second mitotic division (Landmann *et al.*, 2014). In pillbugs, *Wolbachia* can transform genetically male pillbugs into females. In several species, symbionts are critical for the development of reproductive organs or general larval growth. Microbes are also critically important for molting and metamorphosis in several species. Many species cannot molt properly without the digestive enzymes produced by symbiotic microbes, and many marine invertebrate need other organisms (bacteria, algae) to provide the signals for settlement and metamorphosis (Hadfield, 2011; Gilbert and Epel, 2015).

The brain and the immune system present their own developmental interactions with microbes. Gut microbes are not only capable of communicating with the adult brain, but they also appear to be critical for normal brain development (Sampson and Mazmanian 2015). In germ-free mice, the brain microglial cells (tissue macrophages that are critical in homeostasis and disease prevention) do not complete their maturation (Erny *et al.*, 2015), and Diaz Heijtz *et al.* (2011, p. 3051) 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." Indeed, there are two major ways to experimentally generate symptoms of autism in mice by manipulating the microbes of the mother. First, mice born from germ-free mothers and who are themselves without microbes have a syndrome that includes obsessive self-grooming and asocial behavior (Debonnet *et al.*, 2014). Second, one can induce such autism-like features in young mice by giving a large immune insult to the mother while she is pregnant. This causes changes in brain development *in utero*, but these alterations only arise if particular types of bacteria are present to augment the immune challenge (Kim *et al.*, 2017). Moreover, several of these symptoms seem to be cured by adding a different set of microbes into the newborn mice's guts (Hsaio *et al.*, 2013). Thus, there is an entirely new region of developmental neurobiology – how the symbionts interact with the developing brain.

And there is another new science of holobiont immunology (Tauber, 2008, 2017; Pradeu, 2012; Gilbert and Tauber, 2016). If the immune system is supposed to kill all that is not "self", then how do these bacteria even enter our body? Just as developmental biology is changing from seeing development as the readout of the genome, so immunology is changing from the view that the immune system exists to defend the organism against the hostile outside world. Certainly that's a part of it (as development also involves the readout of the genome), but it's far from being the whole picture. The defensive role of the immune system appears to be a subset of a much larger function in mediating our relationships, both positive and negative, with microbes. Just like the immune system of the bobtail squid, the mammalian immune system allows certain microbes

entry, while preventing the penetration of other bacteria and fungi. Not only are microbes needed for the maturation of the gut lymphoid tissue; microbial colonization is also critical for the normal development of T-lymphocytes and B-lymphocytes in the intestinal mucosa (Wesemann *et al.*, 2013) as well as for inducing the specific lymphocyte populations that balance the immune response at mucosal surfaces (Ohnmacht *et al.*, 2015). Lee and Mazmanian (2010, p. 1768) conclude, "Multiple populations of intestinal immune cells require the microbiota for their development and function." Different types of T cells are made depending on which bacteria colonize our guts (Ardeshir *et al.*, 2014). The immune system is a holobiont property; it's not merely the host's immune system. It's the holobiont's immune system. So this means that we should no longer consider ourselves genetically pure. Our immune system facilitates the entry of some microbes and excludes the entry of others.

We complete each other

This has major implications for evolutionary biology (Roughgarden *et al.,* 2017). First, the "tree of life" has become like real trees – full of symbionts. In addition to the genetic lineage provided by our reproductive parents, there are also genetic lineages provided by the symbionts we acquire from our mother and from our environment (Margulis and Fester 1991; Margulis and Sagan 2003). These microbial lineages interact with the eukaryotic lineage in many different ways. Indeed, the microbial lineages can provide selectable genetic traits (Douglas, 2010; Gilbert *et al.*, 2010; Kikuchi *et al.*, 2012; Moran and Yun, 2015), and they are involved with species formation (Brucker and Bordenstein, 2103). Second, if (as evolutionary developmental biology postulates) changes in development are critical for making evolutionary changes in anatomy and physiology, those changes in development could also entail symbionts. Such symbiont-mediated changes in development may even be responsible for such evolutionary transitions as the origins of animal multicellularity (Dayel *et al.*, 2011; Alegado *et al.*, 2012), the mycorrhizal symbiosis that enabled plants to live on land (Heckman *et al.*, 2001), the origin of mammals (Dupressoir *et al.*, 2011; Lynch *et al.*, 2011), and origins of herbivory in insects and vertebrates (Gilbert, in preparation).

We have numerous genomes whose products interact to generate our phenotypes. Monogenomic organisms are in the clade of Cryptid vertebrates whose other members include Nessie, Sasquatch, and the Abominable Snowman. It is dubious that any exist. Therefore, zoology (as well as plant sciences) should deal with this fact. Physiology, developmental biology, immunology, neurobiology, and evolutionary biology each have to concern themselves with this "new imperative for the life sciences" (McFall-Ngai *et al.*, 2013). Developmental biol-

ogy can no longer be seen as the read-out of the zygote genome. Development entails "becoming with" others (Haraway, 2008), generating a body consisting of physiologically connected ecosystems. Developmental biology has also to consider co-development, the body as a constructed niche (Laland *et al.*, 2008; Gilbert *et al.*, 2012). That is the challenge for our field – to study the developmental biology of holobionts.

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