12-1-2019

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Recommended Citation

Collens, Adena; Kelley, Emma; and Katz, Laura A., "The Concept of the Hologenome, an Epigenetic Phenomenon, Challenges Aspects of the Modern Evolutionary Synthesis" (2019). Biological Sciences: Faculty Publications, Smith College, Northampton, MA.
https://scholarworks.smith.edu/bio_facpubs/82

Published in final edited form as:
J Exp Zool B Mol Dev Evol. 2019 December ; 332(8): 349–355. doi:10.1002/jez.b.22915.

The concept of the hologenome, an epigenetic phenomenon, challenges aspects of the modern evolutionary synthesis

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Abstract

John Tyler Bonner's call to re-evaluate evolutionary theory in light of major transitions in life on Earth (e.g. from the first origins of microbial life, to the evolution of sex and the origins of multicellularity) resonate with recent discoveries on epigenetics and the concept of the hologenome. Current studies of genome evolution often mistakenly focus only on the inheritance of DNA between parent and offspring. These are in line with the widely accepted Neo-Darwinian framework that pairs Mendelian genetics with an emphasis on natural selection as explanations for the evolution of biodiversity on Earth. Increasing evidence for widespread symbioses complicates this narrative, as is seen in Scott Gilbert's discussion of the concept of the holobiont in this series: organisms across the tree of life coexist with substantial influence on one another through endosymbiosis, symbioses and host-associated microbiomes. The holobiont theory, coupled with observations from molecular studies, also requires us to understand genomes in a new way -- by considering the interactions underlain by the genome of a host plus its associated microbes, a conglomerate entity referred to as the hologenome. We argue that the complex patterns of inheritance of these genomes coupled with the influence of symbionts on host gene expression make the concept of the hologenome an epigenetic phenomenon. We further argue that the hologenome challenges aspects of the modern evolutionary synthesis, which requires updating to remain consistent with Darwin's intent of providing natural laws that underlie the evolution of life on Earth.

Keywords

holobiont; symbiosis; microbiome; evolutionary theory; epigenomics

Introduction

A common response to the question, 'how many chromosomes are in a human cell?' is 46, but this is inaccurate, as human cells actually have at least 47 when we include the circular chromosomes in our mitochondria. Similarly, every plant cell has three genomes that act in concert: in the nucleus, mitochondrion and chloroplast. These widely studied endosymbiotic

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events demonstrate how genome interactions change the evolutionary trajectories of all organisms involved. In highly intimate endosymbiotic relationships, symbiogenesis creates a new individual from multiple lineages (Martin & Kowallik, 1999; Mereschkowsky, 1910; Nowack & Melkonian, 2010). Clearly in the case of mitochondria and plastids, host and symbiont genomes evolve and function together following endosymbiosis, changing the template for evolutionary processes.

Similar to the connections among genomes following endosymbiosis, the interactions between symbionts and hosts more broadly led to the concept of the hologenome (e.g. Rosenberg, Koren, Reshef, Efrony, & Zilber-Rosenberg, 2007; Zilber-Rosenberg & Rosenberg, 2008) and challenge our traditional understanding of an individual's genome. Because symbionts can impact patterns of expression and inheritance of host genomes, we argue that these relationships are inherently epigenetic as they are consistent with Denise Barlow's broad definition of epigenetics as "all the weird and wonderful things that cannot [yet] be explained by genetics" (McVittie, 2006). We do not mean to suggest that hologenomes are outside of evolutionary theory but instead, as Bonner (2019) reminds us, consideration of microbes as well as plants and animals provides valuable insights that expand evolutionary theory. Here, we discuss data on genome interactions between hosts and symbionts, highlighting the varying degrees of intimacies, to emphasize the importance of the hologenome concept and its implications for evolutionary biologists.

The hologenome

The concept of the hologenome (e.g. Rosenberg et al., 2007; Zilber-Rosenberg & Rosenberg, 2008) emerges in part from the concept of the holobiont (e.g. Gilbert, 2019; Gilbert et al., 2010; Herre, Knowlton, Mueller, & Rehner, 1999) because inherited symbionts change host gene expression and genomes over time. Symbioses are very common in nature (e.g. Gilbert et al., 2010; McFall-Ngai, 2002) and, as a result, many eukaryotes (e.g. plants, animals, ciliates, amoebae) contain heritable symbionts that "contribute to the anatomy, physiology, development, innate and adaptive immunity, and behavior and finally also to genetic variation and to the origin and evolution of species" (Rosenberg & Zilber-Rosenberg, 2016). To realistically encompass the genetic 'individual' created by a host and its microbiome, Rosenberg et al. (2007) first introduced the hologenome as the sum of the genomes of a host and its symbionts, in essence, a conglomerate genome. Within a hologenome, complex patterns of inheritance and epigenetic relationships drive the evolution of both a host and its symbionts (e.g. Gilbert et al., 2010; Herre et al., 1999; Zilber-Rosenberg & Rosenberg, 2008). In other words, eukaryotic genomes evolve in concert with the vast number of microbial symbionts (e.g. microbiomes) harbored within lineages, though at varying scales of interrelatedness.

Before exploring the various degrees of intimacy between hologenome partnerships, we will address a few misconceptions about hologenomes themselves. Some have questioned the concept of the hologenome as a unit of selection because of the variation in both the nature of interactions and patterns of inheritance among its members (reviewed in Moran & Sloan, 2015). To view a hologenome as a single evolving unit would be inaccurate, as interactions between members are not necessarily mutualistic and hence, a hologenome may have negative or neutral consequences for one or more of the organisms involved (see 'intimacy' section below). Additionally, there is confusion between the concepts of the hologenome and the metagenome: the hologenome implies an interaction between a host and its symbionts whereas a metagenome can also describe a community within a non-living environment like soil (Bordenstein & Theis, 2015). Douglas and Werren (2016) go further to suggest that the focus should not be on the individual players; we should instead focus on host-microbe interactions through a wider ecological lens. We disagree with this view and instead believe that the concept of the hologenome expands our views on the genetic complexity of 'individuals'. As the hologenome has been extensively reviewed elsewhere (e.g. Brucker & Bordenstein, 2013; Dale & Moran, 2006; Zilber-Rosenberg & Rosenberg, 2008), we provide only a few examples here to explore how different hologenomes function along a continuum of intimacy as a way to exemplify the power of the concept.

Hologenomes: A Spectrum of Intimacy

Hologenomes vary in intimacy and complexity, as the impact of symbiotic relationships on the survival of its members can be seen to vary across a sliding scale (Fig. 1). Here, we array interactions based on the degree of intimacy and the potential impact on fitness for the organisms involved. Hologenome symbioses range from the least intimate hologenomes, like the human microbiome (Kumar et al., 2014; Stilling, Bordenstein, Dinan, & Cryan, 2014), to moderately intimate hologenomes, including bacteria that provide heat resistance in plants (Gilbert et al., 2010; McLellan et al., 2007), to the most intimate hologenomes like those in aphids that have outsourced portions of the production of their protein building-blocks to their symbionts (Fig. 1; Wilson et al., 2010). Because the microbiome may be able to react more quickly to the environment than the host, microbiomes have the potential to impact host adaptation and evolution (Romano, 2017; Rosenberg & Zilber-Rosenberg, 2016; Stilling, Bordenstein, et al., 2014; Stilling, Dinan, & Cryan, 2014), though at varying degrees depending on the intimacy of the relationship such as the pattern of inheritance.

At the less intimate end of the spectrum, host genomes interact with and respond to relationships with other organisms in the holobiont and are not fully interdependent (Fig. 1 a & b). One example of this type of casual symbiosis includes digestion adaptations in the human gut microbiome (Fig. 1a, Postler & Ghosh, 2017). For example, gut symbionts (e.g. *Bifidobacterium*) fluctuate in response to changing levels of lactose and impact the expression of the human *LCT* gene, a gene that also contributes to lactose digestion (Blekhman et al., 2015). Further, expressions of immunity-related human genes like *HLA-DRA* and *TLR1* have been respectively linked to the abundance of *Selenomonas* in the throat and *Lautropia* on the tongue (Blekhman et al., 2015). Another 'relaxed' hologenome relationship is the bioluminescent predator defense in squid. The squid allows colonies of a bioluminescent bacteria, *Vibrio fischeri,* to colonize and illuminate its light organ to ward off predators (Fig. 1b, Gilbert et al., 2010; McFall-Ngai, 2002). Genetic mechanisms in the squid's immune defense system are hypothesized to regulate this bacterial defense mechanism, allowing the colonization of the light organ by helpful *V. fischeri* and preventing against pathogenic bacteria (McFall-Ngai, 2002).

As we move to the right on the spectrum of symbiotic intimacy (Fig. 1 c–i), products expressed by each member of the holobiont are essential to host and endosymbiont survival. For example, extracellular fungi express genes that inhibit a heat shock protein in the Christmas cactus *Opuntia leptocaulis*, preventing the cactus's cells from deteriorating in hot environments (Fig. 1c, Gilbert et al., 2010; McLellan et al., 2007). Additionally, the survival of an amoebae host ensures the survival of its amoebae-resistant bacterial symbionts (Greub & Raoult, 2004; Loret & Greub, 2010). Pathogenic bacteria *Legionella pneumophila* has adapted its surface protein expression and other aspects of its genome to avoid digestion and instead to survive inside both free-living amoebae and human macrophage hosts (Fig 1f, Greub & Raoult, 2004).

In some cases, acquired symbiont genomes can increase host fitness by changing the methods of metabolic function and inflicting harm on non-hosts. Single-celled foraminifera switch from heterotrophy to phototrophy in nutrient-poor environments by stealing chloroplasts from their food (Fig. 1h, Clark, Jensen, & Stirts, 1990; Jauffrais et al., 2016; Pillet, de Vargas, & Pawlowski, 2011). Other holobionts make themselves more dangerous by inflicting harm on non-hosts. *Caedibacter taeniospiralis*, a bacterial symbiont of ciliate *Paramecium tetraurelia*, release a toxin that kills ciliates without the bacterium while genetically protecting their host from the toxin's harm (Fig 1g, Grosser et al., 2018). *Caedibacter taeniospiralis* also up-regulates heat shock genes and metabolism enzymes for an additional fitness advantage to *P. tetraurelia* hosts (Grosser et al., 2018).

Some of the most intimate symbiotic relationships involve bidirectional interactions whereby host and symbiont genomes each provide different pieces of genetic pathways necessary for survival (Fig. 1 i & j). This is seen in sap-eating insects and their bacterial symbionts where gene pathways for metabolism, replication, transcription and translation are derived from products of endosymbiotic bacteria that are vertically inherited (Fig 1j, Bennett & Moran, 2013; Gilbert et al., 2010; Husnik et al., 2013; Provorov & Onishchuk, 2018). For example, *Buchnera* protein HisC can replace the function of the branched-chain amino acid transaminase in the aphid, while phenylalanine 4-monooxygenase and aspartate transaminase in the aphid may replace Tyrosine A and Tyrosine B enzymes absent in *Buchnera* (Wilson et al., 2010). The hologenome of the aphid and bacteria require each to contribute pieces to the other's genome for the organisms to survive, and inheritance is vertical in this intimate relationship.

The genetic dependencies in hologenomes can be complicated by interactions among many players. The endosymbiotic *Wolbachia* bacteria in pea aphids gained the wCle gene from another endosymbiont, *Cardinium* or *Rickettsia,* through lateral gene transfer (Nikoh et al., 2014). This wCle gene synthesizes biotin for the host, which is now essential to the aphid's survival and ability to reproduce (Nikoh et al., 2014). Some eukaryotic symbionts even live within other symbionts, like a Russian nesting doll. *Neoparamoeba* sp. parasitize salmon and other marine animals, and the amoeba themselves are host to *Perkinsela* sp., a kinetoplastid that has evolved in tandem with the amoeba, possibly due to metabolic relationships (Nowak & Archibald, 2018).

Some vertically-inherited symbiont phylogenies trace the evolution of their hosts from vertical inheritance for more than 100–200 million years (Dale & Moran, 2006; Douglas, 2011). This intimacy can also be observed in parasitic wasps (*Asobara tabida*). These wasps pass down their endosymbiotic bacteria through their eggs to the next generation because, without the bacteria, the wasp ovaries cannot develop properly (Fig. 1i, Gilbert et al., 2010). The wasps are trapped in an epigenetic hostage situation, ensuring the *Wolbachia* are passed onto future generations. Intriguingly, there are parallels in the mechanisms that the *Wolbachia* uses to destroy cells the wasp as bacterium *Legionella pneumophila* uses to burst its host cells. *Wolbachia,* the wasp's bacterial symbiont, programs a similar apoptosis in the wasp's ovaries, ensuring any non-hosts are unable to reproduce (Pannebakker, Loppin, Elemans, Humblot, & Vavre, 2007).

The epigenetic implications of the hologenome

The hologenome is, by definition, epigenetic; interactions within the hologenome can both lead to changes in gene expression without changes in DNA sequences (a textbook definition of epigenetics) and be interpreted in light of Denise Barlow's definition of 'weird and wonderful things' (see above and McVittie, 2006). Regardless of the definition one adheres to, the impact of symbionts on host genomes is clearly outside of our traditional Mendelian view of transmission genetics.

The connection between the hologenome and epigenetics has been suggested by others either because of the influence of symbionts on host genetics (e.g. Douglas, 2011; Moran & Sloan, 2015; Zilber-Rosenberg & Rosenberg, 2008), or in light of the intergenerational impacts of symbionts on human phenotypes (e.g. Romano, 2017; Stilling, Dinan, et al., 2014). This interaction led Stilling, Dinan, et al. (2014) to introduce the term 'holo-epigenome' to explicitly acknowledge the epigenetic qualities of genomic interactions between hosts and symbionts.

Some canonical epigenetic functions have been observed in the human hologenome. For example, the diverse community of microbes within the human gut has been found to modulate host DNA methylation, changing patterns of gene expression (reviewed in Cureau, AlJahdali, Vo, & Carbonero, 2016). Further, in a preliminary study on the microbiota of pregnant women, significant differences in DNA methylation patterns were found based on the dominant bacteria that made up their microbiota (Kumar et al., 2014). As just one example, women whose predominant microbiotic fauna were in the phyla *Firmicutes* experienced differential methylation with regards to lipid metabolism and the inflammatory response, with downstream implications for obesity and cardiovascular disease (Kumar et al., 2014).

Symbiont-expressed microRNAs (miRNAs), have also been identified as a potential epigenetic mechanism between the microbiome and the host genomes (Liu, Du, Huang, Gao, & Yu, 2017; Williams, Stedtfeld, Tiedje, & Hashsham, 2017; Xue et al., 2011). Small non-coding RNAs of symbionts can regulate gene expression by repressing the translation of target mRNAs from the host genome (Cannell, Kong, & Bushell, 2008). For example, miRNA-10a levels, which coordinate the innate immune response, were shown to be downregulated by the presence of certain microbiota in mice (Xue et al., 2011). This is thought to promote homeostasis and prevent an immune response to commensal gut bacteria (Xue et al., 2011). Recent evidence also indicates that the miRNA-coordinated epigenetic communication between the host and microbiome is reciprocal, with host genetics able to shape the gut microbiome (Liu et al., 2017; Williams et al., 2017). For example, host extracellular miRNAs secreted by epithelial intestinal cells of mice may be regulating bacterial gene expression and ultimately bacterial growth within the intestine, (Liu et al., 2017; Williams et al., 2017), serving as a potential mechanism for molecular communication within the hologenome.

The concept of the hologenome is at odds with some aspects of Neo-Darwinism

The interwoven, epigenetic relationship between the genomes of host and symbionts complicates our current understanding of evolutionary theory. Today, when students open introductory biology textbooks, they will likely find the definition of evolution referred to as the Modern Evolutionary Synthesis, a combination of Darwin’s natural selection and Mendel’s particulate inheritance genetics. As a main proponent of this evolutionary lens, Mayr (1980) rejects the effect of any “soft inheritance”, defined as “the belief in a gradual change of the genetic material itself, either by use and disuse, or by some internal progressive tendencies, or through the direct effect of the environment” (Mayr, 1980). In contrast to this view, we argue that by excluding soft inheritance, the Modern Evolutionary Synthesis cannot account for the robust observations of epigenetics resulting from symbiotic relationships involving diverse lineages from across the Tree of Life (Table 1). Instead, we believe understanding evolution through the hologenome provides a more complete depiction of genome evolution, one that expands the traditional views on evolutionary theory.

Our discussion of how hologenomes epigenetically shape evolution is only one component of the broadening of the Modern Synthesis. New information from current explorations of non-genetic inheritance calls for a multidimensional reshaping of the Modern Synthesis. Because of our newfound appreciation of complex epigenetic mechanisms and their impact on evolution, we suggest use of a more inclusive and dynamic theory called the Extended Modern Synthesis – a conceptual expansion to the classical Modern Synthesis theories that includes an understanding of “soft inheritance” (Danchin et al., 2011; Pigliucci & Finkelman, 2014). As new discoveries broaden our understanding of evolutionary theory (Mendelian inheritance, natural selection), Novick and Doolittle (2019) implore us to expand our evolutionary theory ‘toolbox’ to account for the complexity of hologenomes and other epigenetic phenomena. Darwin’s original theory was built on understanding the natural world he observed. By excluding certain truths, like those we observe from symbiosis, we stray from Darwin’s original aim of an evolutionary model that captures the whole of nature (Raoult & Koonin, 2012). We need to remain open-minded and allow new evidence to reshape our theories. As Bonner (2019) points out, evolution has been evolving since the beginning of time. If our goal is to truly understand it, we must continue evolving our understanding of evolution as well.

Acknowledgements

We are very grateful to members of the Fall 2018 *Epigenetics* class at Smith College for conversation and inspiration. L.A.K. is supported by NSF grants DEB 1651908 and DEB-1441511.

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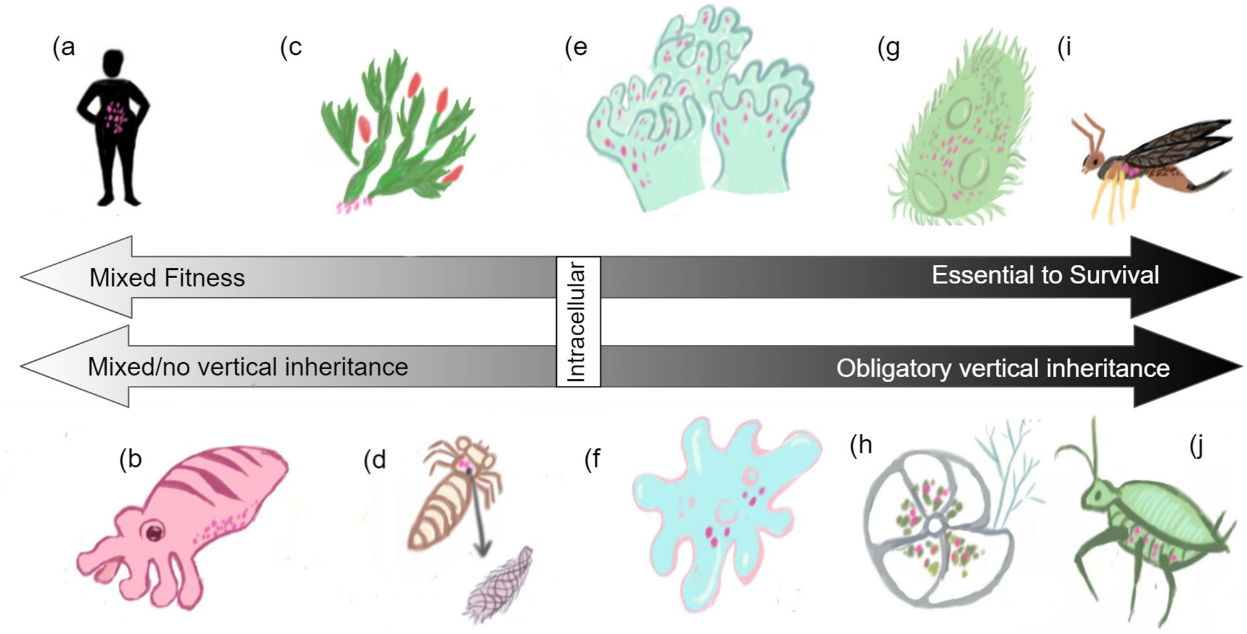

Figure 1:

Symbiotic relationships exist on a spectrum of intimacy and contribute to the concept of the hologenome. This spectrum is simply a tool to compare relative intimacies of symbiotic relationships. a) Human health is dependent on the microbiomes in the gut and other places on the body (Postler & Ghosh, 2017); b) Animals acquire fitness advantages from bacterial symbionts like *Vibrio fischeri* which cause *Euprymna* to glow (Gilbert et al., 2010); c) Symbiotic fungi provide a protein inhibitor that prevents Heat Shock Protein (HSP-90) in the Christmas Cactus *Opuntia leptocaulis*, allowing the plant to maintain its cells in various heat conditions (Gilbert et al., 2010; McLellan et al., 2007); d) Diversity of the communities of eukaryotic excavate symbionts in termite guts reflects the environment of their host communities (Duarte et al., 2018); e) Hermatypic coral are dependent on dinoflagellate *Symbiodinium* for 95% of their energy (Gilbert et al., 2010). f) *Acanthamoebae* are hosts to amoeba-resistant-bacteria like *Legionella pneumophila* which have evolved to resist digestion and become resistant to human macrophages (Greub & Raoult, 2004; Loret & Greub, 2010); g) Ciliate *Paramecium tetraurelia* hosts bacteria *Caedibacter taeniospiralis* in its cytoplasm which can kill non-host *P. tetraurelia* and protect current hosts from its killer mechanism (Grosser et al., 2018); h) Single-cellular foraminifera can ‘steal’ chloroplasts from the diatoms and algae they eat to use for photosynthesis -- a phenomenon known as kleptoplasty (Clark et al., 1990; Jauffrais et al., 2016); i) Parasitic wasp *Asobara tabida* needs *Wolbachia* bacteria to develop into adulthood and form its ovaries properly (Gilbert et al., 2010). j) *Acrythosiphon pisum*, otherwise known as pea aphids, need endosymbiotic bacterium *Buchnera aphidicola* to make essential amino acids it doesn’t get from its diet of sap (Wilson et al., 2010).

Table 1:

Example of hologenomes exist among organisms in many eukaryotic clades, in diverse lineages of eukaryotes (See also figure 1).

Host Clade	Host(s)	Example symbiont	Reference
Opisthokonta	*Homo sapiens*	Various including *Bifidobacterium*	(Blekhman et al., 2015; Romano, 2017; Stilling, Dinan, et al., 2014)
	Euprymna (Bobtail squid)	*Vibrio fischeri*	(Gilbert et al., 2010)
	Asobara tabida (Wasp)	*Wolbachia*	(Gilbert et al., 2010),
	Acrythosiphon pisum (Pea aphid)	*Buchnera aphidicola*	(Wilson et al., 2010)
	Corals	*Symbiodinium* (Alveolata)	(Pillet et al., 2011; Rosenberg et al., 2007)
	Termites	Various Excavata	(Duarte, Nobre, Borges, & Nunes, 2018)
Plantae	*Opuntia leptocaulis* (Christmas cactus)	*Paraphaeosphaeria. Chaetomium*, (Fungi)	(Gilbert et al., 2010; McLellan et al., 2007)
	Legumes	Rhizobia (Bacteria in root nodules)	(Gage, 2004; Oldroyd, Murray, Poole, & Downie, 2011)
Amoebozoa	*Acanthamoeaba*	Diverse bacteria	(Greub & Raoult, 2004; Loret & Greub, 2010)
	Neoparamoeba sp	*Perkinsela sp.* (Excavata)	(Nowak and Archibald, 2018)
Rhizaria	Foraminifera	Dinoflagellates, diatoms	(Jauffrais et al., 2016; Pillet et al., 2011)
Alveolata	*Paramecium tetraurelia*	*Caedibacter taeniospiralis*	(Grosser et al., 2018)