

Title: Holobiont: is the whole more than the sum of its parts?

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Summary

Biology has traditionally operated on the organism as the natural unit for understanding life and population for understanding evolution. Advances in sequencing technologies have recently made it possible to uncover the contribution of multiple organisms and mobile genetic elements to the operation of conventional “organisms.” This dawning realization has led to a reconceptualization of the organism as a community of multiple interacting partners, often referred to as the holobiont. Recasting organisms as holobionts has the advantage of bringing to the forefront the myriad contributions of the diverse entities making up the holobiont, but also puts forward new challenges that need to be addressed to make this reconceptualization applicable to modern biology. Here we provide an operational definition of the holobiont and use it to address the evolutionary processes driving the formation and maintenance of these compound entities. We discuss the main challenges that need to be faced in order to make the holobiont stance a useful paradigm across multiple scales and fields in biology.

Background

Many biological studies regard the organism as an entity composed of a single genotype. This assumption is embedded in most models currently used in biology. Organisms are, however, a composite of different partners that together form the biological entity. The contributions and interactions of these partners dictate the properties of this combined entity, which can be called the holobiont. The conventional ‘individual’ organism is therefore a conglomerate, deriving from a suite of complex interactions with other organisms that define the genotype and phenotype of the holobiont.’

We define the holobiont as a symbiotic community with a compound fitness that can be operated upon by natural selection as well as other evolutionary processes. This definition entails that the holobiont provides an additional fitness advantage as a whole, and that natural selection embeds the capacity to form, and exploit, the holobiont as an evolutionarily advantageous feature for its constituents. The holobiont exhibits multi-level symbioses that persist (or recur) in space and time enough for natural selection to operate on it. Evolution is acting on very different time scales for different organisms in a given holobiont. The holobiont therefore constitutes a special case of biological organization in which the selectable phenotype is the concerted activity of the meta-organism, whereas its genetic encoding is distributed among several lineages, which could span multiple domains of life. Selection will be acting on the holobiont as a whole as well as on each member, and the selective processes for individual components will proceed across different time- and spatial scales, with the joint holobiont fitness as a unifying trait. The holobiont concept

can be in principle deployed across all scales of life, from a bacterium and its entourage of interacting mobile genetic elements, to a forest with all its constituents, but it has been mostly used when approaching self-contained systems, such as those embodied by animal or plant and their symbiotic organisms. Heretofore, we refer broadly to these self-contained systems when discussing the holobiont and its implications for biology. For example, gut microorganisms, that associate with animals form endosymbiotic networks of intergenomic associations with the host. Bacteria residing in animal intestinal tracts have the ability to produce vitamins which directly benefit the host. Gut bacteria play a crucial role in human health, such as supplying thirteen essential nutrients, synthesizing vitamin K, and assisting in the digestion of cellulose. Along similar lines, the sea anemone *Exaiptasia pallida* and its endosymbiotic algae belonging to the family Symbiodiniaceae. In Florida, the vast majority of *E. pallida* hosts are Symbiodiniaceae belonging to the genus *Symbiodinium* while Bermuda anemones host *Breviolum* algae. Goulet et al. (2005) compared natural and lab-produced host-symbiont genotypic combinations. These produced three different net oxygen fluxes and three different responses to elevated temperatures (Goulet et al. 2005). This study illustrates the importance of identifying the holobiont partners in interpreting physiological results and the effects of perturbations.

Plant associated microbes including archaea, bacteria, fungi, picoeukaryotes, and viruses have been shown to affect plant growth, nutrition, development, susceptibility to disease, resistance to heavy metals, and the degradation of xenobiotics, thus contributing to the fitness of the whole holobiont (plant plus associated microbes). Distinct microbial communities could be found in different plant organs and are referred as: rhizosphere, which is the microbial community found in roots and closely associated soil, phyllosphere in leaves, microbes residing within plant tissues form the endosphere, whereas there are also microbial communities associated to flowers (anthosphere) and fruits. In a single plant, those microbial communities are distinct, although they may share many members. Furthermore, the plant species, genotype and soil type could lead to different plant-associated microbial communities where those microbial assemblages are the result of root morphology, exudates and rhizodeposits. A deep knowledge of how those microbial associations impact whole plant fitness could lead to improvements in plant food production and protection to plant diseases by manipulating the associated microbes.

Conceptual Diagram of the Holobiont

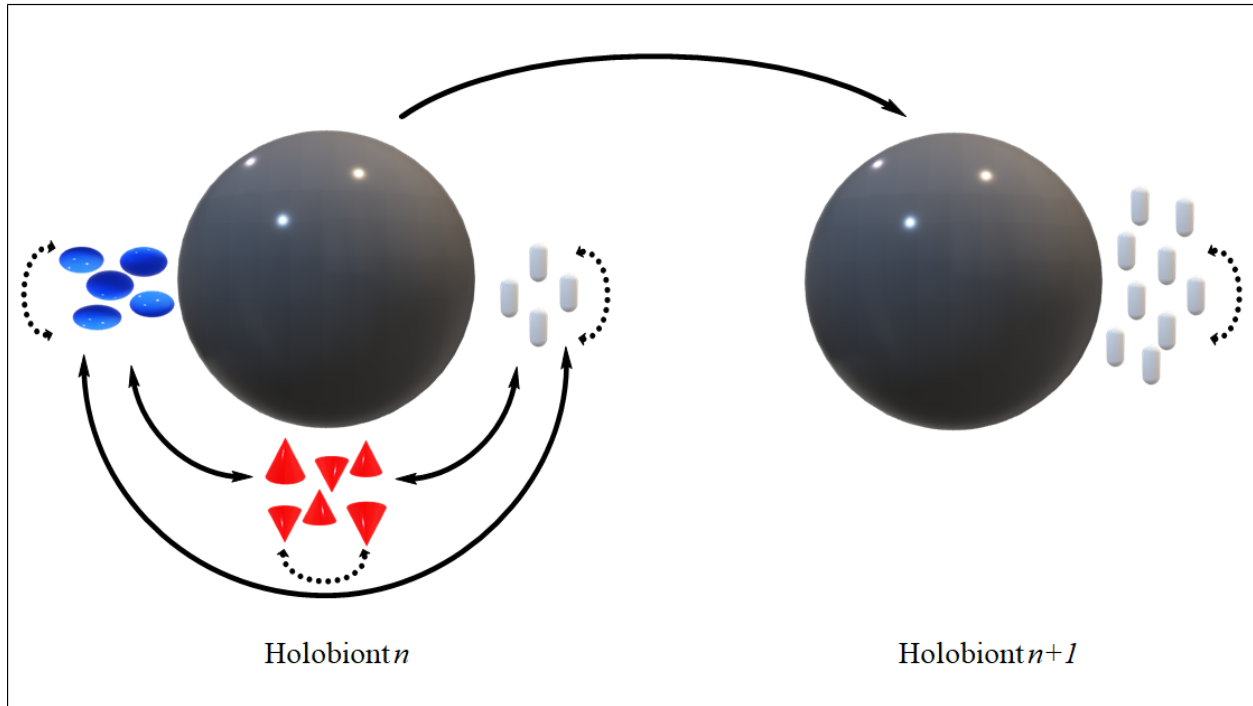


Figure 1. Conceptual diagram of the holobiont. The selective process acts on the holobiont as a whole as well as on each symbiont, and selection for individual components will develop across different time- and spatial scales, with the collective fitness of the holobiont as a unifying trait. The relatively large population size and the short generation time of the resident (white) and transient (blue and red) symbionts compared to the host permit different evolutionary rates and development of novel observable phenotypes within a short period of time.

Relevance

In many holobionts, some of the organisms are microscopic (e.g. bacteria, viruses) and/or are morphologically indistinguishable (e.g. dinoflagellate algae). To identify and distinguish between the partners involved requires molecular methods that are now feasible and financially accessible. “Omics” approaches from metagenomics, metabarcoding, metatranscriptomics, metaproteomics and metabolomics have revealed the great diversity of microorganisms associated with macroorganisms, and the complexity and physiological importance of these interactions for the long-term survival and adaptability of all the different participants. This new understanding enables us to shift our current vision of organismal physiology and evolution towards the more integrative concept of the holobiont.

As we face novel changes due to global changes in environmental conditions (e.g. climate change), organisms are subjected to environmental changes at an accelerated rate. Whether the organisms that constitute the holobiont react to the perturbations, react in similar ways, or at a similar rate, will affect how the whole holobiont deals with the potential perturbations. A holistic perspective on the organism will also enable us to assess the relative and overall fragility of holobiont components, and will illuminate interventions, such as manipulating the members of the

holobiont as a means to engineer enhanced resilience and adaptability of the holobiont as a whole.

Adopting the holobiont motif will also enable us to explicitly incorporate emergent community phenotypes that confer greater fitness to the holobiont and its diverse and multi-tiered encoding across multiple genetic lines into our understanding of the processes driving the evolution of organisms. This will lead to the generation of new evolutionary models capable of taking into account the inherent complexity of these interactions.

Research questions and challenges

Two fundamental questions about holobionts are: (i) What are the mechanisms that make a holobiont a coherent entity and (ii) what factors contribute to holobiont phenotype, evolutionary forces acting on it, and responses to those forces that are exclusive to the holobiont? These questions raise a number of challenges.

One challenge is that as our awareness of the complexity of holobionts grows, our ability to address and assess all the components becomes more difficult. For example, for nearly a century, research on coral symbioses has been dominated by studies on the mutualism between corals and dinoflagellate algae from the family Symbiodiniaceae (e.g. Boschma, 1925). Only relatively recently has the bacterial consortium garnered attention (reviewed in Knowlton & Rohwer, 2003; Thompson et al. 2015), and studies on the bacteria are descriptive (Hernandez-Agreda et al. 2017) or correlative (Shirur et al. 2016) with the vast majority of the publications focusing just on the bacterial complement. Little work has focused on concurrently studying the effects of stressors on both the Symbiodiniaceae and the bacteria (Shirur et al. 2016). Similarly, studies on the viruses within coral holobionts lag behind their bacterial counterparts. Either descriptive or manipulative studies assessing the multiple components of the holobiont are rare (e.g. Shirur et al. 2016). Investigating the multitude of layers in symbioses will necessitate a cross disciplinary collaborative effort.

A growing number of studies have shown the potential of the holobiont framework in crops systems. A better understanding of how each member of the community contribute to the fitness of the holobiont could be harnessed to improve production, resistance to diseases or treatment of diseases.

Another challenge that needs to be addressed is the need to identify temporal and spatial boundaries that provide an operational definition of holobionts. Although we propose a working definition of holobiont in this paper, community-driven discussions are needed to generate a consensus of when to adopt the holobiont stance, constrained by the need to ensure that the consortium making up the holobiont satisfies minimum temporal and spatial continuity.

Further, we need to define methods to carry out evolutionary experiments within a holobiont framework, since traditional evolutionary experiments have not taken into account the multifaceted and diffuse nature of the holobiont. Potentially, preserved specimens from previous evolutionary experiments could be further studied through the lens of the holobiont framework. Additionally, evolutionary models that take into account the specific properties of holobionts will need to be developed. These unique properties include a substantial amount of population replacement, genetic exchange and turnover, which are not typically addressed by standard evolutionary models. The development of evolutionary models for holobionts requires

experimental work to determine rates, amounts, boundaries and conditions for lateral gene transfer and population dynamics, such as partial replacement of the members of a microbial consortium. Additionally, it is essential to define and measure the impact of relevant interactions among holobiont members to differentiate and estimate singleton and holobiont fitness.

Reconceptualizing when and how communities assemble and disassemble, and how often this cyclic process takes place, will allow for tests of natural selection that may be deployed across all scales of life. Furthermore, although some holobionts include obligate symbioses, in multiple obligate symbioses, many symbionts are acquired every generation (horizontal symbiont acquisition). Therefore, there is a mechanism for acquisition of novel symbionts but it is unclear how holobiont specificity can develop and be maintained. These mechanisms may generate similarities across generations, or they may promote gene flow among hosts. Addressing the challenge of determining the mechanisms of specificity will be an important advancement towards understanding symbioses. In other symbioses, with transfer of symbionts from parent to offspring (vertical symbiont acquisition), there is a mechanism for symbiont heredity but not necessarily for novel symbiont acquisition. In a study on the coral *Stylophora pistillata*, it was found that the lateral symbiont acquisition was punctuated with horizontal Symbiodiniaceae acquisition illustrating that multiple acquisition strategies may address the specificity and selection of the holobiont unit (Byler et al. 2013).

Roadmap

Most of the technological innovations (e.g. metagenomics) required to push forward a holobiont-based approach to biology are already in place, but there is a need for dissemination of the concept and its utility for it to effectively permeate across multiple biology disciplines. We believe that a key development in this respect will be the identification, study and ultimate adoption of model holobionts, in much the same way as model organisms have been used to push forward different areas of biological research. While model holobionts may overlap to some degree with current model organisms, and might benefit from the buy-in of those communities, this is not necessarily a certain outcome. In fact, most of the research performed to date on the holobiont concept has been carried out in non-model organisms, mostly invertebrates, with a relatively simple network of associations. Some examples are corals, squids with tight bacterial partners, and cohesive microbial communities, such as kefir. As the holobiont paradigm becomes more established, we expect researchers working on conventional animal and plant models, where the microbiome contribution has already become largely established, to gradually expand their view of model organisms to include many other components of the holobiont, such as invertebrate, fungal and viral communities coexisting with vertebrate hosts and their microbiomes.

A further step in the holobiont research roadmap will come with the involvement of phylogenetics and modeling communities interested in adapting current 'organismal' models of evolution to gradually take into account several of the unique properties of the holobiont perspective. Among these, we anticipate that research quantifying the amount of gene flow, both through mobile genetic elements and partial population replacement, within a population will be instrumental in providing modelers with quantitative estimates for parameters that define holobiont population dynamics and that can be used to generate models of evolution including this additional layer of complexity. Another interesting venue for research will be the development of protocols for

experimental evolution studies that consider the holobiont as a unit. Whereas current experimental evolution setups make use of well-defined and controlled germ-lines to assess differential fitness, the need to concurrently assess multiple individual contributions to holobiont fitness, and to track the subsequent distributed fixation of alleles, as well as the 'soft' spatial and temporal boundaries of holobionts will require creative approaches for the design of experimental protocols.

Holobiont research will also necessitate for an integrative approach to meta-omics research, where datasets and protocols often use different standards (e.g. literally excluding other components of the holobiont to focus on one), as well as from an overall shift in the perception of organisms fundamentally as symbiotic communities.

How does the holobiont concept reintegrate biology?

The study of holobionts requires integration at multiple levels and multiple taxonomic fields. Holobionts often include multiple species from different phyla and even domains. Redefining organisms as holobionts capitalizes on the awareness from the organismal scientific community as well as collaborative efforts between scientists from multiple interconnected disciplines. Therefore, to fully comprehend holobionts, one must have knowledge about the different entities making up the holobiont. To assess the holobiont's fitness, a multi-faceted approach incorporating different aspects such as gene expression, physiological assessment, ecology, etc. may be warranted. Such diverse taxonomic, discipline and technique require a collaborative effort between individuals with different skill sets (microbiology, evolutionary biology, developmental biology, molecular biology, ecology, bioinformatics, computational science, biological engineering, agronomy, and more).

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