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# Interactions within the Holobiont: On the Holobiont's Interactions of Its Microorganisms

Tamar Schneider*

I address the question of how we should understand the holobiont and offer to look at it from the perspective of interactions. The debate about the holobiont centers on two issues: where to place its boundaries and what are the criteria for distinguishing inside from outside. By shifting the focus from degrees of cohesion of the host-symbiont interactions to the heterogeneity of interactions, I suggest a different perspective on interactions and their role in shaping the interacting agent (e.g., host-organism/microorganism/holobiont). I focus on the notion of mutuality of interactions by thinking about the holobiont through microbial interactions, using the case study of quorum sensing between bacterial cells. I conceptualize interactions as constitutive, placed on a scale between constitutive and contextual of each interacting agent. Constitutive interactions in this view are not interactions between individuals composing a third individual (i.e., symbionts within a host organism). Instead, the interactions are constitutive of each of the interacting organisms in interdependence relations. Furthermore, I argue that this interdependence involves the environment as an active participant which affects the nature of the interactions through environmental modifications.

**Keywords**

holobiont • interactions • biological individuals • ecological communities • quorum sensing

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## 1 Introduction

Over the last three decades, studies in microbiology have exposed a world of diverse and dynamic interactions. Through metagenomic sequencing, complex bacterial communities became

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*The Cohn Institute for the History and Philosophy of Science and Ideas, Humanities Faculty, Tel Aviv University, Ramat Aviv, Tel Aviv 6997801, Tamisch0106@gmail.com
https://orcid.org/0000-0001-8045-4691

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visible and proved important for many biological phenomena. As a result of discovering the connection between microorganisms and organisms' survival, the notion of the holobiont has become prominent and has been suggested as a biological individual. The view of the holobiont as an individual, commonly known as the Hologenome Theory, focuses on the interactions and relations between the host and its symbionts in the host's development and evolution (Zilber-Rosenberg and Rosenberg 2013; Bordenstein and Theis 2015). Today, the holobiont is at the heart of the debate on the nature of the biological individual, a debate which is connected to the same question about the nature of the individual organism.

I address the question of how we should understand the holobiont and examine this question from the perspective of interactions. The debate about the nature of the holobiont centers on two questions: *where* to place its boundaries and *what* the criteria distinguishing inside from outside are. Two main views relate to these questions: one is that the holobiont is indeed a biological individual, and its borders include symbiotic interactions and exclude harmful interactions (Zilber-Rosenberg and Rosenberg 2008, 2013; Bordenstein and Theis 2015; Dupré and O'Malley 2009; Lloyd 2017). The other view considers the holobiont as an individual only in special cases where the host-microbe interactions are obligatory (loyal) and vertically inherited. All other types of interactions between hosts and microorganisms, according to the latter view, should be considered as an ecological community mixed from different individuals (Godfrey-Smith 2013; Douglas and Werren 2016; Skillings 2016). Thus, the former sees the holobiont as a biological individual, and the latter looks at the holobiont as an ecological community.

I argue for a different way of thinking about the holobiont through interactions, namely considering it to be an individual that is also an ecological community. The holobiont is a unique ecological community, an assembly of host-microbial and microbial interactions. It is an individual in Pradeu's sense of a physiological individual that includes its microbial interactions, but also in this same sense, these interactions are ecological (Prudeu 2016; Skillings 2016). By shifting the focus from the degrees of the cohesion of the host-symbiont interactions to the heterogeneity of interactions, I suggest a different perspective on interactions and their role in shaping the character and nature of the holobiont. Furthermore, by looking at the holobiont's heterogeneous interactions rather than their cohesion, I offer a different set of questions. Instead of asking about the boundaries and the criteria distinguishing the inside from the outside, we need to ask about the interdependent nature of the interactions between the organisms composing the holobiont and the interactions' role in determining the characteristics of those organisms.

I demonstrate my perspective on interactions by describing studies on bacterial molecular interactions, particularly quorum sensing. Thinking about molecular interactions, I wish to show the role of the interactions in the materialization of the bacterium properties and function. Here the bacteria change their own gene expression (and sometimes their genes!) in coordination with other bacterial cells through releasing and sensing molecules (Keller and Surette 2006). In other words, the interactions occur through molecular exchange between bacterial cells. The molecules released from the bacterial cells to a small-scale environment create modifications that accumulate to influence the mode of bacterial proliferation and function. Thus, diverse bacterial communities interact and coordinate their gene expression to perform their functions mutually and simultaneously. The individual bacterium not only determines these interactions but, also, the interactions determine the nature of each individual bacterium.

Thus, I examine through this perspective the interactions between microbes, cells, and the host composing the holobiont. Here I put an emphasis on the small-scale interactions which create small-scale environmental modification. Then, I examine the significance of the small-scale environmental modifications on the larger scale organization (i.e., the interactions within the holobiont in its environment and the interactions between holobionts). In each case, the focus on the interacting agent (i.e., bacterium) should be through its interactions with other agents (bacteria) in its environment. Thinking about interactions and the way they constitute the agent's function and characteristics will give a better understanding of the heterogeneous nature of the holobiont and its relations with its environment.

## 2 How to Understand the Holobiont?

The holobiont is an entity with fuzzy boundaries because it is constructed out of the relations and interactions between a host and an interchangeable microbial composition. That alone makes it hard to delineate and distinguish those interactions that are part of the entity and those that are not. To make this distinction, different claims are made regarding the nature of the interactions and the relations within the holobiont. Thus, certain types of interaction, such as symbiotic or obligatory, are usually considered to be part of the entity while harmful interactions are not. Definitions of this sort join the philosophical debate about the nature of the biological individual, resulting in the debate about whether the holobiont should be considered a biological individual. Thus, the question of how we should understand the holobiont becomes the question of whether an organism should include its microbiome or whether the microbiome should be defined separately from the host organism. Either way, the host-microbial heterogeneous interactions pose challenges.

Thomas Pradeu (2016) points to the distinction between the physiological individual and the evolutionary individual while also examining their connectedness and relations in the different fields of biology. In terms of evolutionary individuality, the evolutionary unit can be the unit of living (i.e., the organism), but is not necessarily that. Thus, he suggests making this distinction clear in each argument with the understanding that when thinking about definitions of organisms or a unit of living, the discussion is of a physiological nature (ibid.). In this regard, the physiological nature of the individual relates to borders and boundaries and degrees of cohesion:

> At the most general level, the problem of biological individuality asks what, in the living world, constitutes a relatively well-delineated and cohesive unit. Biological boundaries are often fuzzy, and biological individuality is often question-dependent, coming in degrees, and being realized at different levels. (Pradeu 2016, 799)

In terms of physiological individuality, the answer to the question of whether or not the holobiont is a biological individual relates to where we wish to place the boundaries and the criteria distinguishing the inside from the outside. Both boundaries and the inside-outside distinction are measured by degrees of cohesion and the nature of the interactions within the holobiont. Here, my interaction analysis relates to the notion of the holobiont as a physiological individual. But I wish to question the nature of the inside/outside delineation.

When considering how to delineate the boundaries and degrees of cohesion, there are two main views in the debate about the holobiont's nature. The first view, which brought this debate to the center of attention, emerged with Ilana Zilber-Rosenberg and Eugene Rosenberg's paper "The Role of Microorganisms in the Evolution of Animals and Plants: The Hologenome Theory of Evolution" (2008). In this paper, Zilber-Rosenberg and Rosenberg see the holobiont as a biological individual and as an evolutionary individual. Others have joined this view, arguing that symbiosis and collaboration between different organisms are prominent and essential for most biological, developmental, and evolutionary processes (Gilbert and Epel 2009; Dupré and O'Malley 2009; Gilbert, Sapp, and Tauber 2012; Zilber-Rosenberg and Rosenberg 2013; Bordenstein and Theis 2015; Lloyd 2017).

The other view comes as a response to the first and considers the holobiont (mainly) an ecological community (Douglas and Werren 2016; Skillings 2016).[1] In this view, as in the first, the center of attention is on the degrees of cohesion and the nature of the interactions between the organisms within the holobiont serving as a criterion for boundary delineation. Thus, necessary interactions for the host's existence are part of the organism, or only such interactions that are consistent and inherited vertically between generations (Godfrey-Smith 2013). Peter Godfrey-Smith (2013) makes a distinction between organisms that are multispecies and Darwinian individuals that are multispecies and argues that some multispecies organisms are Darwinian individuals and some are not. A similar view is held by David Queller and Joan Strassmann (2016), who examine the holobiont's degrees of cohesion by looking at levels of cooperation and conflict between the organisms within the holobiont.

Both views address the questions of boundaries and the distinction of the inside from the outside; the first view delineates the boundaries to include both the host and microorganisms in symbiotic interactions while the second delineates the boundaries to include only the obligatory and inherited symbionts. In the first view, the criteria for distinguishing inside from outside examine the symbiotic interactions (inherited or acquired from the environment) that are part of the organisms' development, reproduction, and survival. Here there are physiological mechanisms, such as the immune system functioning as a discriminatory system (Pradeu 2012; Tauber and Gilbert 2016). Supporting this view is the notion of the holobiont as a hybrid individual composed of the interactive association between the host and its symbionts. This view considers the interactive association between the bacteria and the immune cells as a structure of developmental scaffolds (Chiu and Eberl 2016). The second view looks at vertical inheritance and high degrees of collaboration or obligation with low or zero degrees of conflict as criteria for an evolutionary process (differently from the first view, not necessarily as physiological mechanisms) that helps in making distinctions between an individual (maybe an organism or multispecies organism) and an ecological community (Queller and Strassmann 2016).

### *2.1 Different aspects of interactions: degrees of cohesion or interdependence*

The question of how to understand the holobiont is silenced by the biological individual debate. In other words, it seems that the debate about the biological individual is the main conceptual tool for understanding the holobiont. Thus, the main questions regarding host-microbial relations focus on degrees of cohesion and levels of dependency between host and microbes in order to delineate the boundaries. However, focusing on this aspect of the relationship imposes binaries such as inside/outside, self/non-self, and part/whole, which might not be helpful. The interchangeable bacterial composition or the interchangeable microbial properties between harmful and beneficial challenges the inside/outside self/non-self binary. For example, the same microorganisms can be considered inside or part of the self in one aspect and non-self in another, depending on their interactions with the host and other microbes.

In their paper "Rethinking 'mutualism' in diverse host-symbiont communities," Mushegian and Ebert (2016) argue that for a better examination of host-symbiont mutualism it is essential to follow various interactions within the microbial ecological communities that play a role in the host-symbiont mutualism but are not necessarily reciprocal with the host:

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[1] I added the reservation because this approach does find some host-microbial relations to be part of an individual in the case of endosymbionts or where the microbes are vertically inherited and obligatory.

> We argue that defining the nature of a relationship between an animal host and a diverse microbial community as mutualism, commensalism, or parasitism poses not only empirical but also conceptual challenges. We propose approaching this question in the larger framework of questions in community ecology and the context-dependency of species interactions. (Mushegian and Ebert 2016, 101)

The host-related microbiomes are heterogeneous, with diverse, dynamic interactions that influence their properties and function in the host. Therefore, like Mushegian and Ebert, I believe that centering only on the aspects of host-symbionts relations misses other aspects of the microbial and host-microbial web of interdependence. Furthermore, I argue that there is a significant aspect of the holobiont beyond the symbiotic/non-symbiotic relations, namely the mutual interactions and interdependence. This aspect enables a wider perspective on the interactions in their different scales of micro, macro, and physiological or ecological systems. Examining the mutuality of interactions from this perspective also includes the background conditions that lead to the interdependency. This type of mutual interaction emphasizes the interdependence between entities and enables conceptualizing the boundaries as vague and dynamic.

The heterogeneity of the interactions includes different relations, such as competitive, collaborative, cooperative, and parasitic ones between diverse types of organisms and cells. Thus, the holobiont is a composition of dynamic interactions between a multicellular organism, which is a macroorganism, and many different species and strains of unicellular organisms—the microorganisms. More so, in the interactions between the cells in the holobiont there are interactions between body cells, between body cells and bacterial cells, and between bacterial cells. These interactions are not static and can change from beneficial to harmful or from competitive to collaborative, depending on the background conditions. Also, the environment or background conditions on a small scale depends on the interactions and the holobiont's surroundings and behavior, such as its habitat and nutrition. Therefore, asking only about degrees of cohesion, even in terms of levels of cooperation and collaboration, is not enough to give a clear understanding of the holobiont.

By thinking about the holobiont through its interactions, as I suggest, we can see advantages in looking at the holobiont as a biological individual from the physiological perspective that is also an ecological community. The holobiont that is featured as the host and microbe complex is an individual in its physiological definition because it involves the host's physiological systems. Without the host, the microbiomes are simply described as microbial communities in their environmental niche. Once these communities are entangled with a host organism, it becomes a holobiont. My point here is that because of the host physiology, the holobiont is a unique ecological community, and because of the microbial ecological communities, the host physiology should be examined from an ecological perspective. If the holobiont is an individual, then it is clearly the case that the holobiont is an individual composed of other individuals. Then, it is important to examine all types of interactions—between the individuals within and around the host that constitutes a holobiont. That is why the notion of an ecological community is helpful, with its focus on interactions and with similar challenges of fuzzy boundaries and heterogeneity.

Thinking of the holobiont as both a physiological individual and an ecological community is an alternative to Pradeu's notion of a physiological individual because here I am looking at the ecological interactions and their interdependency instead of the degrees of cohesion and dependency. Most of the debate about the holobiont focuses on the host-symbiont degrees of cohesion in an attempt to determine the boundaries. Both accounts of the physiological individual and the evolutionary individual look at the interactions as criteria for distinction and the fuzzy boundaries as a challenge to solve. However, I focus here on the bacterial interactions with a different motivation, focusing instead on patterns of mutual exchange through interactions and their dynamics of interdependence to understand the nature of the holobiont and its dynamic boundaries. Thus, the notion of reciprocity of interaction and interdependence defines the boundaries by their vagueness, rather than by their demarcation.

## 3 Thinking About Organisms Through Their Interactions

By looking at the holobiont through interactions, I offer a different set of questions to understand its nature. Instead of asking about the boundaries and inside-outside distinction criteria, I suggest asking how to think about the role of interactions in shaping the properties and characteristics of the interacting agents. Is the nature of the individuals composing the holobiont determined by their interactions with each other? Which interactions constitute, and which are contextual to the interacting individual? And what are the environmental conditions and relational dynamics influencing the interactions?

First, I elaborate the important clarification on the distinction between interactions and relations and the possibility of confusing them. Interactions require mutual exchange between two or more agents, and relations refer to the different positions of the agents to each other (such as spatial or temporal relations). In this sense, we can think about interactions as a mutual exchange between agents that are in some form of relations. Thus, we can have relations of conflict with the interactions of exchanging force or relations of two friends sitting at a table looking at their phones with no interaction between them. The relations refer to the agent's positions, and the interactions refer to the agents' *mutual acting of exchange*. The relational domain is the background conditions that shape the agents' positions. For example, in social structure, the workplace is the relational domain of co-workers, as marital institution is the relational domain of the married couple. Thus, interactions occur between agents that are in some form of relation within a relational domain. The relations or the relational domain influences the interactions, their iterations, and strength.

The notion of interactions as constitutive of the individual's nature is taken from an interactionist approach to the development of social cognition. In this approach, social cognition is developed by social interactions. The idea is that the social cognition that influences social interactions is also developed by social interactions leading to the individual's ability not only to understand others but also to an understanding *with* others in a social context (De Jaegher, Di Paolo, and Gallagher 2010). Understanding with others means more than understanding verbal explanations; it becomes a pragmatic ability to act appropriately (ibid.). The definition of social interactions that constitute the development of social cognition involves engagement between agents:

> [S]ocial interaction as a co-regulated coupling between at least two autonomous agents, where: (i) the co-regulation and the coupling mutually affect each other, constituting an autonomous, self-sustaining organization in the domain of relational dynamics and (ii) the autonomy of the agents involved is not destroyed (although its scope can be augmented or reduced. (De Jaegher, Di Paolo, and Gallagher 2010, 442)[2]

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[2] The notion of autonomy in the definition means a self-sustaining networking of processes under precarious conditions: a self-sustaining identity. The self-sustaining identity applies to both agents and the relational dynamics of their coupling. This definition excludes situations of coercion (De Jaegher, Di Paolo, and Gallagher 2010).

Interactions mean mutual engagement between entities mutually affecting each other. This mutuality, though, excludes cohesion and is constitutive of the agents by being a part of a self-sustaining organization in a domain of relational dynamics.[3]

In the conceptualization of the holobiont as a physiological individual, the question of levels and degrees of cohesion is at the center. My motivation in my interaction analysis is to shift this perspective to look at the interdependence between organisms that are not in cohesive relations or regardless of them. That is, I address the nature of interdependence and not the levels of cohesion as essential in the inquiry and understanding of the holobiont. Here I make the analogy of the interactive explanation of social cognition, which belongs to an individual but is also the result of its interactions with others, to the microbial molecular interactions that constitute the microbial functions on a small scale.

### *3.1 The case of quorum sensing*

We can understand the organisms' characteristics/traits by understanding their interactions with other organisms. The symbiosis relations between the Hawaiian squid *Euprymna scolopes* and the bacteria *Vibrio fischeri* operate and maintain the light organ within the squid, which is essential for the squid's camouflage at night in shallow waters. These symbiotic relations are the results of different types of interactions occurring, during the early developmental stages, between the squid's immune cells and the bacteria. However, there are also interactions among the bacteria's individual cells that determine the act of switching the light on and off. Thus, the squid and the bacteria collaborate every night and part ways in the morning, but for that to happen an interactive pattern needs to be established in the early stages of the squid's development.

The juvenile squid harvesting bacteria for the first time goes through the developmental process and morphogenesis of its light organ. This process is triggered by molecules released from the bacteria *V. fischeri* that activate the squid's immune response to induce the apoptosis of the epithelial cells that cause the complete loss of the ciliated, resulting in the light organ's morphogenesis (Koropatnick et al. 2004).[4] These immunogenic molecules released by the bacteria also activate the immune cells to recognize *V. fischeri* as a symbiont, not letting other bacterial species in (Brennan et al. 2014).[5] Thus, the *V. fischeri* and the squid's immune cells form their mutualistic, self-sustained domain of relational dynamics during the development of the light organ and the elimination of non-mutualistic bacteria. Also, in these relations they interact in a constitutive way that shapes their unique characteristics: the squid develops its light organ, and the bacteria loses its flagellum.

However, the development of the light organ and the recognition of the bacteria by the squid's immune system is not enough for the completion of the light organ. There is another important set of interactions that need to take place for the light to go on. These interactions, known as quorum sensing, refer to the molecular signaling between bacterial cells that triggers

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[3] A domain of relational dynamics in a social context can be social institutions, such as work or school, and the different roles within them, such as teacher and students, or co-workers and cohort. In the case of interacting organisms, the relational dynamics can be the environmental and topographic landscape surrounding the host and its symbionts and the different parts each organism has, such as immune cells, blood cells, and bacteria.

[4] *Vibrio fischeri* bacteria release a fragment of their peptidoglycan and lipopolysaccharide (LSP) surface molecules, which are considered pathogenic, in their niche in a juvenile Hawaiian squid *E. scolopes*. The LSP triggers the morphogenesis of the light organ in the squid (Koropatnick et al. 2004).

[5] The role of the sheathed flagellum rotation in the release of immunogenic LPS can indicate the importance of immune modulation by the bacteria. The symbiosis between the squid and the *V. fischeri* is constructed by the immune response to the bacteria trigger, which activates the immune system's two important responses: cell apoptosis in the development of the light organ, and the elimination of non-mutualistic bacteria (Brennan et al. 2014).

their gene expression to activate this function simultaneously. Quorum sensing is thought to be some form of communication between bacterial cells to orchestrate their behavior and function as a group rather than isolated cells. In the case of the light organ, the light on and off switch has a significant impact when the light comes from the cells of an entire bacteria colony simultaneously.

Quorum sensing is a name given to extracellular molecular signals between bacterial cells within and between bacterial communities, used to coordinate their different functions collectively. These molecular interactions between bacterial cells happen through sensing and releasing extracellular chemicals called autoinducers (AIs), which then translate the information into internal changes in their gene expression (Miller and Bassler 2001). This 'chemical language' between bacterial cells seems to be diverse and composed of more than one type of molecule. Melissa Miller and Bonnie Bassler write in their review:

> We now know that a vast assortment of different classes of chemical signals are employed, that individual species of bacteria use more than one chemical signal and/or more than one type of signal to communicate, that complex hierarchical regulatory circuits have evolved to integrate and process the sensory information, and that the signals can be used to differentiate between species in consortia. It seems clear now that the ability to communicate both within and between species is critical for bacterial survival and interaction in natural habitats. (Miller and Bassler 2001, 166)

A single bacterial cell does not function by itself without a sufficient quorum of kin cells and possibly also with other groups of neighboring colonies. Thus, understanding of quorum sensing as a general phenomenon in bacterial life has changed the perception of bacteria from individuals to social entities (Keller and Surette 2006). Bacterial communities are interdependent on each other and their environment for their functions. One of the manifestations of such interdependence is the microbial ability to act simultaneously to produce an environmental impact. Another example is the cross-feeding of one species on the metabolites secreted by another. The relations of interdependence can be in different forms, such as collaboration or competition, and the molecular exchange is responsible for the regulation and synchronization between bacterial cells. Thus, the interactions help in the regulation of activating or deactivating different physiological functions, such as mating, proliferating, biofilm formation, secretion of toxins such as antibiotics, activating virulence, bioluminescence, and horizontal gene transfer (Ng and Bassler 2009; Perez et al. 2012).[6]

Furthermore, the process of exchanging molecular signals between bacterial cells works through small environmental modifications. Thus, the systematic structure of bacterial interactions is embedded in the molecular compound of the environment and the environmental topography. The molecular signals depend on the numbers of cells and their composition as well as the environmental conditions where the exchange takes place. Thus, the mutualistic nature of bacterial interactions connects the bacteria with their host environment through a chain of interdependencies. The spatiotemporal relations are the domain where the molecular interactions occur. These relations influence the quorum and the molecular exchange (i.e., the interactions) to determine the activation of different bacterial functions (Even-Tov et al. 2015).

The interactions between bacteria are such that it is difficult, and maybe impossible, to distinguish them from the interactions between the bacterial cells and the environment. In the

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[6] For more about quorum sensing in the Bonnie Bassler Lab research see https://scholar.princeton.edu/basslerlab/research.

case of quorum sensing, or other molecular signals such as metabolic interactions, the environment is an active part of the interactions (Konopka 2009). The microbial interspecies and intra-species molecular interactions establish a variety of functions at the level of the individual cell, but in connection with neighboring cells and as a community. Whether molecular sensing is restricted in activating genes only in specific quorum or in a specific composition, it is a mechanism that constructs the bacterium as part of its community and environment. Thus, the molecular interactions are the mutual exchange of molecules between bacterium cells that depend on the relational domain, affect the bacterium gene expression and constitute the bacterial colony's function. The characterization of the molecular interactions is on a continuum where one end marks the interactions constitutive of the bacterium, while the other is the contextual interactions. The role of these molecular interactions is dynamic and can move on this continuum depending on their numbers and relational domain (i.e., background conditions). In the next two sections, I will elaborate on the constitutive-contextual continuum role of the interactions, and then on the environmental role.

### *3.2 The role of interactions on a continuum between contextual and constitutive*

Interaction, as distinguished from relation, requires mutual exchange between two or more interacting agents. The process of mutual exchange is important in this distinction because it requires feedback between the giver/receiver and receiver/giver. Each side in the interaction goes through some changes by receiving and giving back and by action and reaction. Here, it is essential to clarify what exactly is given and received, as well as the domain where these exchanges occur. The interactions can exchange forces, words, things, or, as in the case of bacterial interactions, molecules. The interactions are also influenced by the relations or relational domain between the interacting agents (i.e., the relations between the agents and their environmental niche).

When thinking about interactions we are used to thinking about the interacting agents and their characteristics that determine the nature of the interactions. Using the interactions view and the case study of quorum sensing, I show that it can also be the other way around: the interactions determine the characteristics of the interacting agents, depending on their intimacy and intensity. When the interactions affect the agent's characteristics and properties, they constitute the agent, and when the interactions are affected by the agents, they are contextual to the agents. The constitutive-contextual roles of the interactions are not mutually exclusive and are on a continuum that also has a feedback loop, depending on the relations between the interacting agents and their background conditions. Thus, this distinction is not a binary; instead, we should think of it on a dynamic scale between the agents determining the interactions to the interactions determining the agents.

In molecular interactions, such as quorum sensing, the exchange of molecules in a certain density determines their gene expression to a specific function. In low density, the bacterial cells continue to release and sense autoinducers from the environment, but with no effect on their gene expression. Without the right quorum, the specific genes for the function will not be activated. Thus, interactions between bacterial cells in high density will determine their gene expression (or even their gene horizontal transmission), and interactions in low density will not. Changes in density and molecular exchange, which reflects on gene expression, form a process that is also connected to the bacterium's life cycle, as shown in the *Vibrio*-Squid example. In the right quorum inside the light organ niche, the light switch turns on. Once it is expelled back into the sand and the density reduced, the light switch is off.

The molecular exchange continues constantly and, depending on the level of iterations, whether high or low, it will create a change within the bacterial gene expression. When in low density, the interactions are contextual, i.e., with no change in gene expression, metabolic path, or function. In high density, the interactions change the bacterial properties and function (in most cases, due to changes in background conditions) and the interactions become constitutive. This is a dynamic continuum between contextual and constitutive interactions, sensitive to environmental conditions (biotic and abiotic) that influence the density of the microbial cells. In the case of the *Vibrio*-Squid symbiotic relations and the *Vibrio* molecular interactions, this dynamic of change in gene expression is daily. But in other cases of quorum sensing, such as lateral gene transfer (LGT), the change is to the genetic sequencing and lasts longer.

The molecular interactions cause modifications in the bacterium properties and characteristics. For the changes to be constitutive, they should last for a period of time and constitute properties or functions. There is another sense of constitutive interactions: that of individuals that compose and constitute together a third entity.[7] However, I am not discussing this latter kind of constitutive interactions. The constitutive interactions I discuss here hold between separate entities that are interdependent by their interactions, which mutually constitute each individual's characteristic and property. Thus, in the case of molecular interactions, the interacting bacteria are interdependent in the sense that their properties and characteristics cannot be defined separately from their interactions.

To better understand the difference in the role of interactions as constitutive and interactions that are contextual it is helpful to think about Salmon's definition of causal interactions between processes (1984). The causal interactions are interactions between processes that modify them. This modification is described by Salmon as leaving a mark that persists:

> Modifications in processes occur when they intersect with other processes; if the modifications persist beyond the point of intersection, then the intersection constitutes a causal interaction, and the interaction has produced marks that are transmitted. (Salmon 1984, 170)

Salmon looked at causal relations as processes, not as singular events, and causal interactions as the intersection between causal relations (i.e., processes). The interactions that are the intersections between processes produce cause and effect simultaneously in both processes (Salmon 1984, 178–183). A mutual exchange is, by itself, an ongoing process of reciprocity between two or more interacting agents. Thus, it seems that mutuality of interactions or reciprocity of causal interactions becomes a meta-process of reoccurring feedback of causal interactions. These processes, as with any process to some extent, are embedded within their environment.

I use Salmon's account of causal interactions to clarify that, in my case, any interactions of mutual exchange are causal interactions that leave some form of a mark. But depending on the strength of the mark or iteration and persistence of the interactions, they can be classified on a continuum between contextual and constitutive. If the mutual exchange iterates and is consistent, then the interactions modify and reshape the agents. Or, as in the case of LGT, the exchange leads to modifications that persist without any iterations. But because it is on a continuum, the modification is also dynamic, and the persistence of a mark can be considered in degrees and levels of time and intensity. Thus, the change in each individual and its persistence define the role of the interactions on the continuum between constitutive and contextual.

Salmon gives an example of the intersection between a pulse of white light and a piece of red glass, which leaves a mark. The mark is where the white light changes into red light, and

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[7] This latter notion of constitutive interaction is, I believe, the framework for examining the degrees of cohesiveness in the host symbionts' interactions to determine whether they represent an individual or a community.

the glass absorbs some of the light and "goes through an increase in energy that remains *for some time after* the intersection" ([my emphasis] Salmon, 170–171). The mark is the indicator of the causal interaction, but there are different durations—it is possible for one mark to persist longer than others. This act of persistence can also be looked at on a scale, depending on the duration of the mark, meaning some interactions leave marks that persist for a long time while some may not leave a mark at all.

Salmon's notion of causal interactions relies on modifications and their persistence. Thus, constitutive interactions are also causal, but not all causal interactions are constitutive. Depending on the type of the mark and its persistence, the interactions can still be causal, but they are contextual and not constitutive.[8] The agents materialize through their constitutive interactions, depending on the interaction's iterations and the persistent of the mark. In other words, the constitutive elements in the interactions are their iterations and the degrees of the persistence of the mark and its significance in reshaping the agent's properties and functions. The distinction is of gradual differences between different types of interactions, depending on the duration of the mark they leave. On one end of the scale, we can have contextual interactions that do not leave a mark, or leave a transient mark, and on the other end we have constitutive interactions that leave a mark for a long duration of time (i.e., through iterations or strength or both).

In bacterial molecular interactions, the persistence of a mark depends on the number of bacterial cells as well as their composition and their environmental conditions. The mark in these causal interactions is the change that each interacting agent undergoes because of the mutual exchange. In other words, the causal interactions are interactions that constitute the functions of the cell, depending on the environmental conditions. Thus, by the gradual differences in the persistence of the mark or the change caused by the interactions, the role of the interactions differs from contextual to constitutive. If the mark is persistent for a long time or continues to occur in mutual and reciprocal interaction, then we can say that the interactions constitute the agent's traits. However, if the mark appears for a short time, the change is transient, and then the interactions are contextual to the agent.

My notion of the constitutive role of interactions demonstrates the interdependence between individuals through their interactions that change or shape their characteristics (e.g., the bacterium gene expression). As such, we can see that quorum sensing between cells in a colony constitutes the characteristics and functions of the bacterium cells through gene expression and repression. The interactions constitute the individual when they are a sustained network of exchange that shapes the individual's traits. Thus, the interactions are essential to the understanding of the bacteria's properties and functions, but for this understanding, we also need to investigate the background conditions further. I elaborate in the next sub-section on the role of the background conditions as the relational domain (e.g., competition, collaboration) and the environmental niche (e.g., molecular composition, substrate, and topography) where the interactions occur.

### *3.3 The reciprocity between interactions and environmental conditions*

The role of the interactions as constitutive or contextual is conditioned by the environment and involves the environment. Bacterial interactions are organized in a sustaining network of processes under precarious conditions (nutrition, space topography, flushing, composition, and density). These conditions are the domain in which the interactions occur, therefore influencing

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[8] Note that the gene expression for the light function changes when the iterations and intensity of the interaction changes. That is, each time, the interactions are the cause of the change. The change is transient in the sense of the reoccurring dynamics of the interactions and not because it fades.

them. So long as the conditions are stable, and the bacterial composition remains, the interactions are organized in a sustaining network of processes. Once the conditions or the bacterial composition changes the network of the interactions changes as well, promoting changes in the bacterial function. Returning to the example of quorum sensing in *V. fischeri*, the interactions change the bacterial function, depending on the molecular composition in the environment. The molecules that cause the change in the bacterial gene expression are called autoinducers (AIs).

These molecules released by the bacteria are present in their immediate environment. In the light organ, the bacteria are in high density, and so are the AIs' molecules, which promote the gene expression for luminescing. When the bacteria are released back into the sand, their density is low, and so is the density of the AIs' molecules. The low composition of AIs' molecules changes the gene expression again, and the bacteria lose their luminescence. Each individual cell interacts with its close environment and changes it by releasing and sensing the AIs. In low density, the molecules sensed are not sufficient to induce changes back to the cell; however, in high density, the AIs levels rise, and their high presence sensed by the cell promotes the expression of the genes for luminescing.

The interactions are embedded within their environments because the mutual exchange occurs through environmental modification of molecular density that activates or deactivates specific genes in the individual cell. Thus, in the examples discussed above, the mutual exchange of molecules between the bacterial cells happens through the environment. Furthermore, the effect of the exchange on the bacterium depends on the molecular composition in the environment. The exchange of molecules in quorum sensing are interactions that do not involve a necessary physical intersection, as in the case of LGT or biofilms. Not every casual interaction also involves a direct physical intersection between the agents.

For example, two bacterial colonies exchange molecular signals and activate the release of antibiotics to the environment that inhibit their growth. They interact with each other through their environment by signaling to each other because of changes in their environmental conditions (Romero et al. 2011). The bacterial cells release molecules into the environment, which immediately changes it to signal other cells; the signal will be 'successful' if the accumulation of the molecules is significant. Quorum sensing happens through signals released from the cell and received by another cell. The combination of the cell-to-cell interactions through the environment results in small-scale environmental modifications that accumulate to influence the environment on a larger scale.

A good example of this is the modification of the environment in the gut or, on a larger scale, of lakes or the ocean (Konopka 2009).[9] Thus, the interactions are not only between cells in response to environmental pressures but also cause environmental modification. The bacteria interact with each other through the environment, which brings the element of bacterial communities as ecological communities with unique bacterial interactions and ecological interactions (ibid.). Thus, the mark on each interacting agent is stronger or weaker depending on several factors, such as who are the agents, and what is the domain or the structure of their relations (i.e., the structure of the colonies, the topography, and conditions of their environmental niche).

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[9] Allan Konopka (2009) explains how the notion of the ecological community in bacteria is different because of two important aspects. First, the meaning of bacterial interactions is by the consumption of substrate from the environment and the emission of metabolic products to their environment, thereby creating small-scale environmental changes. However, these changes in microns accumulate to meters in density stabilized marine water. The second is the bacterial transference of genetic material, which brings in the element of metagenomics and suggests a unique property to bacterial communities, which is the community metagenome.

Interacting with the environment or through environmental modification implies the role of the environment as a middle, interacting agent. The intersection happens between the bacterial cell and another neighboring cell and between the bacterial cell and the substrate it is living on. Consequently, on the one hand, a substance containing bacterial colonies intersects with the colonies, thus going through changes by the bacteria, which are primarily affected by the metabolic pathways of the bacteria living on it, and on the other hand, the bacterial colonies go through changes affected by the molecular composition of the substance.

The interaction between bacterial cells through quorum sensing involves a direct interaction of each cell with its surrounding environment. Thus, there are different kinds of interacting agents that can be divided into biotic interacting agents (i.e., organisms) that interact with each other, sometimes intersecting directly and sometimes interacting through environmental modification. Interactions through environmental modifications between two or more organisms mean that each of them is also interacting with the substances in its close environment. This view shows the importance of the environmental conditions, not only in the establishment of the relational dynamics between the interacting agents but also as the abiotic component of the environment directly interacting with the agents. Therefore, in thinking about the interactions as constituting the agents' properties or functions, we need to consider also the environmental conditions.

Interactions between bacterial cells shape the nature of the bacteria if they are a part of a systematic network that is sustained and maintained in environmental conditions. The interactions constitute the nature and characteristics of these cells, depending on a certain quorum. Also, the type of characteristic (i.e., promoting a function or repressing it) depends on both the density and the environmental conditions in their niche, such as the example with *V. fischeri* and the bioluminescence. Significant changes in the environment or the interacting agents (i.e., bacterial density and composition) can affect the systematic network of interactions and thus change the nature and characteristics of the individual cell. Thus, the number of cells, the environmental conditions (topography, acidity, fluids, temperature, and other environmental molecules), and the diversification (i.e., crosstalk quorum sensing between strains and species) will determine whether the interactions constitute or are contextual to the bacterial cell.

## 4 Are Interactions a Better and More Useful Way of Thinking about the Holobiont?

The holobiont is a heterogeneous entity connected to its environment and is composed of interactions with microbes from the environment. The heterogeneity of the interactions composing the holobiont means that the interactions are dynamic and can change as well as the relations between the host and its diverse community of microbes (Bordenstein and Theis 2015). In this complexity, in most cases, the borders between what is the holobiont and what is its environment are fuzzy and might be of less importance than the characterization of the interactions in the different layers of the holobiont.[10]

For example, the *E. Scolopes* (the lightening squid) adapts to its habitat by changing its morphology through interactions with *V. fischeri*. The *V. fischeri* is clearly not an obligatory symbiont and lives partly in the sand and partly in the squid, depending on the sun or other

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[10] The layers of the holobiont are not a synonym for levels. Instead, they refer to the layers within the web of interactions that continues from the inside to the outside or from the outside to the inside.

environmental illumination (Rudy and Lee 1998).[11] Also, the bacteria *V. fischeri* go through morphologically and functional alterations, such as the loss of their flagella and motility, which is needed in the initial colonization but not needed later in the light organ (Lupp and Ruby, 2005). The flagella release virulence molecules that activate the squid's immune response and induce apoptosis and participate in the morphogenesis of the light organ (Koropatnick et al. 2004). Thus, without the bacterial molecular interactions of quorum sensing to regulate their virulence through flagellation, the development of the light organ and the initial colonization will not occur (Wolfe et al. 2004). All these types of interactions constitute their interacting agents, meaning they are causal interactions that leave a mark through an exchange of molecules as well as a direct intersection.

In thinking about the holobiont and its properties through interactions, the question we need to ask is: What is the nature of the mutual exchange, and do the interactions determine (constitute) the individual trait or are they background influence (contextual)? This line of questioning changes the framework from that of looking at the interactions as markers of degrees of cohesion and boundaries to a wider framework of questions concerning the web of mutual interactions that include the background conditions. The latter, I argue, is better because it enables an inquiry into a variety of interactions similar in method to that of an ecological community and ecosystem.

Pradeu offers the perspective of the physiological individual following the immune system's patterns of response as the boundary of the immunological entity or the immune-self. Here the physiological individual, composed of interactions between the host and microbes, is not considered an ecological community. In this view, there is a clear distinction between microbes belonging to the individual and the microbial communities that do not. However, such a distinction does not fit the interchangeable nature of the host-microbiome relations. Thinking about the holobiont through its interactions emphasizes the importance of its bacterial environment as well as the dynamics between its different close, distant, obligatory, and temporal constituents.

This framework emphasizes the interdependence between the interacting agents and the role of the background conditions. Thus, such an examination portrays the holobiont as a physiological individual that is also an ecological community (i.e., the microbiomes entangled with the physiological systems of a host). Such conceptualization better addresses the holobiont because the holobiont does not fit neatly into either of these definitions. Firstly, the holobiont constructs around a host organism and therefore is not a 'typical' ecological community, such as the soil microbiome. Secondly, the physiological systems in the host organisms involve different microbial communities (microbiomes), which should be studied as ecological communities (Mushegian and Ebert 2016; Skillings 2016). Thus, looking at the holobiont as an individual that is also an ecological community addresses the discrepancy of a host that is a part of an ecological community but also provides the environmental niche for these microbial communities.

Additionally, my analysis of the interactions has implications on the microbiome's definitions or characterization. In most microbiome studies today, the characterization is mainly by taxonomic composition (Lynch et al., forthcoming). The interactionist approach looks at the bacterial properties as determined by their activity and interactions (i.e., on a continuum between constitutive and contextual interactions). This emphasis is different from the view that regards the organisms' properties and characteristics as only affecting the interactions but not shaped or developed by them. Thus, in the latter view, the microbial taxonomic composition holds the potential for the microbial properties, while my view adds the factor of the interac-

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[11] At the end of the night, after sunrise, the light organ expulses 90% of the bacteria back into the sea. By the end of the day, a new colony of *V. fischeri* has grown in the light organ and is ready to illuminate the squid during its nighttime foraging activity (Rudy and Lee 1998).

tions and background conditions as the materialization of these properties. For example, in my analysis of interactions, the taxonomic composition of the microbiome is not sufficient in understanding the microbiome function in the holobiont without the examination of the microbial web of interactions and background conditions.

Finally, there is a conceptual advantage in looking at the holobiont as an individual and an ecological community, namely the placing of the holobiont as a boundary concept between disciplines in biology, such as immunology, microbiology, and ecology (Löwy 1992). This boundary concept can help clear up some of the issues by way of the possibility of their examination from different perspectives. For example, debates in the ecology of borders and part/whole relations of lakes or forests can be applied to the holobiont as an ecological community. Such an analogy can help clarify an alternative conceptualization for boundaries as well as the conceptualization of ecosystem health. Another example is the debate about invasive species in ecology that resemble the pathogenic/non-pathogenies properties. When we think about the holobiont in ecological terms we can borrow the terminology and debates from ecology to re-examine those concepts and metaphors related to organisms and the body. This is the unique and novelty in thinking of the holobiont as a boundary concept between physiology and ecology.

## 5 Summary: Thinking about the Holobiont and its Properties through Interactions

In this paper, I have suggested an alternative way of thinking about the holobiont, which is not through the question of whether the holobiont is a biological individual. By accepting both positions of the holobiont—as a biological individual (i.e., physiological individual) composed of individuals, which is also an ecological community—I offered a framework for looking at the holobiont through its interactions. In my analysis of interactions, I suggested thinking about interactions and their role in constituting the agent's nature and characteristic that is taken from social and cognitive studies. To demonstrate this way of thinking in regard to the holobiont, I used bacterial interactions called quorum sensing. Then, by using Salmon's concepts of causal interactions, I showed that the role of interactions as constitutive of the agents and contextual to the agent is on a continuum depending on their systemic iteration, background conditions, and the persistence of the change in each agent. From this perspective and inquiry, I have argued, the interacting entity is defined/materialized by its interactions and environmental conditions and in its actions and interactions modifies its environment.

Understanding the holobiont through its microbial interactions leads to the understanding that its properties are defined by its mutual interactions in the environmental niche. The important conclusion of my argument is the portrait of the holobiont as a biological individual that is also an ecological community composed of layers of different interactions. I accept the argument for the view that the holobiont is a biological individual and give a conceptualization of what it means to look at the holobiont *also* as an ecological community. The bacterial interactions are what determine their properties and functions (biofilm, virulence, luminescence, and more). Because these interactions determine the microbial properties they affect the hosts' biological systems and their development, such as the immune system and the digestive system. Furthermore, in a global view of the holobiont, the interactions between holobionts change their biological nature through the exchange of symbionts, such as infections, hygiene, vaccination, and the production of antibiotics resulting in the antibiotic resistance crisis. Thus, it is not the separation and distinction of the inside from the outside that defines the holobiont; instead, it is the connection and mutuality of the interactions of its parts.

Thinking about the holobiont through interactions allows the understanding that the holobiont is defined/constituted/materializes by its interactions and background conditions. Thus, there are two ways in which the holobiont is determined by interactions, one as a community of microbes and host, and the other its constitutive interaction as a whole. The constitutive interactions and their nature are conditioned by the environment and the different positioning and relations between the interacting agents. Thus, to understand the agent's nature, we need to follow its interactions with other agents, their relations, and the environmental conditions affecting or shaping the relations. The individual, in that sense, becomes an ecological individual embedded in its environment, depending on its interactions with other individuals.

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