A Mixed Self: The Role of Symbiosis in Development

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Abstract:

Since the 1950s, the common view of development has been internalist: development is seen as the result of the unfolding of potentialities already present in the egg cell. In this paper I show that this view is incorrect, because of the crucial influence of the environment on development. I focus on a fascinating example, that of the role played by symbioses in development, especially bacterial symbioses, a phenomenon found in virtually all organisms (plants, invertebrates, vertebrates). I claim that we must consequently modify our conception of the boundaries of the developing entity, and I show how immunology can help us in accomplishing this task. I conclude that the developing entity encompasses many elements traditionally seen as "foreign", while I reject the idea that there is no possible distinction between the organism and its environment.

Keywords: development; symbiosis; organism; self; organogenesis; internalism; bacteria.

Since the 1950s, developmental biology has been dominated by an internalist perspective (Lewontin 2000; Oyama 2000; Gilbert 2002). According to this conception, the organism is merely the product of the successive divisions of the egg cell. In consequence, according to this view, only "self" cells (that is, those bearing the organism's genome) interact to induce developmental pathways, and, as a whole, constitute the organism. This common view offers an answer to the question of the boundaries of development raised in this issue of *Biological Theory*. From a spatial point of view, development is seen as the development of the endogenous organism, meaning that everything and only that which comes from the inside belongs to the developing entity. In other words, according to this view, the distinction between what is internal and what is external amounts to the distinction between the endogenous and the exogenous. From a temporal point of view, this conception holds that development lasts from fertilization to adulthood (that is, the reproductive capacity): development is finished when the potentialities contained in the egg cell have been unfolded, giving rise to the expected form of the organism.

Several biologists and philosophers of biology have offered a critique of this common view. They include developmental biologist Scott Gilbert (Gilbert 2001, 2002, 2005; Gilbert and Epel 2009; see also Gilbert, this volume), and the proponents of the "development systems theory", or DST (Oyama 2000; Oyama et al. 2001; Griffiths 2009; see also Pradeu 2010b), who reject both developmental internalism and

the idea that development is accomplished at an early period of life.

In this paper, I show that the common view must be abandoned because of recent advances demonstrating the role of the environment in development. I focus on a fascinating example, that of developmental symbioses. I show that symbioses, and particularly bacterial symbioses, are indispensable to normal development, and that this phenomenon is virtually ubiquitous. From these observations, I deduce that a new definition of the boundaries of development and a new conceptualization of what a biological individual is are necessary. Using recent results in immunology, I show that every organism is heterogeneous, that is, made of entities of different origins, but that it is possible nonetheless to establish what its spatial boundaries are.

The analysis offered in this paper backs up the "ecological developmental" perspective ("eco-devo") defended by Scott Gilbert (Gilbert 2001; Gilbert and Epel 2009), which insists on the decisive influence of the environment on development. At the same time, I hope to take the eco-devo perspective one step further, in clarifying the question of how to delineate the developing organism. In addition, my conception bears some similarities with the developmental systems perspective, but also some differences, which I will describe in detail.

I start with a very classic preliminary definition of *development*: development is the set of mechanisms that lead an organism from the egg cell to adulthood (itself defined as the reproductive capacity). Thus understood, development includes

key embryological stages, such as cleavage, gastrulation, cellular differentiation, and organogenesis. As my argument proceeds, it will become clear how I depart from this classic definition, and how this relates to the reconceptualization of the boundaries of development.

The acquisition of bacteria that play a role in development

This section demonstrates the necessity of symbioses, in particular bacterial symbioses, for normal development to be accomplished, and explores the different ways for the acquisition of these bacteria.

Yet, before showing the decisive role of bacterial symbioses in development, it is necessary to define "symbiosis". I take "symbiosis" to refer to any long-lasting interaction between two organisms of different species, this interaction being evolutionarily beneficial for one partner, and either beneficial or neutral for the other partner. In the first case, the symbiotic interaction can be called "mutualism", while in the second case it is sometimes called "commensalism". This definition is not accepted by every specialist of symbiosis. Some conceive of symbiosis as any long-lasting interaction between two organisms of different species (e.g. McFall-Ngai 2002). The advantage of this second definition is that it takes into account the fact that it is sometimes difficult to determine whether an interaction is evolutionarily beneficial, neutral, or detrimental, in particular because a given interaction may switch from one state to the other. The drawback of such a definition is that,

in my view, symbiosis is so frequent in nature that it ceases to be a useful, productive concept (for a conception of symbiosis similar to the one I express here, see, for example, Hooper and Gordon 2001).

With such a definition of symbiosis in mind, let us now examine how and when these symbiotic interactions are established. The person who has undoubtedly contributed the most to our knowledge about the role of symbiotic bacteria in development is Margaret McFall-Ngai (see McFall-Ngai 2002; McFall-Ngai, Henderson and Ruby 2005). Her work has proved crucial to the adoption of an ecological perspective in developmental biology, that is, a perspective that acknowledges the importance of environmental factors on development (Gilbert 2001, 2002, 2005; Gilbert and Epel 2009). Following Margaret McFall-Ngai (2002), we can distinguish two main modes of acquisition of symbiotic bacteria: one *vertical* (transovarian acquisition), the other *horizontal* (environmental acquisition).

Transovarian acquisition (vertical acquisition)

In the case of a transovarian acquisition, the symbiotic bacteria are transmitted by the mother, in or on the gamete. This is a vertical (parent-offspring) transmission. In several well-documented cases, the bacteria interact directly with the host's cells during embryogenesis and can have strong effects on development. Transovarian acquisition occurs mainly in invertebrates. In most (but not all) cases, the bacteria involved are intracellular.

The best-studied case of transovarian acquisition of a symbiotic bacterium involved in development is the *Wolbachia*-arthropods endosymbiosis, often described as a model for such an acquisition (O'Neill et al. 1997). The prevalence of this bacterial endosymbiosis is very high; for instance, it is estimated that 70% of insects possess intracellular *Wolbachia* bacteria (McFall-Ngai 2002).

The influence of the bacteria *Wolbachia* on the host lies at the intersection of reproduction and development. A striking demonstration showed that *Wolbachia* is indispensable to oogenesis in the parasitic wasp *Asobara tabida* (Dedeine et al. 2001); more recently, it was shown that one strain of *Wolbachia* is indispensable for the production of daughters in the wasp *Asobara japonica*: Kremer et al. 2009). More generally, the bacteria *Wolbachia* have an influence on sex determination, sex ratios, and the viability of gametes (O'Neill et al. 1997). A well-known case is that of cytoplasmic incompatibility, in which male arthropods infected with *Wolbachia* can reproduce only with females infected with *Wolbachia*, guaranteeing the efficient spreading of the bacteria (O'Neill et al. 1997).

In many cases of vertically acquired developmental symbioses, the symbiont protects the developing egg, which amounts to exerting an immune function before the complete maturation of the host's immune system. This phenomenon is particularly well documented in cases of aquatic hosts, including lobsters or shrimps. For example, a monospecific association with the bacterium *Alteromonas* was shown to be indispensable to the embryo of the shrimp

Palaemonmacrodactylus. The bacterium turns out to produce an antifongic substance, without which the host is rapidly killed by the fungus *Lagenidium calinectes*, a well-known pathogen of many crustaceans (Gil-Turnes et al. 1989). More generally, there is nowadays a growing interest in the phenomenon of symbiont-mediated protection against pathogens and its evolutionary consequences, in arthropods and elsewhere (Brownlie and Johnson 2009; Jaenike et al. 2010). Sometimes, the vertically acquired host-symbiont association is not monospecific: on the contrary it is an association between a host and a *consortium* of bacteria (for examples in squids or cuttlefishes).

Environmental acquisition (horizontal acquisition)

The second main mode of acquisition is environmental acquisition. In this case, bacteria come from the surrounding habitat, at each generation of the host, and therefore acquisition is said to be horizontal. The difference with the first mode is that the symbionts do not interact directly with the host in the very first steps of its embryogenesis. The symbionts are acquired after hatching or birth. Even so, it is important to note that, in many cases, the horizontally transmitted symbionts come from the parents. For example, newly hatched termite juveniles acquire symbiotic bacteria by being fed by the feces of their parents (Abe et al. 2000: 64).

The most frequent situation seems to be one in which consortia of extracellular bacteria colonize some epithelia of the host, for example the gut. Yet evidence exists for monospecific (as opposed to consortial) symbioses, as well as

for intracellular (as opposed to extracellular) symbionts. It is noteworthy that environmentally acquired symbioses are not necessarily less specific or evolutionarily more recent than transovarian symbioses.

Two models of environmental acquisition of developmentally important symbionts have been particularly well studied: the association between the squid *Emprymna scolopes* and the bacteria *Vibrio fischeri*, and the association between mammals and their numerous gut symbionts (McFall-Ngai 2002).

The Hawaiian bobtail squid *Emprymna scolopes* hunts at night; it emits light from an organ situated in the centre of the mantle cavity, mimicking the moonlight and thus hiding its shadow from potential predators. This light, which is crucial to the survival of the squid, results from the mutualistic association between the squid and the bacterium *Vibrio fischeri*. Strikingly, it appears that the squid can modulate the intensity and direction of the light, and that the "bacterial" light organ can even by itself perceive light! (Tong et al. 2009). For its part, the bacterium *Vibrio fischeri* receives carbon and nitrogen from the squid.

The way *E. scolopes* acquires the bacterium *Vibrio fischeri* is very interesting (Nyholm et al. 2000). The still immature light organ facilitates bacterial colonization through ciliary motion and mucus shedding. Then a selection process occurs, in which only *Vibrio fischeri* bacteria are retained. Subsequently, the bacteria *Vibrio fischeri* induce apoptosis in the host, leading to the elimination of the recruiting structure that made colonization possible in the

first place. Importantly, therefore, the development of the light organ starts endogenously, before any contact with bacteria, and with no known function other than promoting the host-symbiont interaction. Thus, it seems that an environmental pressure (the useful *Vibrio fischeri* bacteria) has, through evolution, favored the emergence of genes involved in the organogenesis of this specific light organ. This may be seen as an example of genetic assimilation (Waddington 1959) or phenotypic plasticity (West-Eberhard 2003). In any case, the association between the squid *E. scolopes* and the bacterium *Vibrio fischeri* is a remarkable example of *co-development* (McFall-Ngai and Ruby 1991). In the squid, the light organ is very immature before bacterial colonization: it accomplishes its development only after colonization by *Vibrio fischeri*, and because of this colonization. *Vibrio fischeri* bacteria furnish tracheal cytotoxin (TCT), which plays a decisive role in the squid's morphogenesis (Koropatnik et al 2004). Moreover, it was shown recently that the symbiotic *Vibrio fischeri* bacteria are actively tolerated by the squid's immune system, meaning that the squid's hemocytes (phagocytic immune cells) have a specific low-level reactivity towards *Vibrio fischeri* (Nyholm et al 2009; McFall-Ngai et al. 2010). Thus, a crucial aspect of the squid's organogenesis is realized only thanks to the presence of specific bacteria. Reciprocally, *Vibrio fischeri* has the capacity of bioluminescence only after it is established within the light organ of the squid. It is only when the bacteria are established there that they induce

transcription of their lux genes, which are the genes responsible for bioluminescence.

The second model of environmental acquisition of developmentally crucial symbiotic bacteria concerns the association between mammals and their gut bacteria. Before analyzing the role of these bacteria in mammal hosts' development, let us say a few words more generally about the importance of symbiotic bacteria for the normal functioning of the organism. In humans, for instance, 90% of the body's cells are bacterial cells, and 98 to 99% of the genes are bacterial genes (Turnbaugh et al. 2007). It is estimated that 10^{14} bacteria live in our gut, with up to 10^{12} microorganisms packed together per milliliter or gram of luminal contents, and more than 1000 species represented (Lee and Mazmanian 2010). Only 7% of our gut bacteria have been successfully cultured in the lab, indicating that most of them cannot survive outside their host. These bacteria, in turn, play critical functional roles in the host, in particular, concerning digestion and immune defense against pathogens (Xu and Gordon 2003). Though the case of mammals is especially well-studied, for obvious health-related reasons, host-gut microbiota associations are also found in non-mammal vertebrates and in many invertebrates, including arthropods (Ryu et al. 2010), and therefore constitute an excellent and widespread example of mutualism. More generally, it is now clear that microbial symbionts playing crucial physiological roles are found in virtually all plants and animals, both invertebrates and vertebrates, and that these associations may have important evolutionary consequences (Zilber-Rosenberg and Rosenberg

2008; Bright and Bulgheresi 2010).

What is the role of symbiotic gut bacteria in the host's development? These bacteria — acquired at birth from the mother in many animals — are often essential to the normal development of the gut itself. The development of the gut is a clear example of a postnatal organogenesis. In the 1990s, studies on germ-free mouse models had shown that the gut of these mice could initiate, but not complete, its differentiation (Bry et al 1996). These first studies then led to a revolution in the understanding of mammal host-symbionts interactions, a revolution originating in the beginning of the 2000s (McFall-Ngai 2002). In a landmark paper published in *Science* in 2001, Hooper and Gordon showed that *Bacteroides thetaiotaomicron*, a prominent bacterial component of normal mouse intestinal microflora, modulates expression of host genes involved in key processes such as the maturation of the intestine, angiogenesis (formation of blood vessels), nutrient absorption and mucosal immunity (Hooper et al. 2001; see also the viewpoint of Hooper and Gordon 2001). In 2002, it was confirmed that *B. thetaiotaomicron* is involved in postnatal organogenesis in mice, more precisely in the developmental regulation of intestinal angiogenesis via the gut's Paneth cells (Stappenbeck et al. 2002). These founding results revealed a previously unappreciated symbiont-dependent mechanism of postnatal development. It is now clear that the presence of some symbiotic bacteria is indispensable for the normal development of the mouse's gut after birth.

Germ-free mice possess an abnormal cellular composition in the form of secondary lymphoid organs, an altered

metabolism, a modified serological composition, and changes in their cardiovascular physiology and neurophysiology (Smith, McCoy and Macpherson 2007). The role of symbiotic bacteria in the development of the immune system is of particular importance. Germ-free mice show defects in the development of their gut-associated lymphoid tissue or "GALT" (the tissue of the digestive tract, playing a major immune role), in antibody production, and they have fewer and smaller Peyer's patches and mesenteric lymph nodes (Round and Mazmanian 2009). During colonization of animal hosts with the ubiquitous gut microorganism *Bacteroides fragilis*, a bacterial polysaccharide (PSA) directs the maturation of the developing immune system, in particular by insuring a normal balance between helper 1 and helper 2 T cells and directing lymphoid organogenesis (Mazmanian et al. 2005). Symbiotic bacteria are also important for the normal development of immune B cells (Lanning et al. 2005). Moreover, symbiotic bacteria are essential to the homeostasis of the gut. In collaboration with the local immune system, they limit the expansion of other bacteria, and prevent inflammation (Mazmanian et al. 2008; Garrett et al. 2010). As Mazmanian and coauthors put it: "the host not only tolerates but has evolved to require colonization by commensal microorganisms for its own development and health." (Mazmanian et al. 2005; see also the general review by Hill and Arthis 2010). In addition, according to impressive data, microbiota could also regulate the development and metabolism of the liver (Bjorkholm et al. 2009), as well as brain development and behavior (Heijtz et al. 2011).

In humans as well, symbiotic bacteria play indispensable

roles, in particular in digestion and immunity, but also in development (Wilks 2007). Contrary to the long-lasting hypothesis that gut bacteria were invisible to the local immune system (a phenomenon sometimes associated with the concept of "immune ignorance"), there is now little doubt that this symbiosis actually is the result of a complex, highly regulated dialogue between the bacteria and the host (Garrett et al. 2010). Arguably, symbiotic bacteria are indispensable to the normal human development after birth, in particular for the development of the gut-associated lymphoid tissue (GALT) and for the development of a functional immune system (Hooper 2004; Turnbaugh et al. 2007; Eberl 2010). The interest in the physiological roles played by symbiotic microorganisms in the human body has led to the "human microbiome project", which aims at offering a detailed analysis of the interplay between the human host and the microscopic world that each of us harbors (Turnbaugh et al. 2007).

In plants as well, symbionts are crucial for development. Important examples include rhizobium-legume symbioses (Stougaerd 2000), mycorrhizae (an extremely frequent symbiotic association between the plant's roots and fungi) (Pivato et al. 2009), and endophytes (an almost ubiquitous situation in which symbiotic fungi live inside the tissues of plants) (Hardoim et al. 2008). In particular, an extraordinary example of horizontally acquired symbiont-mediated organogenesis is found in leguminous plants (Kereszt et al. 2011). In these plants, nitrogen fixation is insured thanks to nodules, the organogenesis of which results from the symbiotic association with soil bacteria of the *Rhizobiaceae* family (Crespi and

Frugier 2008; Kondorosi and Kondorosi 2004). It was recently demonstrated that antimicrobial peptides (AMPs) are actually used, in this case, to promote a beneficial irreversible terminal differentiation (Van de Velde et al. 2010; Wang et al. 2010; Kereszt et al. 2011), which suggests that the immune system can resort to usually destructive mechanisms to facilitate a developmentally indispensable symbiotic association.

Taken all together, these data about the two main modes of acquisition, that is, the transovarian and the environmental (and, of course, the series of intermediates between these two extremes in a spectrum), drive to the conclusion that developmental symbioses appear to be the rule in nature, not the exception. In virtually every organism where they have been investigated, symbiotic bacteria playing a crucial role for the development of the host have been found (mammals, arthropods, crustaceans, amphibians, virtually all plants, etc.; see, for instance, the interesting example of the earthworm: Davidson and Stahl 2008).

I now turn to the second part of this paper: what do these recent data about the intricate relationship between the developing host and its symbionts tell us about the spatial and temporal boundaries of the developing entity?

Establishing the boundaries of the developing entity

The data analyzed in the first section of this paper amply demonstrate that “all development is co-development” (Gilbert 2002). Developmental internalism, as it has been defended for

decades, is wrong: it is simply not true that the organism is the set of constituents originating from the egg cell. Instead, every organism is the genetically heterogeneous product of endogenous and exogenous constituents. Gut microbiota, for instance, is part of my body, and even an indispensable part of it. To resort to the appealing and widely used language of the "self", one can say that every self is a mixed self from its inception, that is, as early as the developmental period, and sometimes, as we have seen, as early as the very first stages of development (Turnbaugh et al. 2007; Eberl 2010). The organism harbors on all its surfaces (gut, skin, lungs, sexual organs, etc.) huge numbers of symbiotic microorganisms, with which it interacts dynamically, as these microorganisms may change during the lifetime of the organism.

How are these symbionts tolerated by the host? In accordance with the self-nonself theory (Burnet 1969), the immune system is usually said to discriminate between self and nonself, and consequently to reject any genetically foreign entity. Yet, to develop normally, the organism actively tolerates (and *must* tolerate) many foreign entities. Therefore, immunoregulation towards symbiotic bacteria (that is, downregulation of a potentially destructive response against these bacteria after a specific interaction) is fundamental, in vertebrates as well as in invertebrates. In *Drosophila*, for example, immunoregulation to symbiotic bacteria has proved indispensable to development (Bischoff et al. 2006), and the same is true in mammals (Mazmanian et al. 2005) and plants (Kereszt et al. 2011). This massive tolerance

of foreign but indispensable bacteria shows that the immunological self-nonself theory is inadequate (Pradeu 2009).

Thus, every organism encompasses microorganisms that are crucial for its development. This view confirms the "ecological developmental" perspective ("eco-devo") defended by Scott Gilbert (Gilbert 2001; Gilbert and Epel 2009), but I think it offers a more precise delineation of the developing organism, as will be clear in what follows. This view also corroborates the developmental systems theory (DST) in that it rejects the conception of the organism as a homogeneous and endogenously defined entity. However, I do not agree either with the alternative conception suggested by at least some versions of the developmental systems theory (e.g. Griffiths and Gray 2001: 207). According to this alternative conception, what develops is, strictly speaking, a developmental system or "DS", which can be defined as the broad association of an organism and its environment. "Developmental systems" are described as close organism-environment associations, or "Œ", in which it is impossible to dissociate the organism from its environment (Pradeu 2010b). In contrast, I suggest that what is needed is a new conceptualization of the organism's boundaries – a claim which clearly does not amount to saying that there is no actual distinction between the organism and its environment.

In my view, the immune system, via its tolerance/rejection activity, is still critical to delineate the organism, but the organism is a heterogeneous entity, made of both endogenous ("self") and exogenous ("foreign") constituents. In other words, the immune system defines a

boundary between the "inside" and the "outside" of the organism, but this boundary is not equivalent to the boundary between the endogenous (that which comes from the inside) and the exogenous (that which comes from the outside) (Pradeu 2010a). In this sense, the phenomenon of immunoregulation highlighted here points to an original solution to the problem of the spatial boundaries of the developing entity, distinct from both the traditional view and developmental systems theory. In addition, it hopefully takes the ecological developmental perspective one step further, by showing that the assertion that the environment influences the organism's development needs to be complemented by a new conceptualization of what the developing organism is, some entities usually seen as "environmental" (microorganisms, in this case) being in fact true *constituents* of the organism itself (O'Hara and Shanahan 2006).

The view defended here emphasizes that the key question is: among many foreign entities, how does the immune system discriminate between those that are useful, or even indispensable, and those that are potentially harmful? What are the biochemical mechanisms of this active, specific discrimination? I suggest the immune system does not respond to nonself, but to the appearance of *unusual* molecular patterns in the organism. When immune receptors interact specifically with molecular patterns that are strongly different from the ones with which they usually interact (be they endogenous or exogenous), an immune response is triggered (Pradeu and Carosella 2006a, 2006b). This makes it possible to explain the phagocytosis of dead cells, the activation of

regulatory T cells, or the immune response triggered against tumor cells, which are genetically "self" cells. Accordingly, in this view, exogenous entities that penetrate the organism progressively and in small quantities may induce a tolerogenic state, and not a rejection response (for a detailed explanation, see Pradeu 2009; Pradeu and Carosella 2006b). This leads to the idea of an extension of the immune tolerance period: within the self-nonself framework, and following the work of Medawar and colleagues (Billingham et al. 1953), it has long been thought that the immune system can tolerate foreign entities for a short early period corresponding to a state defined as "immature", usually the fetal or the immediately postnatal period, and then will reject every foreign entity (Burnet 1969). If the view defended here is correct, then the immune tolerance can occur throughout the life of the organism, though the degree of immune tolerance is probably higher in early periods than in later ones.

On this basis, before concluding I would like to suggest a daring, still to be proven hypothesis. This hypothesis states that the mechanisms that enable the tolerance of commensal and symbiotic bacteria are partly *developmental* in nature. These mechanisms may be considered "reactivations" of developmental constituents and processes. Evidence for this hypothesis can be found in *Drosophila*, where the homeobox gene Caudal (that is, a gene regulating development, and more precisely morphogenetic patterns) plays a critical role in maintaining the gut-bacteria homeostasis in the adult (Ryu et al. 2008). Moreover, key components of the "Toll" pathway are involved both in development and in immune responses, effector

responses as well as immunoregulatory responses (Lemaître and Hoffmann 2007). In mammals, the formation of isolated lymphoid follicles (ILF, which are organized clusters of naïve lymphocytes in the lamina propria of the intestine) is induced by intestinal commensal flora after birth. In the adult, tissue genesis is symbiont-mediated (Eberl 2007). Several researchers suggest that homeostasis between the intestinal immune system and bacterial flora is ensured through development-like mechanisms, that is, mechanisms reminiscent of those used during fetal development (e.g. LTα, LTβR, members of the tumor necrosis factor family, RORγt) (Bouskra et al. 2008; Eberl and Lochner 2009), and it has been suggested that the formation of inducible lymphoid tissues should be seen as a "recapitulation of a fetal pathway" (Eberl 2005; see Figure 1). Thus, resident bacteria may be tolerated in part because they induce some particular developmental-like mechanisms, giving rise to the idea of a co-organogenesis that lasts throughout life.

In any case, the view defended here – which insists upon the possibility and even the necessity for any organism to constantly integrate foreign entities – strongly argues in favor of the study of the ontogeny of the immune system, of the gut immune system and of the acquisition of microbionts in early development (Palmer et al. 2007). It also provides an argument for those who claim the necessity of articulating immunology, ecology and developmental biology (Schulenberg et al. 2009; Pradeu and Alizon, in preparation), though many immunologists are probably not yet fully ready for this articulation. I suggest that the immune system, if no longer

understood as that which fights every foreign entity, helps instead to establish the partially open, highly regulated, spatial boundaries of the organism.

The conception defended here has consequences for the problem of establishing the *temporal* boundaries of development as well. The question of whether development lasts throughout life (as claimed, in particular, by Oyama 2000; Oyama et al. 2001; Griffiths 2009; West-Eberhard 2003; Gilbert 2010) or not naturally depends on the definition of "development" one adopts. I believe that the word "development" is too broad and equivocal to make a precise answer to the problem of the temporal boundaries of development possible. It may be useful to dissolve the concept of development, and to replace it with a series of mechanisms that are characteristic of the construction of the embryo, or embryogenesis. These mechanisms include, in particular, cleavage, gastrulation, cellular differentiation, and organogenesis (e.g. Love 2008). Therefore, I suggest reframing the question of the temporal boundaries of development by asking: *when* do processes commonly seen as typical of "embryogenesis" occur? Are they all limited to the embryonic period *per se*? Or do some of them reoccur later in life, or even occur throughout the lifetime of the organism? The above showed clearly that organogenesis can occur at the embryonic, the postnatal and the adult stages, in the context of intimate interactions with symbionts. Therefore, not only does the view defended here demonstrate the importance of symbionts-dependent development, but it also prompts us to suggest that organogenesis lasts throughout life, though in a much narrower way during adult

life than during embryonic life. In other words, there exists a quantitative difference in organogenesis between early life and adult life, but true manifestations of organogenesis can occur in adulthood in many different organisms.

Conclusion

In this paper, I have argued in favor of an extension of the classical conception of boundaries of development. *Spatial* boundaries of development are redefined in so far as symbiotic microorganisms constitute a real organ (a "part") of the developing organism (O'Hara and Shanahan 2006). The *temporal* boundaries of development are redefined in so far as organogenesis, usually seen as one of the most fundamental aspects of embryogenesis, can occur throughout the organism's life, on the basis of intimate interactions with symbiotic microorganisms. If the view defended here is correct, then the idea of developmental autonomy is a myth, for development is always co-development, that is, it results from the co-construction of living things belonging to distinct species. Every organism is "mixed" and heterogeneous, and not homogenous or "pure". A well-understood convergence of today's microbiology, immunology, ecology and developmental biology leads us to better understand the organism as the unity of such a plurality.

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Figure caption:

Figure 1: Development of isolated lymphoid follicles (ILFs)

Gram-negative commensal intestinal bacteria induce the production of CCL20 and β-defensin 3 through the NOD1 pathway. CCL20 and β-defensin 3 bind to the receptor CCR6 borne by lymphoid tissue inducer (LTi) cells in cryptopatches. When activated, cryptopatches recruit CCR6+ B cells, which accumulate and form immature isolated lymphoid follicles. Tumor-necrosis factor α (TNFα), produced by dendritic cells and macrophages, facilitates the transformation into mature isolated lymphoid follicles. These mature ILFs then generate IgA-producing B cells, inhibiting the commensal bacteria. This is a negative feedback loop: bacteria stimulate the production of ILFs, which in turn inhibit bacteria. The development of isolated lymphoid follicles offers an example of organogenesis occurring during adult life. (Adapted from Eberl and Lochner 2009).