Opinion

CellPress

Microbial priming of plant and animal immunity: symbionts as developmental signals

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The functional similarity between root and gut microbiota, both contributing to the nutrition and protection of the host, is often overlooked. A central mechanism for efficient protection against pathogens is defense priming, the preconditioning of immunity induced by microbial colonization after germination or birth. Microbiota have been recruited several times in evolution as developmental signals for immunity maturation. Because there is no evidence that microbial signals are more relevant than endogenous ones, we propose a neutral scenario for the evolution of this dependency: any hypothetic endogenous signal can be lost because microbial colonization, reliably occurring at germination or birth, can substitute for it, and without either positive selection or the acquisition of new functions. Dependency of development on symbiotic signals can thus evolve by contingent irreversibility.

Microbiota as a signal for immunity maturation

In Europe, the arrival of swallows and storks, returning from Africa, announces spring to everyone. They give the tempo of the 'martenitsa' tradition in Bulgaria, a celebration of the spring where people exchange white and red tassels, called martenitsi, in early March. Martenitsi are then pinned on clothes until one sees a stork or a swallow, marking the return of spring. The martenitsa is then suspended on a tree (Figure 1) as a gift to Nature's divinities, which are expected to make you safer and happier in the coming spring.

Biotic components can give information about the environment and often represent more integrative indicators of environmental conditions than punctual physicochemical measurements (e.g., daytime temperature or day-length as proxies for spring). For example, plants are commonly used as bioindicators to monitor the presence of pollutants in water. We show here how multicellular organisms similarly use their microbial symbionts (microbiota, see Glossary) in a martenitsa-like way as a signal to set the maturation of immunity and possibly other developmental

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Keywords: axeny; immune system; gut microbiota; MAMPs; mycorrhiza; neutral dependency; rhizosphere.

0966-842X/
 http://dx.doi.org/10.1016/j.tim.2014.07.003

Glossary

Antibiosis: a biological interaction where one organism releases metabolites that are detrimental to one or more other organisms.
Axenic: an environment or an organism that is devoid of microbiota, in other words that is microbiologically sterile.
Contingent irreversibility: an evolutionary mechanism proposed by Maynard Smith and Szathmáry [58] that forces previously independent units to become interdependent without evolution of new functions nor progress, simply by mutational drift (Figure 3). This neutral mechanism, not requiring any positive selection, is often irreversible (ratchet mechanism) because it is unlikely that reversions will restore the previous independence.
Cytokines: small secreted proteins important for cell–cell signaling in animals at low concentrations. They were initially identified in immunity, in which they shape the immune response.
Endophytes: organisms that diffusely grow within living plant tissues, without apparent symptoms of infection.
Immunity: the ability of the organism to resist unwanted, harmful microbes from entering and developing within its tissues. The immune system is the sum of the biological structures and physiological mechanisms taking part in this process.
Induced systemic resistance (ISR): broad-spectrum primed defensive capacity manifested throughout the whole plant, acquired upon local induction by beneficial microorganisms.
Jasmonic acid (JA): plant hormone, structurally similar to animal prostaglandins, with key roles in regulating plant immune responses.
Lymphocyte: white blood cell participating in the vertebrate immune system. The diverse functional types include natural killer cells involved in innate immunity, and cells involved in adaptive immunity such as T cells (that mature in the thymus) and B cells (that produce antibodies). Some T cells (such as regulatory T cells and natural killer T cells) secrete cytokines.
Microbe-associated molecular patterns (MAMPs): conserved microbe-specific molecules such as lipopolysaccharides, peptidoglycans, flagellin, or chitin. Sometimes referred to as pathogen-associated molecular patterns (PAMPs), they are recognized by the innate immune system of animals and plants, but are also developmental signals in plants and animals.
Microbiota (or microbiome): community of microorganisms that live in a specific ecosystem, here mostly referred to the community in close association with a host plant or animal.
Mycorrhiza: symbiotic association between a soil fungus and a plant root, often mutualistic, in which plant photosynthates are exchanged for mineral resources acquired by the fungus from the soil.
Priming: the propensity of a cell, organ, or an organism to react more efficiently to environmental stresses upon appropriate prior stimulation. We focus here mainly on the priming of defenses against biotic stresses, generally of systemic character (see ISR).
Rhizosphere: the portion of soil that surrounds the root and is modified by it. It differs from the bulk soil, especially by its high and differentiated microbial diversity.

Figure 1. Microbial martenitsa. The Bulgarian pagan martenitsa tradition uses the arrival of swallows and storks, migrating back from Africa, as a proxy for the beginning of spring. People exchange white and red tassels, the so-called martenitsi, and wear them pinned on their clothes. When one sees a stork or a swallow, one must hang the martenitsa on a blossoming tree, as seen on this picture. This gift to nature's divinities is supposed to bring luck and success during the next spring. We suggest that an analogous biological mechanism occurs when microbial colonization primes immunity after germination or birth: microbes are proxies for the relevant time of maturation of the immune system, bringing successful defenses against pathogens. This signal allows timely protection of the host, representing a type of microbial martenitsa (photography courtesy of Jilly Bennett).

processes. We first draw a parallel between the priming of plant immunity induced by microbiota surrounding the root and the triggering by gut microbiota of postnatal development of the animal immune system. We then propose an evolutionary framework for the recruitment of symbionts as developmental inducers, and for the use of inter-kingdom rather than endogenous signals for development.

Rhizosphere microbiota warning plants

The soil surrounding roots, termed the rhizosphere, is enriched in dead cells and root secretions, and harbors diverse bacterial and fungal taxa [1]. Rhizosphere microbiota differs from the bulk soil community, and a subset of these microorganisms even enter the root and live as endophytes [2]. This rhizosphere microbiota is extremely dense and diverse, with $>10^{10}$ microbial cells per gram and $>10^{6}$ taxa [3]. These microbes can be pathogenic or commensal, but most are mutualistic, paying back the host root with nutrients or protection [4,5]. Among other rhizosphere mutualists, mycorrhizal fungi, which form a dual organ associating fungal hyphae and root tissues, are perhaps the best-studied example and have coevolved with land plants since their origin [6]. They provide mineral resources, collected by their soil mycelia, to the root, and receive photosynthates as a reward. Rhizospheric and root endophytic microbes, including mycorrhizal fungi, also protect roots against soil pathogens by competition for space and food, direct antibiosis, and most importantly by inducing plant defense mechanisms that are effective against pathogens [7] (Figure 2).

Mycorrhizal fungi must deal with the plant immune system to colonize the root successfully. A molecular dialog between the symbionts modulates host defenses and triggers a symbiotic program for mycorrhizal development. This modulation acts in two ways. On the one hand, it enhances local tolerance to the mycorrhizal fungus. For example, small secreted fungal peptides injected into root cells [8,9] block specific regulators of plant defense signaling locally, resulting in a partial local desensitization that allows colonization. On the other hand, mycorrhizae also put other tissues or organs of the plant in a warned state, known as 'priming', which allows earlier and enhanced defense responses to pathogen attack compared to non-mycorrhizal (NM) plants [7]. During attack by soil pathogens, primed plants accumulate more pathogenesis-related proteins, callose, and phenolics compared to NM plants [10], and this early and strong reaction is pivotal for successful defense [11]. Priming also spreads systemically in distant parts of the root system and shoots, conferring induced systemic resistance (ISR). Primed plants are thus more efficiently protected than NM plants against foliar pests such as fungal parasites and insect herbivores [10,12].

Other rhizosphere microbes prime local resistance and ISR as well [13]. Plant immunity relies on the recognition of general features of microbial pathogens: the so-called microbe-associated molecular patterns (MAMPs) which include lipopolysaccharides, peptidoglycans, flagellin, and chitin. In addition, damage to host tissues during colonization by pathogens releases damage-associated molecular patterns (DAMPs) that are recognized in plant immunity [14]. MAMPs and DAMPS activate signaling cascades orchestrated by phytohormones such as salicylic acid, ethylene, and jasmonic acid (JA), three major regulators of inducible plant defenses [15]. Beneficial microorganisms also possess MAMPs that trigger immune responses and also may result in the priming of defenses [16]. Several rhizosphere bacteria have been described to induce ISR by way of MAMPs [17], including isolates from diverse bacteria such as *Pseudomonas* and *Bacillus* spp. [17,18]. ISR is also reported for endophytic fungi that colonize root tissues diffusely such as *Trichoderma* spp. [19], sebacinales, and non-pathogenic *Fusarium* strains [20]. Beyond elicitors, the molecular mechanisms involved in priming remain poorly understood, but may include elevated levels of key regulatory proteins such as mitogen-activated protein kinases, transcription factors, and epigenetic modifications [11,21]. Precise molecular crosstalk among the diverse signaling pathways likely explains the apparent paradox between systemic priming of defenses and local desensitization that promotes the establishment of the beneficial microbiota [7,16]. Remarkably, ISR by

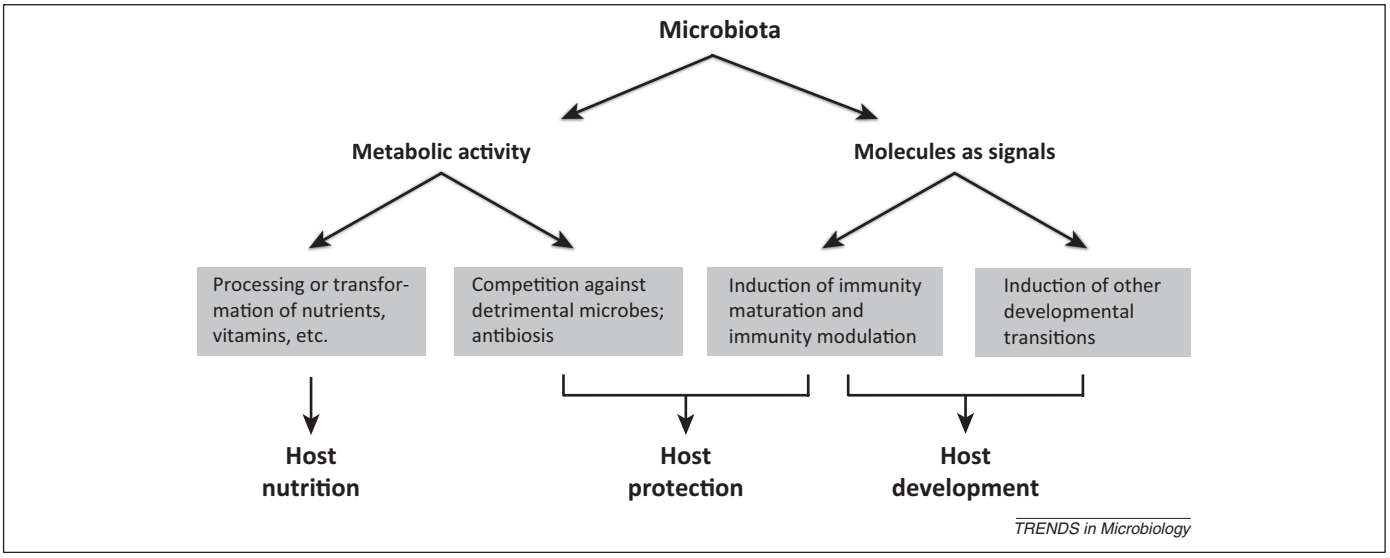

Figure 2. Microbial symbionts provide both their metabolic activity and their molecules as signals to hosts. Protection by microbes results from both pathways: microbial activity can actively contribute to protection by direct interaction with the pathogens, and microbial molecules can also be a signal that triggers host immunity maturation (among other developmental transitions).

diverse beneficial soil microorganisms frequently relies on common mechanisms, for example the priming of the JA signaling pathway [12,19]. The structure, metabolism, and function of JA are strikingly similar to those of animal prostaglandins which are potent immunomodulators of inflammation in animals [22].

Fine-tuning defenses through priming instead of a direct activation is a cost-efficient mechanism to improve resistance. Priming fitness benefits outweigh their costs under pathogen pressure [23]. Additionally, priming may increase tolerance to abiotic stresses [24]. Although the fitness costs of priming remain to be assessed, field-grown mycorrhizal plants show a higher number of fruits than NM plants, especially with increasing stress severity [25], and sometimes the quality of the offspring, rather than the quantity, is enhanced [26].

Gut microbiota warning animals

The animal gut is reminiscent of the rhizosphere [4] because this environment is also modified by the vicinity of the host, its secretions, dead cells, and leakage products. As in the rhizosphere, hyper-diverse microbiota colonize the gut which differ from environmental communities [27]: for example, the human gut harbors 10^{14} bacterial cells from >1000 species, representing 10 times our own cell number and 100 times our own gene number [28]. Although some components of the gut microbiota can be adverse, most are mutualists aiding in digestion and protecting the host against potential pathogens [29]. Similarly to rhizosphere microbiota, gut microbiota protect the host not only by competing for space and food with potential invaders, but also by shaping host defenses [30] (Figure 2). In vertebrates at least, both the innate and adaptive immune systems require microbial interactions during their postnatal development [30,31].

Germ-free (GF) mice were instrumental in discovering the complex role of gut microbiota in the development of the immune system. GF mice are axenically raised in a sterile environment, thus preventing skin and gut colonization by microbiota. Compared to normally colonized individuals, they display several immune defects [32]. Indeed, gut-associated lymphoid tissues that contain immune cells, such as the Peyer's patches, mesenteric lymph nodes, and isolated lymphoid follicles, are less numerous and underdeveloped in GF mice. These mice also have reduced secretion of IgA immunoglobulins that protect against pathogens and modulate the immune response directed against the normal gut microbiota [33]. The production of IgA by lymphocytes is induced by cytokines (peptides active in cell signaling) produced by intestinal epithelial cells upon recognition of bacterial MAMPs by Toll-like receptors [34,35]. Indeed, IgA production in GF mice can be rescued by intake of bacterial MAMPs (such as lipopolysaccharides) or intake of entire gut bacteria, such as *Bacteroidetes fragilis* or *Escherichia coli*, which also rescues normal lymphoid tissue development [32,36]. Finally, some segmented filamentous Clostridiales were shown to activate the production of specific cytokines by lymphocytes [37] that activate other immune cells and induce the production of antimicrobial peptides. Remarkably, the injection of complex microbial stimuli in colonized mice leads to a significant increase of inflammation mediators, including prostaglandins, but not so in GF mice [31]. Thus, the transition from a GF to a colonized state modifies the host inflammatory responses, shaping its immunity, and altering the way in which the host perceives and reacts to environmental stimuli [31]. Gut bacteria and their MAMPs thus shape the maturation of gut immunity.

As in roots, immunomodulation encompasses local tolerance, thus allowing the settlement of mutualistic microbiota. A group of lymphocytes that moderate immune responses, the regulatory T cells, are under the influence of gut microbiota and their MAMPs [38]. For example, ingestion of *B. fragilis* or simply its polysaccharides induces production of anti-inflammatory cytokines by regulatory T cells ([39] and references therein). Short-chain fatty acids, produced as fermentation waste products by

gut bacteria, also trigger the development of regulatory T cells [40,41].

Moreover, as in plant priming, immunomodulation is systemic. For example, gut microbiota and specific MAMPs systemically enhance the potential of T cells to proliferate under suitable signals [36]; in mice lung, microbiota reduce the abundance of invariant natural killer T cells (iNK-T), lymphocytes that trigger inflammation upon detection of bacterial antigens [42]. The later reduction of iNK-T abundance is mediated by systemic repression of a cytokine stimulating iNK-T accumulation. In developed countries, dysfunction of this pathway may contribute to the current increase of allergic responses: by reducing exposure to microbiota, the hyper-hygienic food is supposed to allow higher iNK-T accumulation [41]. Thus, gut microbial signals activate or repress immune cells and thereby contribute to mature animal immune systems after birth. This is reminiscent of rhizosphere microbiota that not only play a role in direct protection but also trigger the development of host immunity and its efficient response (Figure 2).

Microbes as developmental signals

Stimulation of host immunity by microbial colonization is not restricted to roots or guts, and occurs in other organs. In plants, leaves are colonized upon their emergence by microbial endophytes that increase protection against pathogens [43], although whether there is an induction of host defenses remains to be established. In mice, skin microbiota control local inflammation and T cell activation, improving skin protection against pathogens compared to GF mice [44], and priming via nasal inoculation of live or heat-inactivated *Lactobacillus* protects mice against otherwise lethal viruses causing pneumonia [45].

Strikingly, microbiota turn out to have other developmental roles. GF mice display increased activity and reduced anxiety behaviors that correlate with differences in gene expression and brain neuronal circuitry, suggesting that gut microbiota indirectly contribute to normal postnatal brain development [46]. Mycorrhizal colonization drastically changes vegetative and reproductive development in plants [47], and rhizosphere bacteria interfere with postembryonic root development [48]: this can in part result from microbial effectors that hijack plant developmental signaling pathways [49]. Bacterial biofilms were discovered to induce the metamorphosis and settlement of co-occurring sessile marine organisms with planktonic dispersion: bacterial cues, which signal empty spaces open to colonization, induce fixation in tubeworm larvae [50] and mobile cells of macroalgae [51], thus triggering major developmental transitions.

Using microbial signaling is understandable when external conditions are perceived, for example in metamorphosis to sessile adult organisms. By contrast, their involvement in more internal developmental processes, which are expected to be autonomously regulated, is less foreseen. However microbial priming of immunity arose by convergence in plant and animal evolution. Considering the primordial importance of immunity, why does it not maturate autonomously, in other words through an endogenous signal?

Microbes are timely signals

First, microbes arrive at the right time for induction of immunity maturation. Axenic or NM plants and GF mice never exist in the wild (Box 1): when plants and animals lose their initial axenic protection, in the seed and the womb (or the egg) respectively, they acquire microbiota immediately and are thus primed in good time. In the same way as the arrival of the storks and swallows coincides with the spring in the martenitsa tradition, gut or rhizosphere colonization indicates contact with the surrounding environment. As with the martenitsa tradition, this interaction prepares the host for a better future. Many animal behavioral traits facilitate microbial colonization throughout life [50], but this facilitation is most striking around birth. Other mechanisms contribute to the initial colonization: for instance, vaginal delivery in mammals favors contacts between the neonate and the microbiota of the mother [52];

Box 1. Axenic organism as heuristic artifacts

Priming of defense and ISR in plants as well as immune maturation during the postnatal development of animals are often viewed as additions to the phenotype of multicellular organisms conveyed by microbial symbionts. Microbiota do indeed take part in these processes (Figure 2 in main text), and NM plants or GF mice were heuristic in discovering this role. It is, however, misleading to see axenic organisms as 'default states': biologically speaking, all standard organisms are colonized and primed to some extent. Axenic organisms, such as GF animals, NM plants, and plants grown in sterile soil devoid of rhizosphere microbiota, are laboratory artefacts [62] that never exist in the wild. As such, they at least lack the functions undertaken, directly or by way of modification of the host, by their microbial partners (Figure 3 in main text), explaining their impaired features [29,46]. These axenic organisms survive in conditions that have never occurred in their evolutionary history, and thus no selection has occurred to favor adaptive or relevant traits for such conditions. Moreover, the mechanism for neutral dependency described in the main text predicts that diverse functions, beyond priming, may have been undertaken by microbial symbionts (Figure 3 in main text). Thus, axeny impacts many traits and likely has a complex influence on phenotype, which should not be overlooked when using axeny as a control state.

On a striking epistemological note, plant researchers often emphasize the impact of microbes as a positive gain for the organism (using the words 'induction', 'priming', or 'elicitation' [17–19,21]), whereas studies on animals tend to emphasize the deficiencies in GF animals [63]. This likely reflects differences in experimental approaches and technical constraints. Axenic plant seedlings are rather easily obtained, and their inoculation with microbes provides direct evidence of the role(s) played by microbial colonization. By contrast, animals become quickly colonized after their first feeding – and when GF organisms were finally obtained much was discovered from their missing or altered functions.

Despite the generality of microbial priming of immunity, there is room for beneficial microorganisms to boost immunity further, for example in the context of sustainable agriculture for plants, or in the design of pre- and probiotic foods for animals. Different microbes may have different efficiencies in priming, and may require specific density and/or conditions for efficient priming [13,64]. Understanding the mechanisms by which microbiota induce immune priming under natural conditions, and selecting the most efficient microbial inoculants, are thus crucial before beneficial microbiota can be optimally adapted to biotechnological applications.

Figure 3. Evolution of neutral dependency by contingent irreversibility. In this process, two initially independent entities begin to interact, and then evolve into dependency, without the emergence of novel functions. **(A)** Two authors, A.B. and M.A.S., playing the role of such entities, are initially stable autonomously. **(B)** They evolve into interacting with one another, irrespective of the outcome, and start to stand permanently close together (red arrow) but still display autonomous stability. **(C)** Then, any loss by one entity of the ability to stand alone can be compensated for by the other. Here, M.A.S. has a modified leg (blue arrow), the equivalent of a loss-of-function mutation, but gains stability from A.B., either without any modification of A.B. or thanks to the emergence of some active reaction from A.B. (e.g., A.B. pushes toward his left to maintain stability) – the equivalent of a compensatory mutation in the other entity. Dependency has arisen here by contingent irreversibility, because M.A.S. has now lost autonomous stability and requires A.B. If A.B. pushes on M.A.S. he may even be himself now unstable without the weight of M.A.S. weight on his left and, in this case, interdependency has emerged. The accumulation of such ratchet steps on both sides leads to systems that are unlikely to revert to independency.

more surprisingly, human breast milk contains abundant oligosaccharides that are not digestible by the infant but favor the establishment of specific microbiota [53]. Similarly, primary metabolites (e.g., malic acid) and/or secondary plant metabolites (e.g., benzoxazinoids) from root exudates shape the diversity of the rhizosphere community [54]. Moreover, the diversity of the microbiota and the common signals used (such as MAMPs and microbial metabolites, see above) allow for redundancy between priming signals and between priming microbes, which thus ascertains the outcome.

A neutral view of how microbes evolved into signals

It is unclear whether a microbial signal is more profitable than an endogenous one. After germination or birth, shifting from reserve- or mother-based resources to autonomous nutrition requires intense modifications. Reasonably delaying the costs associated with immunity maturation may allocate resources to such modifications. In plants at least, analysis of mutants provided strong evidence for high costs of constitutive resistance expression ([55] for review), but we are not aware of any similar demonstration in animal models. Quantifications of costs and benefits for early versus late maturation of immunity remain to be investigated. Until then, the respective advantages of endogenous versus microbial signals remain speculative.

It is also possible that microbial signals are not optimal, but simply contingent. There is increasing evidence that biological dependency can arise by neutral evolution. A neutral ratchet-like process was recently proposed to account for the evolution of many complex cellular functions and molecules at the cellular level [56,57]. When two initially independent entities permanently interact, such as two subunits of a protein complex or two species, redundancies become unstable: a mutation in one can be complemented by the presence of the other, or even by a complementing mutation in the other (Figure 3). Independency is then lost without any gain of function or positive selection; moreover, the accumulation of such ratchet steps makes the reversion to independency more and more unlikely, and can result in reciprocal dependency [58]. This contingent irreversibility explains how some pathogens permanently infecting their host secondarily evolved into essential partners [59].

Immunity maturation may have followed such a pathway, where any hypothetical ancient endogenous signal may have been lost by mutation because it has become redundant with microbial colonization. Mutational drift is likely to have occurred in some ancient symbioses, for example in the 400 Ma mycorrhizal symbiosis [6]. Obviously only the endogenous signal can be lost (e.g., by mutational drift) because microbes are always present. Furthermore, multicellular organisms may have never evolved any endogenous signal and, because their ancestors lived in the continuous presence of microbes, there was no selective pressure to evolve an ontogeny without them. Thus, external microbial signals may not be better than endogenous ones – instead, dependency may simply have arisen by contingent irreversibility, without positive selection, in the maturation of the immune system.

Concluding remarks: microbial martenitsa

Multicellular organisms are often viewed as autonomous units. It is more and more recognized that microbes shape

Box 2. Outstanding questions

- To what extent do other multicellular organisms (e.g., macroalgae) also undergo defense priming?
- What are the chemical signals that underlie inter-kingdom communication?
- What is the basis of the differential efficiencies of microorganisms in triggering defense priming?
- Can hosts manipulate or select the first colonizing microbiota to maximize priming efficiency?
- Is there a cost to constitutive defenses in young individuals, making microbial priming less costly?
- How is suppression of local immunity (that allows colonization by beneficial microbes) combined with systemic priming of immunity at the molecular level?

crucial functions such as nutrition, metabolism, and protection [60], and development now joins the list (Figure 2). In interactions that regularly bring the partners together, one partner can recruit the other as a signal, exactly as birds are used in the martenitsa tradition (Figure 1). This role, currently absent from textbooks on symbiosis, is especially important and takes various forms for immunity maturation. It implies an increased interdependence between partners – which is not necessarily an optimization or a progressive evolution, but could emerge through neutral evolution (Figure 3).

It has been proposed that the holobiont, in other words the animal or plant with its associated symbionts, emerges as a relevant target for natural selection [61]; conversely, axenic hosts do not occur in nature and are not realistic organisms (Box 1) even at the developmental level. However, several issues remain pending (Box 2) and deserve further study to understand the evolution of developmental dependency of multicellular organisms on their symbionts.

Acknowledgments

We are grateful to Martin Heil (CINVESTAV, Irapuato, México), Luis Sampedro (Misión Biológica de Galicia, CSIC), Mathilde Simonson (Institut de Biologie de l'Ecole Normale Supérieure) and two anonymous referees for critical reading of the manuscript. Many interesting papers could not be cited due to space limitations, for which we apologize.

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