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Life history and eco-evolutionary dynamics in light of the gut microbiota

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The recent emergence of powerful genomic tools, such as high-throughput genomics, transcriptomics and metabolomics, combined with the study of gnotobiotic animals, have revealed overwhelming impacts of gut microbiota on the host phenotype. In addition to provide their host with metabolic functions that are not encoded in its own genome, evidence is accumulating that gut symbionts affect host traits previously thought to be solely under host genetic control, such as development and behavior. Metagenomics and metatranscriptomics studies further revealed that gut microbial communities can rapidly respond to changes in host diet or environmental conditions through changes in their structural and functional profiles, thus representing an important source of metabolic flexibility and phenotypic plasticity for the host. Hence, gut microbes appear to be an important factor affecting host ecology and evolution which is, however, not accounted for in life-history theory, or in classic population genetics, ecological and eco-evolutionary models. In this forum, we shed new light on life history and eco-evolutionary dynamics by viewing these processes through the lens of host–microbiota interactions. We follow a three-level approach. First, current knowledge on the role of gut microbiota in host physiology and behavior points out that gut symbionts can be a crucial medium of life-history strategies. Second, the particularity of the microbiota is based on its multilayered structure, composed of both a core microbiota, under host genetic and immune control, and a flexible pool of microbes modulated by the environment, which differ in constraints on their maintenance and in their contribution to host adaptation. Finally, gut symbionts can drive the ecological and evolutionary dynamics of their host through effects on individual, population, community and ecosystem levels. In conclusion, we highlight some future perspectives for integrative studies to test hypotheses on life history and eco-evolutionary dynamics in light of the gut microbiota.

All animals live in intimate association with communities of microorganisms, known as microbiota, composed of bacteria, archaea, anaerobic fungi, protozoa and viruses. The vast majority of these microbes reside in the gut, where they are in continuous and intimate contact with host tissues, and where they can outnumber the surrounding host cells by at least an order of magnitude (Bäckhed et al. 2005, Amato 2016). The existence of these microbes has first been reported several centuries ago, but until recently they remained largely understudied, and thus unknown, essentially because they are difficult to extract and to cultivate in the laboratory. A decade ago, the advent of sequencing technologies finally opened up this frontier (Gilbert et al. 2015). The emergence of powerful genomic tools, such as high-throughput transcriptomics and metabolomics, applied to the older technology of gnotobiotics, have led to a detailed understanding of how microbiota shapes many aspects of host physiology (Hooper et al. 2012). Combined to whole-genome shotgun metagenomics and metatranscriptomics, which allow precisely determining the taxonomic composition and functional profiles of gut microbial communities, it is now possible to estimate how variations in gut microbiota can affect the host and be affected by various environmental and dietary conditions (Rampelli et al. 2015).

Evidence has accumulated that the gut microbiota is not just a random set of microorganisms, but rather a complex community that plays a critical role in host physiology and behavior (Engel and Moran 2013, Douglas 2015, Amato 2016; Fig. 1). In particular, gut symbionts provide their host with metabolic capabilities not directly encoded in the host genome, such as digestion of plant polysaccharides (David et al. 2014) or detoxification of food-borne toxins (Kikuchi et al. 2012, Kohl et al. 2014), and contribute to the normal development of the host, e.g. by fostering the maturation of the immune system (Belkaid and Hand 2014). Dysbioses in the gut microbial community have recently been associated with diseases such as obesity, diabetes or inflammatory bowel disease, and new therapies based on fecal transplants are progressively emerging to prevent or cure such diseases (Belkaid and Hand 2014). Consequently, the gut microbiota has started to be studied intensively and is a burgeoning field of scientific research. So far, most studies have focused on

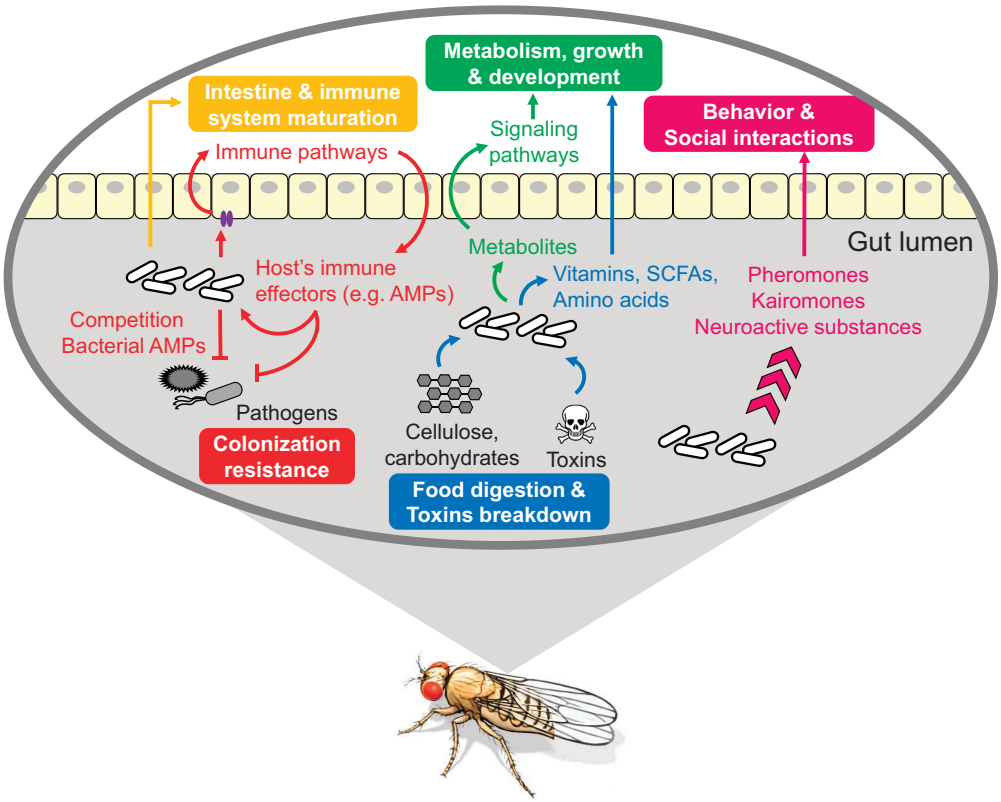

Figure 1. Contributions of the gut microbiota to host phenotype. The gut microbiota (white rods) contributes to the normal development of the intestine and the immune system (yellow). In addition, by interfering with host immune pathways, and by competing with other microbes, gut symbionts enhance colonization resistance and thus protects the host against invading pathogens (red). Gut microbiota also has a crucial role in food digestion (e.g. breaking down non-digestible substrates) and in the provisioning of important nutrients to the host. Moreover, it helps in the degradation of either food-borne or environmental toxins (blue). The production of secondary metabolites by gut bacteria can interfere with host signaling pathways (e.g. insulin pathway), resulting in various effects on host physiology and developmental processes (e.g. growth, fat metabolism) (green). By producing neuro-active substances that directly act on host brain, or pheromones/kairomones that mediate inter- or inter-species communication, gut microbiota further influences host behavior and social interactions (purple).

the bacterial component of the microbiota, while there is a relative lack of research on other gut microorganisms, such as archaea, fungi or viruses (Ogilvie and Jones 2015). In this review, the term 'gut microbiota' thus mainly refers to the bacterial gut microbiota.

Despite its impact on host physiology and behavior, the gut microbiota is rarely accounted for in life-history theory, as well as in classic population genetics-, ecological- and eco-evolutionary models. Contrary to the genome, which is largely static, the microbiome is highly flexible, and can respond rapidly to changes in host diet or environmental conditions, e.g. through changes in the taxonomic composition of the community (David et al. 2014). It may thus represent an important source of metabolic flexibility for the host. As such, the gut microbiome is sometimes referred as "the third malleable genome" (Carroll et al. 2009), and is increasingly hypothesized to play a role in host ecology and evolution (Bordenstein and Theis 2015, Gilbert et al. 2015). An emerging theory suggests that animals should no longer be seen as autonomous entities, but rather as a biomolecular network composed of the host plus its associated microbes, i.e. the 'holobiont', the collective genome of which being referred to as "hologenome" (Bordenstein and Theis 2015). Animals may therefore be considered as polygenomic entities, in which variation in the hologenome can lead to variation in phenotypes upon which natural selection, and genetic drift, can operate. This theory, however, is still highly debated. While most biologists agree that microorganisms likely play an important role in host evolution, the idea that hosts and its associated microorganisms form a primary unit of natural selection, and represent two components of a unified genome, is more controversial (Moran and Sloan 2015). Especially, how gut symbionts evolve, and whether they undergo natural selection to benefit their host, is still far from being evident (Moran and Sloan 2015). Indeed, the gut microbiota is a complex, heterogeneous and variable community of microbes, which is assembled anew in each host generation through different transmission routes. At one extreme, gut symbionts can be directly transferred from mother to offspring, but most of the time they are randomly picked up from the environment (Moran and Sloan 2015, Shapira 2016). Hence, contrary to endosymbionts, vertical transmission of gut microbiota is rare and imperfect. The symbiotic part of the hologenome within the host–symbiont association is thus "labile", i.e. it can be lost between generations (Shapira 2016). As such, the evolutionary interests of hosts and symbionts are not necessarily aligned, and microorganisms may tend to evolve selfish traits, at the expense of the holobiont (Moran and Sloan 2015, Wasielewski et al. 2016). Gut microbes are also not mutualistic in the truest

sense; some of these organisms can be pathogenic to some extent, while others might directly interfere with mutualistic microbes (e.g. through toxins), and their status can change with environmental conditions (Callens et al. 2016, King et al. 2016; Fig. 2). Variations in the gut microbiota composition are thus not always adaptive for the host, as evidenced by dysbioses-induced diseases. Overall, understanding the gut microbiota, and its role in ecology and evolution, is relatively more complicated than intracellular symbionts because gut symbionts evolve in the 'grey zone' of symbiotic interactions, i.e. are not strictly vertically, nor horizontally transmitted, are facultative symbionts, and can be mutualistic or pathogenic depending on environmental conditions.

The molecular dialog between hosts and gut symbionts starts to be deciphered, highlighting a crucial role of host immune pathways in the acquisition and control of gut microbiota (Vavre and Kremer 2014, Tasiemski et al. 2015). Although the study of these mechanisms is still in its infancy, it may provide an opportunity to better understand host–microbiota interactions, as well as their ecological and evolutionary impacts. Shapira (2016) recently highlighted the multilayered structure of the gut microbiota, composed of both a core microbiota, under host genetic and immune control, and a flexible pool of microbes modulated by the environment, which likely differ in their contribution to host fitness and in constraints on their maintenance (e.g. the core microbiota is expected to contribute to more essential functions, and to be more reliable transmitted across generations). Based on this framework, and after a review of the diverse functions ensured by gut symbionts, we will demonstrate how gut microbiota can mediate life history, and drive the ecological and evolutionary dynamics of their host, through effects on individual, population, community and ecosystem levels (Fig. 3). We will finally delineate particular future perspectives for integrative studies to test these hypotheses.

The pivotal role of gut microbiota in life history mediated via effects on host physiology and behavior

Host nutrition and metabolism

The gut microbiota plays a prominent role in the host nutritional ecology, either by aiding in digestion, or by providing nutrients that are limited or lacking in the diet (Engel and Moran 2013; Fig. 1). For instance, herbivorous insects and mammals lack the appropriate enzymes to digest plant cell wall material and resistant starches, and thus rely on their gut symbionts to convert these indigestible compounds into absorbable short-chain fatty acids (SCFAs) (Feldhaar 2011, Douglas 2015, Amato 2016). In herbivorous animals, e.g. termites, mutualistic gut symbionts also compensate for the low amount of nitrogen provided by plants, either by recycling nitrogenous waste products excreted by the host, or by fixing nitrogen from the atmosphere (Hongoh et al. 2008, Thong-On et al. 2012).

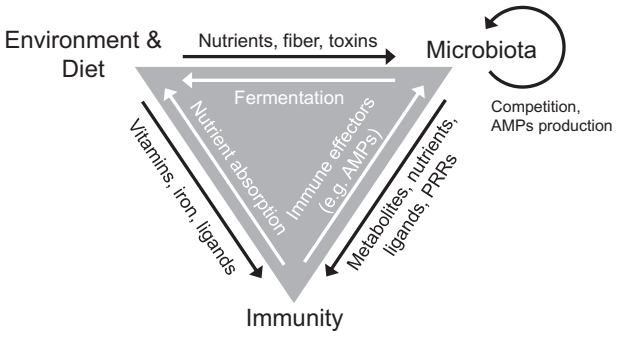

Figure 2. A complex network of interactions between the environment, the immune system and gut symbionts mediates the composition of the resident gut microbiota (adapted from Belkaid and Hand 2014). Arrows indicate fluxes of nutrients, metabolites, vitamins, etc. and biotic processes within the host (e.g. fermentation or competition).

The enzymatic degradation of food by bacteria, associated with the signaling and epigenetic roles of bacterial metabolites, have strong impacts on host metabolism, especially on energy storage (Bäckhed et al. 2004, Tremaroli and Bäckhed 2012, Douglas 2015). In mice, the gut microbiota increases both fat deposition and metabolic rate, through an increased processing of polysaccharides and interactions with host metabolic pathways (Bäckhed et al. 2004). Interestingly, the gut microbiota differs between obese and non-obese mice (Sommer and Bäckhed 2013), with more genes encoding for carbohydrate metabolism enzymes and providing a greater capacity to extract energy from the diet in the microbiome of obese mice (Turnbaugh et al. 2006, 2009). Gut microbiota transplants towards germ-free animals further result in the transfer of the donor phenotype (obese or lean), with a higher fat deposition in mice receiving the gut microbes from obese donors (Turnbaugh et al. 2006), highlighting the pivotal role of gut microbiota in host metabolism. In *Drosophila*, germ-free animals have elevated levels of lipid and glucose, together with reduced basal metabolic rates, indicative of enhanced energy harvesting (Douglas 2014, Newell and Douglas 2014, Ridley et al. 2012). These effects have been linked to altered insulin signaling in the host (Shin et al. 2011). From an ecological and evolutionary point of view, microbial effects on host metabolism and fat deposition can be important in areas with highly variable food resources or extreme weather (Amato 2016), as host adipose tissues play an important role in thermoregulation (Kozak et al. 2010). In the same vein, cold exposure leads to a marked shift in the gut microbiota composition of mice, and transplantation of 'cold' microbiota to germ-free mice increases tolerance to cold by promoting white fat browning, leading to increased energy expenditure and fat loss. Transplant experiments further showed that, during prolonged cold, the gut microbiota is responsible for altered intestinal gene expression promoting increased gut absorptive surface, thus increasing caloric uptake (Chevalier et al. 2015).

In addition to contributing to food digestion, the gut microbiota participates, through its enzymatic activities, in the metabolism of xenobiotic bioactive molecules, such as diet-derived toxins, human-crafted poison or therapeutic drugs (Carmody and Turnbaugh 2014; Fig. 2), e.g. in herbivorous species which strongly depend on their gut symbionts to escape toxins produced by the plant they consume. In desert woodrats, for example, specialization of some populations on the highly toxic creosote bush is mediated by gut microbes: a disruption of the gut microbiota with antibiotics results in their inability to consume creosote toxins (Kohl et al. 2014). In the same way, gut symbionts enable

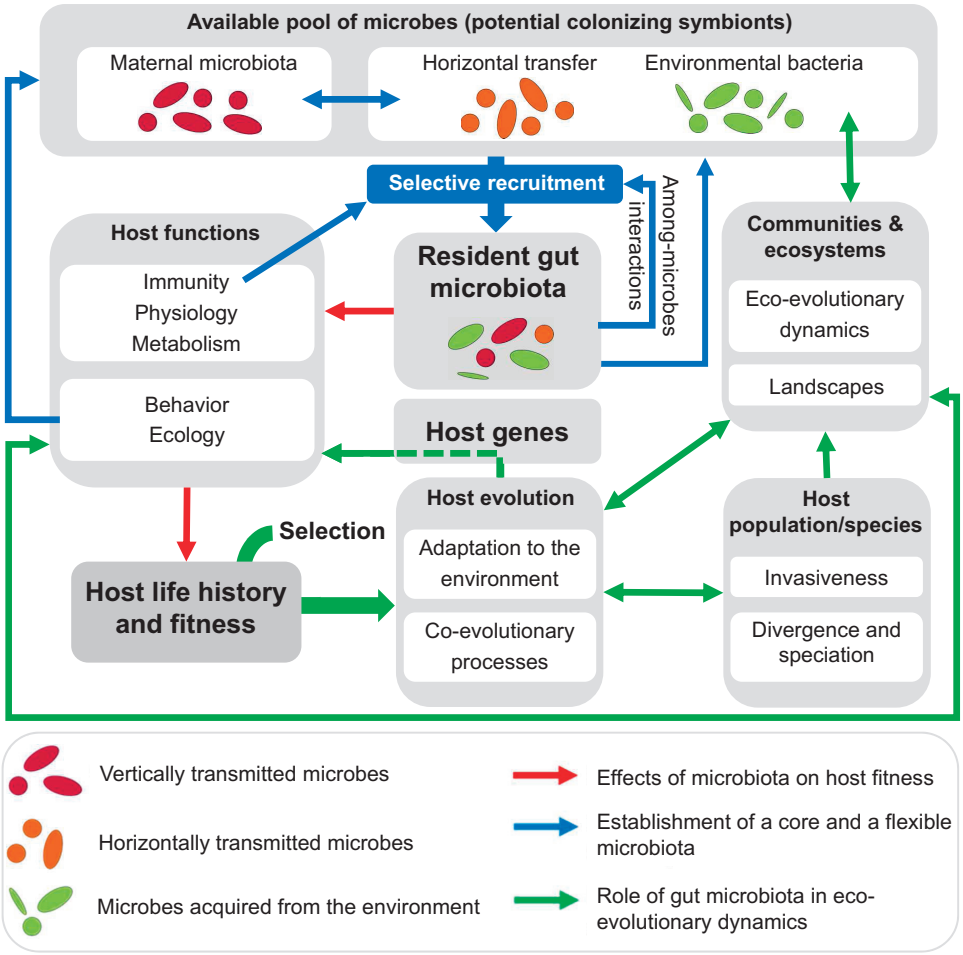

Figure 3. The gut microbiota as a key factor of eco-evolutionary dynamics in natural systems. The gut microbiota has large impacts on host physiology and behavior, and consequently on host life history and fitness (red arrows). Unlike intracellular symbionts, which are strictly vertically transmitted to the embryo, gut associated microbes are mainly acquired during and after birth via maternal transmission and horizontal transfer from either conspecifics or the surrounding environment. From this pool of microbes available to the host, only a certain proportion is selectively recruited in the resident gut microbiota, depending on both host factors (genetic background, immunity) and a complex interaction network between host, microbes and environmental factors (blue arrows). The gut microbiota is thus composed of both a flexible pool of microbes, dependent on environmental diversity and external conditions, and a core microbiota linked to host genetics. This duality makes the gut microbiota a source of both phenotypic plasticity, through fast variations in gut microbiota composition within the life cycle of the host, and evolution, through selection on symbiont-mediated traits. Adaptation to local environmental conditions can result in a rapid evolution of host genes (especially immune genes) involved in acquisition, control and tolerance of beneficial symbionts, allowing for a co-inheritance of nuclear genes and microbes, which is a pre-requisite for evolution to occur. At the population and species levels, such evolution can contribute to population divergence and speciation, or increase the invasiveness of the host species. By mediating the interactions of the host with the rest of the community (e.g. parasites, host plant, predators), and by acting as an ecosystem engineer that contributes to shaping the biotic and abiotic environment of the host, gut symbionts can further affect eco-evolutionary dynamics and regulate community and ecosystem functioning (green arrows).

the coffee berry borer to feed on coffee berries while avoiding the otherwise toxic effects of caffeine (Ceja-Navarro et al. 2015). Such bacterially derived detoxification contributes to local niche adaptation, and provides the host with food or a habitat that would otherwise be inaccessible (Engel and Moran 2013, Shapira 2016).

Host development and maturation of the immune system

There is increasing evidence that bacterial symbionts influence host processes once thought to depend solely on the genetic program of the host, including development, morphogenesis and cell proliferation (Sommer and Bäckhed 2013, Gilbert et al. 2015). As such, host species are often highly dependent on the presence of commensal intestinal bacteria to achieve a normal development.

In both invertebrate and vertebrate species, gut bacteria apparently promote host growth and development either indirectly through their role in nutrient provisioning, or directly through interference with host physiology, e.g. by providing signals that stimulate developmental processes (Engel and Moran 2013; Fig. 1). In mice and zebrafish, gut microbes are required for a proper development of the gut (reviewed by Gilbert et al. 2015). Indeed, germ-free animals have smaller and less functional intestines, and these defects can be reversed by the introduction of bacteria later during animal development (Bates et al. 2006). The gut microbiota

also contributes to the homeostasis of the intestinal tissue, by regulating the balance between cell renewal and death (Broderick and Lemaitre 2012, Engel and Moran 2013, Sommer and Bäckhed 2013). For example in *Drosophila melanogaster*, the gut microbiota apparently promotes stem cell proliferation and epithelium renewal, a process essential to the defense against bacterial infection. However, in mutant flies unable to control the population of commensal bacteria, an excessive proliferation of intestinal stem cells can be observed, suggesting that the host response depends on bacterial load and the composition of the microbiota (Buchon et al. 2009).

Animal–bacteria interactions contribute at the organism or tissue level to the development and maturation of the immune system along the life of an individual (Fig. 1). Some host species can be highly dependent on the presence of intestinal bacteria, as exemplified by germ-free mice which present an undeveloped mucosal immune system, a reduced epithelial cell turn-over, resulting in a lower ability to regain tissue homeostasis following injuries of the intestine as well as structureless immune organs (lymph nodes and spleen; Rakoff-Nahoum et al. 2004). Germ-free individuals suffer from serious immune defects, and are more susceptible to infections than colonized animals (Belkaid and Hand 2014).

The crosstalk between intestinal immunity and microbiota is particularly well described in mammals thanks to the development of very powerful models such as germ-free mice. A brief overview of this inter-relation is given in Box 2 and Fig. 4. Gut symbionts have the remarkable ability to promote and calibrate both innate and adaptive host immunity, thus promoting their own containment and limiting pathogen invasion (Hooper et al. 2012, Honda and Littman 2016, Thaiss et al. 2016). In humans, imbalance in the gut immune system homeostasis has a negative impact on health, causing severe and/or chronic pathologies. Intestinal dysbiosis during maturation of the immune system is correlated with a failure of the immunological tolerance that subsequently leads to exacerbated local auto-inflammatory disorders such as inflammatory bowel diseases (IBD), but also extra-intestinal inflammatory and autoimmune disorders such as type 1 diabetes or rheumatoid arthritis (Kahrstrom et al. 2016, Kataoka 2016). The system becomes even more complex when taking into consideration the environmental/ecological context. In high-income countries, overuse of antibiotics, changes in diet, and 'over-hygienic conditions', can have selected for a gut microbiota that lacks the resilience and diversity required to establish balanced immune responses. This phenomenon referred to as the 'hygiene hypothesis' (Box 1) might explain the dramatic rise in autoimmune and inflammatory disorders, like IBDs such as Crohn disease (Belkaid and Hand 2014). Understanding the ecological, environmental influences on gut immunity is another frontier for immunologists to cross by exploring immuno-ecological concepts that would bridge the gap with immunology concepts developed by ecologists.

Protection against parasites and pathogens

Increasing evidence demonstrates that the gut microbiota plays a crucial role in host resistance against invading pathogens within the intestine, a process referred to as colonization resistance (Kamada et al. 2013; Fig. 1). Consequently, loss or

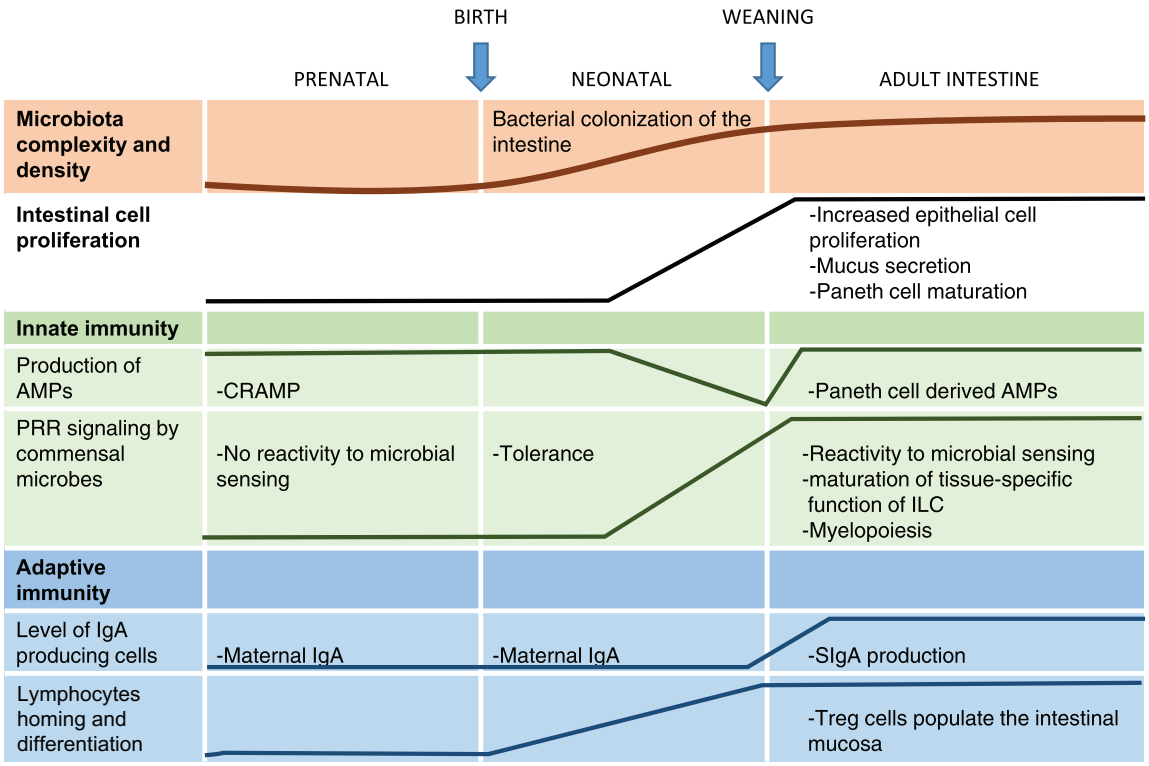

Figure 4. Gut microbiota and host immunity. Schematic representation of immune system development and maturation before birth, after birth and after weaning in mammals (adapted from Renz et al. 2012).

Box 1

AMPs (Anti Microbial Peptides). Key components of the innate immune system that rapidly eradicate or incapacitate pathogenic agents such as viruses, bacteria, fungi, attempting to invade and proliferate in multicellular eukaryotes (Zasloff 2002, Bulet et al. 2004, Maroti et al. 2011). They have also been evidenced to shape, control and confine the symbiotic microflora into specific anatomic compartments (gut, bacteriomes, skin…), thus contributing to the symbiostasis of both invertebrates and vertebrates (Salzman et al. 2010, Gallo and Nakatsuji 2011, Login et al. 2011, Franzenburg et al. 2013, Tasiemski et al. 2015). In metazoans, the evolution of AMPs has been shown to be driven by recurrent duplications (i.e. creation of paralogs) and balancing/positive selection to face and kill new and/or altered bacterial pathogens that can be encountered in a novel habitat and/or that have rapidly evolved to escape the immune response (Tennessen 2005, Gosset et al. 2014, Unckless et al. 2016).

PRR (Pattern Recognition Receptor). Receptors expressed by invertebrate and vertebrate cells. PRRs sense/recognize conserved microbial molecules called microbe associated molecular patterns (**MAMPs**). MAMPs recognition by PRRs induces, via an intracellular transducing pathway, an immune response (AMPs, cytokines production, cell migration….) of the cell expressing the PRR. This response can be 1) deleterious by eliciting an inflammatory response; 2) beneficial by eliminating pathogens or more surprisingly by contributing to various aspects of host development mediated by commensal stimulation of host PRRs. Understanding how the same molecules can achieve such divergent and opposing responses remains a challenging unanswered question.

SIgA (Secretory IgA): is the most abundant class of antibodies found in the intestinal lumen in most mammals. SIgA produced by plasma cells, is secreted across the epithelium into the lumen. It serves as the first line of defense in protecting the intestinal epithelium from enteric toxins and pathogenic microorganisms. SIgA promotes the clearance of antigens and pathogenic microorganisms from the intestinal lumen by blocking their access to epithelial receptors, entrapping them in mucus, and facilitating their removal. SIgA has also the capacity to directly quench bacterial virulence factors, and influence composition of the intestinal microbiota (Mantis et al. 2011).

T_{reg} cells: comprise a large proportion of the T cells of the intestine in mice and humans. They play an important role in maintaining immune tolerance to dietary antigens (Stefka et al. 2014) and to the gut microbiota as well as suppressing tissue damage caused by immune responses against some bacteria such as *Clostridium rodentium* (Josefowicz et al. 2012).

Hygiene hypothesis: is a term coined by Strachan (1989) based on reasonable clinical epidemiological evidence showing that children from families of lower socio-economic status or brought up on farms have a decreased incidence of autoimmune or allergic diseases. Exposure to oro-faecal microorganisms and helminthes during childhood leads to deviation/tradeoff of the immune responses that reduce the development of such atopies in adults.

perturbation of gut microbial communities are often associated with increased infectivity of pathogenic bacteria, with evidence from diverse species including both invertebrates and vertebrates (Engel and Moran 2013, Kamada et al. 2013, Belkaid and Hand 2014). For example, in bumblebees, gut microbes were recently shown to protect their host against *Crithidia bombi*, a natural and highly virulent parasite (Koch and Schmid-Hempel 2011). In the mosquito *Anopheles gambiae*, a clearance of gut microbes with antibiotics enhances infections with *Plasmodium falciparum*, the causative agent of malaria (Beier et al. 1994, Dong et al. 2009, Meister et al. 2009, reviewed by Engel and Moran 2013). Microbiota-mediated resistance to pathogens has also been observed in vertebrates, e.g. in mice, in which germ-free or antibiotic-treated individuals are more susceptible to various enteric pathogen infections (Kamada et al. 2013, Belkaid and Hand 2014). A diverse gut microbial community is expected to be more difficult for an opportunistic microbe to invade – as more diverse ecological communities can be more resistant to invaders (Romanuk and Kolasa 2005, Beisner et al. 2006, Byun et al. 2013), so that individuals with high gut microbial diversity should be more resistant to invading pathogens. This hypothesis is supported, e.g. in the desert locust *Schistocerca gregaria*, in which gut community diversity correlates negatively with colonization success of the pathogen *Serratia marcescens* (Dillon et al. 2005).

Direct interactions between commensals and pathogens, such as competition for shared nutrients and ecological niches, have been shown to play an important role in colonization resistance (Kamada et al. 2012, Khosravi and Mazmanian 2013; Fig. 2). By consuming common limited resources, such as organic acids, amino acids or other nutrients, the indigenous gut microbiota contributes to limit the growth and the survival of competing pathogenic bacteria. For example, commensal strains of *E. coli* were shown to suppress the growth of entero-hemorrhagic *E. coli*, through competition for proline. This phenomenon of colonization resistance was strongly attenuated by the addition of proline in the medium (Momose et al. 2008). As a result of competition, microbes have evolved mechanisms to outcompete each other, such as the production of antimicrobial substances that inhibit the growth and the survival of other bacteria within the gut (Kamada et al. 2013). This chemical warfare further contributes to colonization resistance against pathogens. For example, *E. coli* produces bacteriocins, a family of antimicrobial peptides (AMPs, Box 1), that specifically targets similar bacterial strains, thus impairing the growth of the related entero-hemorrhagic pathogen *E. coli*. Similarly, in mosquitoes (Cirimotich et al. 2011) and leeches (Tasiemski et al. 2015), production of antimicrobial substances by gut symbionts provides protection against invasive bacteria. Microbiota-derived metabolites, such as

SCFAs, also contribute to prevent pathogen infection by altering the intestinal environment (e.g. pH) to inhibit the growth of pathogens, or by down-regulating the expression of virulence genes (Gantois et al. 2006).

In addition to these direct effects in invasive microbes, the gut microbiota capacity to control infection is also associated with its ability to mediate host immune responses (Kamada et al. 2013). In mice, individuals treated with antibiotics or raised under germ-free conditions have significantly impaired immune responses, and are thus more susceptible to pathogens (Belkaid and Hand 2014). As discussed previously, commensal bacteria produce signals that can enhance expression of host defense genes, such as AMPs, which have the dual effect of promoting their own containment and to limit pathogen invasion (Belkaid and Hand 2014, Tasiemski et al. 2015). In mice, the loss of microbiota-induced antimicrobial lectin leads to increased bacterial dissemination and susceptibility to bacterial pathogens (Kamada et al. 2013, Belkaid and Hand 2014). Another example is that of segmented filamentous bacteria, which promote T-cell differentiation in the mice intestine and are involved in the clearance of pathogenic bacteria (Gaboriau-Routhiau et al. 2009).

Behavior and social interactions

Some parasites are known to manipulate the behavior of their host to improve their own transmission. A famous example occurs in crickets, in which hairworms induce suicidal behavior and jumps into water, so that the parasite can complete its life cycle (Thomas et al. 2002). Accumulating data now indicate that symbiotic bacteria can also affect host behavior (Archie and Tung 2015, Cryan and Dinan 2015, Shapira 2016; Fig. 1). In vertebrates, the gut microbiota communicates with the central nervous system – through neural, endocrine and immune pathways – and thereby influences brain function and behavior (Heijtz et al. 2011, Archie and Tung 2015). Studies in germ-free animals and transplant experiments have highlighted a role for the microbiota in modulating stress responses and stress-related behaviors relevant to psychiatric disorders, such as anxiety and depression (Cryan and Dinan 2015). In germ-free mice, stress exposure induces an exaggerated release of adrenocorticotropic hormone and corticosterone compared with control mice with a normal microbiota. The stress response in the germ-free mice can be partially reversed by colonization with fecal matter from control animals (Sudo et al. 2004). Reciprocal microbiota transplant between mice strains differing in their microbiota composition further reveals that the behavioral profile of recipient mice is similar to that of the donor strain (Bercik et al. 2011). These data clearly demonstrate that the microbial content of the digestive tract has a direct impact on the host behavior.

In addition to effects on the nervous system, gut microbes produce chemical signals used in social communication, thus affecting social behavior (Archie and Tung 2015; Fig. 1). As bacterial communities can be shaped by social contacts, family relationship, genotype, environmental condition or host health status, they have the potential to communicate important information about their host (Lizé et al. 2013). For instance, correlations have been observed between host traits (e.g. dominance rank, social group membership), the bacterial communities living in scent glands, and the volatile compounds emerging from these glands (Archie and Tung 2015). In *Drosophila melanogaster*, gut bacteria mediate olfactory cues involved in social attraction, kin recognition and mating preferences (Lizé et al. 2013). A striking example is described in an experiment in which a population of inbred flies was divided in two groups reared on two different diets (Sharon et al. 2010). Within one generation, populations developed a mating preference for flies grown on the same food, a preference that was abolished by an antibiotic treatment. Infection experiments confirm the role of the gut microbiota in this mating preference, and especially of the bacteria *Lactobacillus plantarum.* Analytical data suggest that these preferences rely on the modification of pheromone profiles through the production of cuticular hydrocarbons which serve as sex pheromones in *Drosophila* (Sharon et al. 2010). This could be an indirect effect of the symbiont or, as in the grass grub beetle symbiont *L. plantarum*, could produce the pheromone itself (Shapira 2016).

The gut microbiota as crucial mediator of life-history strategies

By affecting functions as important as nutrition, metabolism, resistance to pathogens and behavior, the gut microbiota necessarily affects life-history traits contributing to host fitness (Fig. 3). Evidence mainly comes from studies comparing germ-free to conventionally reared individuals, in particular in arthropods, which reveal that gut bacteria have an overwhelming influence on growth, development, reproduction and survival. In the fruit fly *Drosophila melanogaster* (Storelli et al. 2011) and in the water flea *Daphnia magna* (Sison-Mangus et al. 2015, Callens et al. 2016), germ-free individuals develop more slowly and are smaller than conventional animals, while in mosquitoes, axenic larvae fail to develop beyond the first instar (Coon et al. 2014). In all these species, inoculating axenic larvae with gut bacteria can restore a normal developmental rate (Storelli et al. 2011, Coon et al. 2014, Callens et al. 2016). Mono-association studies in *Drosophila* (Shin et al. 2011, Storelli et al. 2011), mosquitoes (Coon et al. 2014) and *Daphnia* (Peerakietkhajorn et al. 2016) further identified single bacterial strains that are sufficient to recapitulate the natural microbiota growth-promoting effect. In *Drosophila*, these bacteria exert their beneficial functions on the host by promoting insulin signaling (Shin et al. 2011, Storelli et al. 2011). In some species including *Drosophila* (Brummel et al. 2004, Ren et al. 2007, Petkau et al. 2014), *Daphnia* (Sison-Mangus et al. 2015, Callens et al. 2016), *C. elegans* (Houthoofd et al. 2002) and termites (Rosengaus et al. 2011), germ-free animals have a reduced lifespan and a lower fecundity.

The effects of gut microbes on host fitness are, however, not always positive. Partnership with symbionts can entail direct costs to the host, arising for example from a tradeoff between allocating resources to symbiosis and reproduction or growth. In addition, the effects of gut microbes on host fitness can depend on environmental conditions. For example, in *Drosophila* (Shin et al. 2011, Storelli et al. 2011) and in *Daphnia* (Callens et al. 2016), the effects of gut microbes on host fitness depend on the nutritional value of the diet. In *Drosophila*, germ-free larvae exhibit reduced growth and slower development than conventionally reared larvae, but only when raised on poor medium. Conversely, in *Daphnia*,

the positive effects of gut microbes on growth and survival are only observed when food is sufficient or abundant, with weaker effects under food limitation, demonstrating the context-dependency of fitness effects on the host. The benefits provided by symbionts may also depend on the presence of other organisms in the environment. For example, in the nematode *C. elegans*, the mildly pathogenic bacteria *Enterococcus faecalis* living in worms provides protection against the more virulent pathogen *Staphylococcus aureus*, crossing the parasitism–mutualism continuum. In an environment in which *S. aureus* virulent infection is common, *E. faecalis* would therefore represent a mutualist for the worm, while being pathogenic in the absence of *S. aureus* (King et al. 2016).

Because gut symbionts interact with various aspects of host physiology and metabolism, their effects on host life history traits can have diverse origins, such as a lower ability of germ-free animals to extract energy from food. Recent studies, however, suggest that bacteria-derived metabolites and RNAs can directly affect life-history traits such as senescence in the host (Heintz and Mair 2014, Clark et al. 2015). For example in *C. elegans*, the bacterial production of nitric oxide (NO), a critical signaling molecule that *C. elegans* is unable to produce, can modulate longevity, probably by regulating host transcriptional responses (Gusarov et al. 2013, Heintz and Mair 2014). In addition, Blaser and Webb (2014) suggest that selection can differentially act on the composition of the gut microbiota, depending on age. First, before and during reproductive life, host genes favoring microbes that preserve host function, e.g. through regulation of energy homeostasis or promotion of fecundity, should be selected. However, after reproductive life, selection for maintaining these beneficial microbes should decrease, especially if the mechanisms involved in the control of gut microbes are costly to the host. Consistently with this, changes in gut microbiota composition in aging flies are responsible for changes in excretory function and immune gene activation in the aging intestine, resulting in a deterioration of the intestinal epithelium and finally in death (Broderick and Lemaitre 2012, Broderick et al. 2014, Clark et al. 2015). In humans, alterations of the composition of the gut microbiota are correlated with aging and measures of frailty, morbidity and inflammation (Chang et al. 2008, Willing et al. 2010, Claesson et al. 2012). Such observations suggest that alteration of microbiota dynamics can contribute to health decline during aging in animals.

Microbiota and the pace of life syndrome

An important goal of life-history theory is to explain the range of variation in life-history patterns exhibited in populations (Stearns 1992). Animal populations can be placed along a fast–slow continuum, with species that mature early, have large reproductive rates and short generation times occupying the 'fast' end of the continuum, and those with the opposite suite of traits occupying the 'slow' end of the continuum (Read and Harvey 1989, Promislow and Harvey 1990, Réale et al. 2010). The optimal position on this continuum strongly depends on ecological conditions, which thus affect the evolution of life-history strategies in natural populations (Stearns 1992). Given the strong correlations and mechanistic linkages between physiology and life history, the pace-of-life syndrome (POLS) hypothesis further proposes that closely related species or populations experiencing different ecological conditions should consistently differ in a suite of metabolic, hormonal and immunity traits that have coevolved with life history (Ricklefs and Wikelski 2002, Wikelski et al. 2003, Martin II et al. 2006, Réale et al. 2010). Low rate of metabolism can be one potential component of a slow life history that can lead to selection for other slow traits, such as low fecundity and late reproduction. Consistently with this, tropical birds are typically long-lived and produce few offspring, develop slowly, mature relatively late in life, and also have a low metabolic rate (Wikelski et al. 2003, Wiersma et al. 2007). Hence, relative to their temperate zone counterparts, tropical birds have a slower pace of life along both physiological and life-history axes of variation (Réale et al. 2010). Réale et al. (2010) proposed to further integrate behavior into this POLS theory. Indeed, there is increasing evidence that personality phenotypes are linked to specific life history and physiological patterns. For example, in mammals, proactivity (i.e. high boldness and aggressiveness) is associated with an increased capacity to acquire and monopolize resources, resulting in higher growth rate and higher reproductive success, while decreasing longevity. At the physiological level, proactive animals are characterized by an elevated adrenaline production and heart rate under stress, compared to non-proactive animals (Réale et al. 2010).

Given that gut microbiota has overwhelming effects on host physiology and behavior (Fig. 1), it can be an important missing piece in the POLS concept. In mammals, the composition of the gut microbiota can affect host aggressiveness and anxiety levels, which drive variation in personality traits, and thus in life history (Amato 2016). Furthermore, by mediating the amount of energy extracted from food and the metabolic rate, gut microbes can directly affect tradeoffs between life-history traits. In this way, a microbiota promoting a high metabolic rate would be expected to coevolve with a fast pace of life. In *Daphnia*, the gut microbiota is an important factor mediating the tradeoff between growth, reproduction and survival (Callens et al. 2016). Indeed, while an increase in food quantity significantly increases growth rate and reproduction while decreasing survival in conventionally reared individuals, this effect is less pronounced in germ-free animals. Another argument supporting the idea that gut microbiota mediates life-history strategies is that gut microbiota composition changes across the life cycle of an individual, in diverse species from insects (Chen et al. 2016) to humans (Kostic et al. 2013). Especially, life-history processes such as growth and reproduction, which require additional energy, have been associated with important shifts in the composition of the gut microbiota, suggesting that gut microbiota acts as a buffer against variation in metabolic demands across the life cycle (Kostic et al. 2013, Amato 2016). In humans and mice, for example, shifts in the maternal microbiota have been associated with adaptation to pregnancy, with an increase in gut bacteria promoting fat deposition and energy harvest, which can help mothers nourish their children (Moeller et al. 2016). Furthermore, the transfer of this maternal gut microbiota to the newborn during vaginal delivery can provide the neonate with immediate access to microbiota that allow maximal energy harvest during the incipient hours of life. Gut microbes may thus be

an important mediator of resource allocation strategies, both within the life cycle of an individual (i.e. the gut microbiota facilitates the adaptive adjustment of resource allocation pattern), and on evolutionary time scales (i.e. the evolution of different life-history strategies across species may be associated with the evolution of different gut microbiota compositions).

The multilayered structure of the gut microbiota and its consequences for host fitness

Vertical versus horizontal symbiont transmission

Unlike intracellular symbionts, which are strictly vertically transmitted to the embryo, gut associated microbes are mainly acquired during and after birth via horizontal transfer from the surrounding environment (Broderick and Lemaitre 2012, Amato 2016). In many species, like the gypsy moth or the cabbage white butterfly, gut bacterial communities are highly dependent of food-related bacteria and are mainly composed of widespread environmental taxa that opportunistically colonize the gut (Engel and Moran 2013). "Generalist" bacteria from the soil were shown to successfully colonize the gut of diverse species including the worm *C. elegans* (Engel and Moran 2013), bean bugs (Kikuchi et al. 2012) and mice (Seedorf et al. 2014). These environmental bacteria thus represent an important pool of genetic and functional diversity for the gut microbiota.

Although gut symbionts are not directly transmitted from the mother to the offspring during embryogenesis, females sometimes display sophisticated mechanisms for inoculating progeny with microbial symbionts via vertical transmission, enabling long-term associations (Engel and Moran 2013). For example, in some insects, like the Kudzu bug *Megacopta cribraria*, juveniles acquire their gut symbionts from bacterial capsules left by their mother (Hosokawa et al. 2008, Ezenwa et al. 2012). In other insects, such as flies and butterflies, the eggshell is contaminated with bacteria derived from adults. In the greater wax moth *Galleria mellonella*, bacteria carrying a fluorescent label cross the gut epithelium of mothers to reach the ovaries to be deposited on the egg surface (Freitak et al. 2014). After hatching, larvae ingest the chorion of embryos and thus acquire the bacteria coating them (Broderick and Lemaitre 2012). In viviparous species, including humans, symbionts can directly be transferred from mother to offspring through direct contamination by vaginal microbiota during parturition, or through the breast milk (Amato 2016). Parent–offspring interactions and parental care further facilitate inter-generational transmission of bacteria. Thus, an element of vertical transmission exists for the mammalian gut microbiota. In rats, most taxa detected in maternal feces can also be detected in their pups (Inoue and Ushida 2003), while in mice, gut microbiota composition is more similar between mother and offspring than between unrelated individuals, regardless of diet or genetic similarities shared by unrelated individuals (Ley et al. 2005).

The transmission of bacteria can also occur horizontally between conspecifics (Engel and Moran 2013). For instance in gregarious insects, such as cockroaches and crickets, exchanges of bacteria occur between individuals defecating and feeding in a common area (Engel and Moran 2013). Such transfer of bacteria can further occur between individuals from different species. For example, gut microbes from humans or zebrafish can successfully establish in the gut of mice (Seedorf et al. 2014). Social behaviors, such as coprophagy and trophallaxis, can greatly facilitate the acquisition and exchange of beneficial microbes. Recently, a distinct resident gut microbiota has been identified in bumblebees and honey bees that is not shared with related solitary bee species, suggesting that a stable association with the host can be facilitated by sociality (Martinson et al. 2011). Koch and Schmid-Hempel (2011) have shown that the successful establishment of this specific microbiota, which protects bumblebees against natural parasites, requires an exposure to the feces of nest mates after pupal eclosion. Transmission of beneficial gut bacteria could therefore represent an important benefit of sociality. The social context also shapes the establishment of mammalian gut microbiota, like in Chimpanzees, in which individuals from the same community have more similar microbiota than with individuals from different communities (Degnan et al. 2012).

Core versus flexible microbiome

Even when acquired independently at each generation, gut communities are not random assemblages of bacteria from the food or local environment (Engel and Moran 2013). The composition of gut microbiota strongly differs from that of the surrounding environment, even in filter-feeding species like *Daphnia*, which are continuously in close contact with the bacterioplankton (Freese and Schink 2011, Berg et al. 2016). From the available microbial pool, bacteria can be selectively recruited in the gut, depending on host immunity and genetic background, as well as on complex interactions between microbes, host physiology and environmental conditions (Spor et al. 2011, Engel and Moran 2013). In a recent paper, Shapira (2016) highlights the multilayered structure of the gut microbiota. On the one hand, a core microbiota of host-specific microbes is assembled from diverse environments and determined by host genetic factors. Although only part of these microbes may be strictly beneficial to the host, they probably contribute to essential functions or provide long-term adaptation to stable features of the niche (e.g. herbivory). On the other hand, a flexible pool of microbes depends on environmental diversity and on external conditions. This flexible pool of microbes can vary within the life cycle of an individual, and can thus exhibit high inter-individual variation. This variation may be either beneficial (e.g. diet-induced shift in the gut microbiota, that may increase digestion efficiency), or detrimental to the host (e.g. in the case of gut microbiota dysbioses responsible for diseases in humans). The gut microbial community thus comprises diverse microbes, some more adapted to their host, others generalists, or transients, representing a broad spectrum of potential contributions to host fitness.

The existence of a core microbiota, modulated by host-dependent processes, has been identified in a number of species, from insects to mice and humans (Shapira 2016). For instance, in *C. elegans*, individuals raised in diverse microbial environments reproducibly assemble similar microbiota (Engel and Moran 2013, Berg et al. 2016). Similarly, in mice, transplant experiments reveal that, despite highly dissimilar input communities, the output gut microbial communities of recipient mice cluster

together, systematically excluding clades that prosper poorly in the mouse gut (Seedorf et al. 2014). An increasing number of studies are stressing the importance of host genetic background in structuring this core gut microbiota. In mice, for example, the gut microbial composition differs between genetic lines (Buhnik-Rosenblau et al. 2011), and between mice that are genetically predisposed or not to obesity (Ley et al. 2006, Turnbaugh et al. 2006). In humans, monozygotic twins living separately exhibit more similar gut microbiota than domestic partners (Zoetendal et al. 2001), and monozygotic twins share more of the same types of microbes than dizygotic twins (Goodrich et al. 2014). Interestingly, the study of Goodrich et al. (2014) revealed that the most heritable bacterial taxa within the human gut microbiota are those associated with important metabolic functions, such as fat deposition and weight gain. There is increasing evidence that host genetic control over the gut microbiota relies on the intercession of the immune system (Ostaff et al. 2013). Shotgun metagenomics data from the Human Microbiome Project has e.g. revealed associations between microbiota composition and host genetic variation, especially in genes involved in immunity pathways (Blekhman et al. 2015). In many species, the balanced relationship with microbes depends on a complex and multileveled intestinal barrier that involves an intricate immune strategy network (Box 2). The intestinal epithelium, as the outermost cell layer, constitutes the first line of defense, ensuring the elimination of pathogens while maintaining a coexistence with mutualistic partners (Belkaid and Hand 2014, Gilbert et al. 2015). AMPs (Box 1), natural antibiotics produced in the gut of most animals, are of particular importance in shaping the gut microbiota, in both vertebrate and invertebrate species (Franzenburg et al. 2013, Ostaff et al. 2013, Tasiemski et al. 2015). When their expression is reduced, either experimentally or in the context of immune deficiencies, it results in a dramatic alteration of gut microbial communities (Ostaff et al. 2013). Although their mode of action does not rely on the recognition of specific molecules at the cell surface of microorganisms, AMPs control symbiosis by selectively killing specific bacterial taxa, while being inoffensive for other ones (Tasiemski et al. 2015). The interplay between the suite of AMPs expressed by the host and profile of AMP susceptibility of different community members thus likely play an important role in shaping the composition of the gut microbiota (Broderick and Lemaitre 2012).

The flexible microbial pool is largely influenced by the diet, which can induce important changes in the microbial composition over short periods of time (Spor et al. 2011). For example, in humans, historical shifts from plant-based diet to meat-based diet have been followed by strong shifts in the gut microbial community, with an increase in animal protein metabolizing bacteria and a decrease in bacteria that metabolize dietary plant saccharides (David et al. 2014). Furthermore, the infant gut community assembly undergoes discrete steps of bacterial succession punctuated by life events. The earliest microbiome is enriched in genes facilitating lactate utilization and genes involved in plant polysaccharide metabolism are present before the introduction of solid food, priming the infant gut for an adult diet. The introduction of solid food in the diet, however, causes a dramatic shift in the community, with an enrichment of genes associated with carbohydrate utilization, characteristic of the adult microbiome (Koenig et al. 2011). The nutritional status of the host can further indirectly alter the structure of the microbiota, by affecting the immune system, through both metabolic requirements and direct sensing of food-derived metabolites (Ostaff et al. 2013). In addition to these diet-mediated effects, the persistence and establishment of exogenous bacteria in the gut depends on among-microbes interactions. These interactions can be direct, including e.g. competition for resources and production of biologically active molecules like AMPs (Box 2), or indirect, mediated by the host immune system (Kamada et al. 2013, Douglas 2015). For instance, one microorganism can stimulate (or suppress) the production of immune effectors with high activity against other microorganisms, so depressing (or promoting) the abundance of the latter, as observed in the tsetse fly (Douglas 2015).

The gut microbiota as a community of interacting species: priority effects, cooperation and selfishness

Recent years have seen an astonishing paradigm convergence between microbiologists, physiologists and evolutionary ecologists working on the gut microbiome regarding microbiome dynamics at the scales of individual hosts and host populations. In mammals (mice and humans mostly), the process of gut colonization has proved very similar to ecological successions (Koenig et al. 2011, Seedorf et al. 2014). The formation of the gut microbial community can be viewed as a colonization process, in which the initial adhesion of early colonizers to host-derived structure shapes the metabolic milieu in a manner permissive for establishing a more diversified collection of bacterial species (Hooper and Gordon 2001). For example, the formation of biofilm modifies spatial structure and chemical environment, thus influencing the establishment of other species (McNally and Brown 2015). In addition, because of syntrophic interactions between community members, what species of bacteria colonizes the gut will have an important effect on the final mixture of fermentation end-products, and will thus determine which syntrophs are likely to flourish, which in turn will affect the growth and activity of other bacterial fermenters (Fischbach and Sonnenburg 2012). Priority effects in community assembly theory suggest that the successional pattern of a community can be strongly influenced by the taxa that are first to establish (Law and Morton 1993). As the gut microbiota develops from an initial maternal inoculation at birth, there is likely a maternal effect on its developmental trajectory (Amato 2016). Supporting this hypothesis, infants delivered via C-section, which are inoculated by skin microbes, exhibit patterns of gut microbial succession different from those of infants born vaginally (Dominguez-Bello et al. 2010). The community assembly may further be affected by host genotype, which determines how susceptible a host is to initial colonization by particular microbes (Amato 2016).

As in other communities, interspecies interactions such as cooperation or competition exist within the gut microbiota, and may have an impact on the structure of the whole microbial community (Porter and Martens 2016). For example, Rakoff-Nahoum et al. (2016) revealed that the prominent human gut symbiont *Bacteroides ovatus* releases large

Box 2

Gut microbiota and host immunity (see also Fig. 4 adapted from Renz et al. 2012)

After birth, developmental (such as formation of intestinal crypt and crypt based Paneth cells) combined with microbial signals issued from the bacterial colonization of the intestine, drive significant changes of the intestinal epithelial cells which start to rapidly proliferate and to produce a complex mucus layer protecting the gastrointestinal tract from potential invasion (Renz et al. 2012). The gut microbiota shapes the innate and adaptive immune system which in turn controls the microbiota from over-proliferation and invasion of the intestinal tissue. Immunity is the guardian of the host gut environment, it coordinates cellular and biochemical responses through the epithelial cells, creating a robust equilibrium between the healthy host and its normal microbiota (i.e. intestinal homeostasis). Many of these mechanisms are controlled by PRR signaling (Box 1) induced by the recognition of the MAMPs produced by commensal microbes. The intestinal reactivity to microbial sensing takes place after birth after a period of tolerance of the microbiota. In the small intestine, Paneth cells derived AMPs (Box 1) such as defensins are induced upon PRR stimulation by commensal microbes. AMPs are important in controlling and selecting microbiota as evidenced in defensin-deficient mice (Salzman et al. 2010). Local concentrations of microbiota-derived metabolites also build the myeloid landscape not only in intestinal tissues, but also systemically (Zhang et al. 2015). Microbiota driven modification in the myeloid cell pool increases the host susceptibility to infection, sepsis, allergy and asthma (Hill et al. 2012, Fonseca et al. 2015, Thaiss et al. 2016). Conversely, immune signals stemming from commensal microorganisms also influence the proper tissue-dependent functioning of innate lymphoid cells (ILC) (Honda and Littman 2016). Tissue resident ILCs integrate signals from microbiota to link and refine the innate and adaptive response at the tissue level. ILC3 1) produces Tumor Necrosis Factor β (TNFβ) which is crucial for microbiota homeostasis and production of IgA, 2) contributes to the differentiation of T cells and B cells and 3) promotes the expansion of T_{reg} cells (Box 1) in the intestinal mucosa. The production of IgA by the gut plasma cells is the result of the mucosal adaptive immunity (Honda and Littman 2016). Plasma cells producing IgA (Box 1) are only generated after birth to provide SIgA to the lumen, maternal SIgA is provided by breast milk during the postnatal period. The gut microbiota modifies the accumulation of IgA-expressing cells as well as the level and diversity of IgA in the lumen (Thomas 2016). Interestingly, some members of the microbiota, such as *Sutterella* species, reduce the level of IgA by degrading them (Moon et al. 2015) while others such as segmented filamentous bacteria (SFB), which colonize the surface of the epithelium, activate their production through the T-cell independent pathway (Ivanov and Littman 2010). The type of bacteria targeted by IgA differs according to the diet of the host (Kau et al. 2015) supporting the trialogue that exists between the host, the microbiota and environmental factors. This trialogue is also sustained by the 'hygiene hypothesis' (Box 1).

amounts of inulin (a dietary fiber) digestion products via a pair of dedicated cross-feeding secreted enzymes that are unnecessary for its own use of inulin. These enzymes allow for cooperation with cross-fed species (e.g. *Bacteroides vulgatus*), which provide benefits in return. Other inulin-degrading bacteria like *Bacteroides fragilis*, however, exhibited a more selfish behavior, generating far fewer inulin degradation products, and did not promote the growth of non-inulin degrading bacteria. Such selfish behavior may thus lead to dysbioses in the gut microbiota, with potential negative impacts on the host's fitness, as exemplified by the microbiota disruption associated with inflammatory bowel disease (IBD) in humans (Tailford et al. 2015). Some bacteria in the gut are able to degrade mucin, a protein that is abundant in the outer layer of mucus covering the gastrointestinal tract epithelial cells. The degradation of mucins results in the liberation of sialic acid, that becomes available as a nutrient source for other bacteria of the community (Tailford et al. 2015). It was shown that *Ruminococcus gnavus*, a mucin-degrading bacteria that is present in the gastro-intestinal tract of 90% of humans, but is over-represented in IBD, does not release sialic acid, but another compound that cannot be used by other bacteria. This 'selfish' behavior of mucosal glycan utilization could contribute to the dysbioses reported in ulcerative colitis and Crohn's disease patients.

Interaction network between bacterial, viral, archaeal and fungal members of the community

Although most information on gut microbiota in the literature concerns bacteria, they are not the only inhabitants of the guts. Other members of the community, such as archaea, fungi and viruses, likely play a crucial role in the structure and functioning of the gut microbiota (Fischbach and Sonnenburg 2012, Ogilvie and Jones 2015). The different members of the community are for example engaged in syntrophic interactions, whereby each microorganism lives off the metabolic or waste products of its predecessor. In such association, the producer is dependent on the activities of the consumer, and vice versa (Dolfing 2014). Even in cases where two communities harbor the same bacterial strains (i.e. the same set of bacterial genes), the functions carried out by bacteria may differ greatly, depending on the presence or absence of other community members, such as archaea (Fischbach and Sonnenburg 2012). As an example, fermentation of dietary fiber involves syntrophic interactions between microbes linked in a metabolic food web: primary bacterial fermenters (i.e. Bacteroidetes and

Firmicutes) generate SCFAs, other organic acids (e.g. formate) and gases such as hydrogen (H_2) and carbon dioxide (CO_2). Accumulation of H_2 inhibits bacterial NADH dehydrogenases, thereby reducing the yield of ATP. This H_2 is removed by means of archaeal methanogenesis, resulting in improved fermentation efficiency (Samuel and Gordon 2006). Furthermore, colonization of germ-free mice with *Bacteroides thetaiotaomicron* (bacterial fermenter), with or without the archaea *Methanobrevibacter smithii*, revealed that the presence of *M. smithii* decreases the carbohydrate fermentation activity of *B. thetaiotaomicron*, while increasing the fermentation of fructans (fructose polymers), the by-products of which are used by *M. smithii* for methanogenesis (Samuel and Gordon 2006). *Bacteriodes thetaiotaomicron–M. smithii* co-colonization also produced an increase in host adiposity compared with mono-associated animals. These findings demonstrate a link between this archaeon, prioritized bacterial utilization of polysaccharides, and host energy balance.

Another component of the gut microbiome that is often neglected is the gut virome, which can be defined as the community of viruses associated with the gut microbial community. Although information on the gut virome is limited compared to that on gut bacteria, current evidence suggests that it may play an important role in modulating gut microbiota structure and function (Ogilvie and Jones 2015). Given the predominance of bacteria in the gut microbiota, the gut virome is usually dominated by prokaryotic viruses, i.e. bacteriophages, which mostly have a temperate lifestyle (i.e. they integrate into bacterial host chromosomes as prophages and propagate through lysogenic cycles, or exist as quiescent episomal elements without lytic replication) (Breitbart et al. 2003, Ogilvie and Jones 2015, Murall et al. 2017). These temperate phages are important for the exchange of genetic material between bacterial hosts (transduction), and themselves encode a rich functional repertoire that confers a range of attributes to their bacterial hosts, including toxin synthesis, production of virulence factors and metabolic flexibility (Minot et al. 2011, Reyes et al. 2012, Modi et al. 2013, Ogilvie and Jones 2015). Metagenomic surveys of gut viruses have revealed an important number of genes involved in energy harvest (e.g. carbohydrate and amino acid metabolism; Reyes et al. 2010, Minot et al. 2011), suggesting that phages may confer important metabolic capabilities to their bacterial host, which may in turn indirectly affect animal host metabolism. The genes encoded by the viral genome may thus expand the niche of gut bacteria, and strongly affect the dynamics of the gut microbial community. Furthermore, phages may constitute a genetic reservoir for bacterial adaptation, safeguarding important functions and facilitating the recovery of the community in case of disruption (Ogilvie and Jones 2015). Consistent with this hypothesis, a study performed in mice revealed that antibiotic treatment leads to the enrichment of phage-encoded genes that confer antibiotic resistance, as well as genes involved in gut colonization and growth, indicating that the phageome becomes enriched for functionally beneficial genes under stress-related conditions (Modi et al. 2013; but see Enault et al. 2017 on the possible overestimation of phage-related antibiotic resistance in viral metagenome studies). Phages may thus be an important factor contributing to the stability and the resilience of the gut microbiota.

The role of the microbiome in host ecology and evolution

Effects of variation in gut microbiota at the individual level

Unlike the host genome, the flexible microbiome can change rapidly as a result of modifications in either the composition of the microbial community or individual microbial genomes, resulting in modified transcriptomic, proteomic and metabolic profiles (Sommer and Bäckhed 2013). Gut microbiota thus represents an important source of metabolic flexibility that can allow its host to rapidly acquire a phenotype that is adapted to current environmental conditions. As such, the gut microbiota might be a key, yet understudied, factor driving fast acclimatization to new environments and resistance to habitat disturbance. This can be particularly important in the current context of global climate change and of intense anthropogenic activities, which impose fast and drastic environmental changes to which organisms do not necessarily have the time to adapt (Gilbert et al. 2015). Contrary to vertically transmitted symbionts, which promote coevolution and optimization of host–symbiont interactions, but can also prevent interactions of symbionts with the environment and reduce their adaptive potential, the gut microbiota has the unique property to be highly flexible and interactive. Through horizontal transfer and recruitment of bacteria from the environment, the gut microbiota represents a huge genetic and functional diversity, with a high potential for adaptation, and provide the host with an almost unlimited set of metabolic functions (Shapira 2016; Fig. 2). The advantage of exchanging symbionts with the environment is illustrated by the bean bug *Riptortus pedestris*, which has developed insecticide resistance through the acquisition of insecticide-degrading bacteria from the soil (Kikuchi et al. 2012). In addition to this ability to recruit environmental bacteria, the gut microbial community can rapidly respond to changes in host diet or environmental conditions through variation in the relative abundance of resident bacteria, a process which is facilitated by the high genetic diversity and the short generation time of gut microbes (Bordenstein and Theis 2015). For example, in the herbivorous desert woodrat, animals fed toxic plants show a shift in the composition of their gut microbial community, with an increase in the abundance of bacterial genes that metabolize toxic compounds, compared to animals fed non-toxic plants (Kohl and Dearing 2012). The responsiveness of the gut microbiota is further increased by the fast evolution of bacteria, through either horizontal gene transfer or mutations that increases both genetic and functional diversity of the microbial community (Dillon and Dillon 2004). For example, in Japanese populations that regularly consume red algae in sushi and other foods, the gut bacterium *Bacteroides plebeius* acquired the capability, through lateral gene transfer from environmental marine bacteria, to degrade the polysaccharides of marine red algae (Hehemann et al. 2010). Mutations in members of the gut microbiota can also change interactions with parasites. For example, experimental evolution of the tripartite interaction between *C. elegans* and two of its pathogens revealed that the low pathogenic bacteria *Enterococcus*

faecalis rapidly evolved the ability to suppress its competitor, the highly pathogenic *Staphylococcus aureus*, through mutations associated with an increased production of antimicrobial superoxide (King et al. 2016).

The microbial facilitation of host dietary flexibility and resistance to pathogens may support host expansion into new habitats. Even in humans, dispersal and the ability to colonize the most extreme regions on Earth might have been mediated by gut symbionts. In this sense, the gut microbiota can contribute to determining the geographical range in which a species will be able to establish. In primates, such as howler monkeys, microbiota of species with distinct ranging patterns suggest that more diverse gut microbial communities are associated with wider geographical distribution, supporting the idea that more flexible microbiota increase colonization abilities (Amato 2016). In addition, when different species, or different populations of an invasive species, come into contact, the opportunity for horizontal transmission of gut symbionts arises, which can result in ad hoc acquisition of new traits, which in turn can enhance the invasive potential of host species (Feldhaar 2011). Such hypotheses, however, remain to be investigated.

Not all variation in gut microbiota composition is however beneficial to the host. Given that the fitness of hosts and gut microbes are not always aligned, a conflict might exist and result in a negative outcome for the parties involved (Wasielewski et al. 2016). The western diet, characterized by a paucity of fermentable carbohydrates, has for example been shown to select for a community of microbes that eat host-derived carbohydrates found in the intestinal mucus layer, resulting in mucus layer thinning. By increasing microbial colonization and translocation into host tissues, such mucus thinning can interfere with the normal absorptive function of epithelial microvilli, and induce inflammation and colitis (Sonnenburg and Sonnenburg 2014, Wasielewski et al. 2016). There is increasing evidence that dysbioses in the gut microbiota are involved in human diseases, such as inflammatory bowel disease and obesity (Belkaid and Hand 2014).

The core gut microbiota as an extended phenotype that promotes adaptation

Although strong empirical evidence is still lacking, the gut microbiota is increasingly hypothesized to contribute to host evolution and adaptation to the environment (Gilbert et al. 2015). The strongest evidence of symbiont-mediated adaptation comes from intracellular, vertically transmitted, endosymbionts in herbivorous species, which have been shown to mediate host-plant specialization. For example, the pea aphid *Acyrthosiphon pisum* encompasses ecologically and genetically distinct host races that are locally adapted to their respective host plants (red clover or alfalfa), while being unable to reproduce on the other host plant. This instance of host-plant specialization has long been attributed to chromosomal loci of the aphid, but recent studies revealed that it is in fact mainly mediated by bacterial endosymbionts (Tsuchida et al. 2004). Information concerning the role of gut microbes in host adaptation is more limited, and would deserve further investigation in future studies. The gut microbiota was shown to mediate the ability of herbivorous species to feed on toxic plants, as exemplified by the coffee borer (Ceja-Navarro et al. 2015) and the desert woodrat (Kohl et al. 2014). Although these studies suggest that gut symbionts constitute a crucial factor mediating host plant specialization in herbivorous species, they do not document the evolutionary processes leading to such adaptation. One of the few studies documenting the role of gut symbionts in the process of adaptation is that of Kohl et al. (2016), which shows that experimental evolution on bank voles *Myodes glareolus* for increased herbivorous capabilities results in the concomitant evolution of gut microbial communities. Another example is that of the western corn rootworm, in which the gut microbiota was shown to mediate adaptation to human-driven landscape changes. This major crop pest, that has been controlled via annual rotation between corn and non-host soybean, has evolved to a "rotation-resistant" variant with a shifted gut microbiota composition that increases tolerance to anti-herbivory defenses of the new host plant (Chu et al. 2013).

Selection on symbiont-mediated traits promoting adaptation to local environmental conditions can result in a rapid evolution of host genes (especially immune genes) involved in acquisition, control and tolerance of beneficial symbionts, allowing for an indirect co-inheritance of nuclear genes and microbes (Vavre and Kremer 2014, Bordenstein and Theis 2015). While genes involved in immune defense are among the fastest evolving in the genome of many species, as a result of a coevolutionary arms race between hosts and pathogens (Decaestecker et al. 2007), genes encoding AMPs have been shown to evolve more slowly than average and to exhibit high rates of non-synonymous polymorphisms (Unckless and Lazzaro 2016). Studies performed in both invertebrates (Unckless et al. 2016) and vertebrates further revealed that these non-synonymous mutations strongly affect the antibacterial activity of AMPs and thus resistance to bacterial infection (Tennessen et al. 2009). In diverse species including *Drosophila*, marine mussels, frogs, birds and humans, AMP polymorphism has been suggested to be maintained through balancing selection, driven by fluctuation in natural selective pressure over time and/or geographical space (Tennessen and Blouin 2008, Unckless and Lazzaro 2016). This may be mediated by shifting diversity of pathogens, as well as by correlated life-history costs of overactive immune systems (Unckless and Lazzaro 2016). For example in frogs, differences in the expression and activity of antimicrobial skin peptide across geographically distinct populations was suggested to reflect current and past encounters of these populations with different skin pathogens (Tennessen and Blouin 2008). As AMPs have a role in the control of gut microbiota, it might also be that balancing selection on AMPs results from fluctuating environmental conditions that exert different selective pressures on the gut microbiota composition. Variation in AMPs could thus contribute importantly to the ability of animal hosts to adapt to changing environments through adaptive changes of their symbiotic communities. In addition to act on immunity, selection can further act on genetically heritable traits or behaviors, such as egg-smearing or coprophagy, which encourages the acquisition and/or vertical transmission of specific beneficial symbionts. Although not acting on the gut microbiota itself, such selective processes would result in heritable microbial traits (Amato 2016).

Gut microbiota can promote divergence of host lineages and speciation

Selection for symbiont-mediated traits adapted to the local environment can lead to dramatic changes in gut microbial communities over short periods of time, within both individuals and populations. Consequently, selective pressure on the host to control and tolerate beneficial symbionts can change, fostering a rapid evolution of host immune or developmental genes. Hybrids of populations exhibiting different gut microbial communities may thus suffer from a decreased fitness, favoring the emergence of post-zygotic barriers and differentiation between populations (Vavre and Kremer 2014, Shapira 2016). Consistent with this hypothesis, hybrids of two closely related *Nasonia* species with distinct microbiota are non-viable, and show altered microbiota. When reared under germ-free conditions, however, hybrid viability is restored (Brucker and Bordenstein 2012). Similarly, in *Drosophila* (Miller et al. 2010) and *Nasonia* (Chafee et al. 2011), hybrid sterility has been associated with the overproliferation of symbionts in male testes, which may reflect perturbation of the interaction between symbionts and genes involved in the control of symbiotic populations (Vavre and Kremer 2014). In addition, symbiont-mediated changes in host behavior, such as mate preference, may reduce gene flow between individuals or populations harboring different microbiota, fostering reproductive isolation (Vavre and Kremer 2014). Thus, divergence of host lineages living in different environments and species diversification may be facilitated by the microbiota.

Coevolutionary dynamics between hosts and their symbionts

Similarities in gut microbiomes are often observed between related species, suggesting a high specificity between hosts and their symbionts (Bordenstein and Theis 2015). A recent study in hominids suggests that multiple lineages of the predominant gut bacteria arose via cospeciation with humans, chimpanzees, bonobos and gorillas over the past 15 million years (Moeller et al. 2016). Especially, the clades Bacteroidaceae and Bifidobacteriaceae have been maintained exclusively within host lineages, and their divergence times are congruent with those of hominids, suggesting that nuclear and gut microbial genomes diversified in concert during hominid evolution. In contrast, for Lachnospiraceae, several between-host-species transfer events occurred since the common ancestor of the Hominidae. Interestingly, the Lachnospiraceae, unlike Bacteroidaceae and Bifidobacteriaceae, are spore-forming and can survive outside the gut, which may enhance their ability to disperse and transfer among host species. These results suggest that gut microbiomes are composites of cospeciating species, which are highly specific to their host, and independently diversifying bacterial lineages, which are less strongly linked to a particular host and can be shared among different host species. Similarly, in *Nasonia* wasps, the pattern of phylogenetic branching of gut symbionts were shown to mirror that of their host, a phenomenon that is sometimes referred to as "phylosymbiosis" (Brucker and Bordenstein 2012). However, as explained by Moran and Sloan (2015), phylosymbiosis should be interpreted with caution. It is indeed tempting to conclude that hosts and their gut symbionts have a shared evolutionary history, and that a coevolution occurred. By definition, coevolution requires that each lineage undergoes evolutionary change due to selective forces imposed by the other lineages. The reciprocal impacts on fitness and speciation are, however, usually not known, hence nothing really proves that cospeciation occurred. At the extreme opposite, organisms that do not interact at all could diversify in parallel if subjected to the same series of geographic isolation events. Another hypothesis is that codiversification reflects unidirectional selection. For example, as host lineages evolve, they may shift in their selectivity to pick-up bacteria from the environment, reflecting an evolution of mechanisms underlying microbial community assembly in the host, e.g. the evolution of host immune genes resulting in the recruitment of different symbionts from the environment. These hypotheses, however, remain theoretical, and would need to be tested experimentally. For example, germ-free animals from different host lineages could be exposed to a same microbial inoculum, to see if the community assembly differs among lineages. Reciprocal transplant experiments between related hosts could also be performed, to measure the impacts on both host and microbial fitness.

Generally, due to the fact that host–microbes interactions are labile and dependent on environmental conditions, the room for host–microbiota coevolution is thought to be more limited than for endosymbionts (Moran and Sloan 2015). Especially, co-evolution can only occur if the host-associated phase is predominant in the symbiont's life-cycle. The strength of selection on microbes to benefit their host, and on hosts to maintain a favorable niche for their symbionts, is expected to depend on the tightness of the mutualistic relation and on how the interests of the host and of the symbionts are aligned. From the symbiont perspective, microbe-mediated protection of the host can be directly favored when microbial fitness strongly depends on host fitness, such as when microbes are vertically transmitted, or when the host selectively recruit beneficial symbionts, e.g. through immunity. From the host perspective, behavioral or physiological traits involved in the recruitment and the maintenance of symbionts will be favored if the benefits provided by the symbionts outweigh any cost (Ford and King 2016).

When hosts and symbionts are tightly linked, both hosts and symbionts may become dependent on their mutualistic partner to ensure some functions, which may lead to gene and function loss, especially if the function is costly to perform. This function must provide an indispensable public good, necessitating its retention by at least a subset of the individuals in the community - one cannot play Hearts without a queen of spades (Black Queen hypothesis, BQH, Morris 2015). Any function that is both costly to perform and leaky (e.g. nutrient acquisition, biofilm matrix deposition, nitrogen fixation) is a potential target for function loss in the framework of the BQH (Morris et al. 2012). Many host-associated bacteria have e.g. lost the capacity to synthesize essential metabolites, such as amino acids, provided by their host or by other microbes of the community. Hosts have also evolved dependency on their gut microbes, sometimes resulting in the evolution of specialized anatomical structures aiming at housing the microbes and facilitating their activity (Engel and Moran 2013, Shapira 2016). Mammalian herbivores and sap-feeding insects have become

dependent on plant-degrading microbes, for which they have evolved specialized gut structures, such as the rumen that serves as a fermentation chamber in cows. Such adaptations arose independently during evolution, resulting in distinct anatomical structures, but resemble each other in microbial composition, suggesting that microbes are adapted to some shared functional characteristics of their niche.

Impacts of gut microbes on the eco-evolutionary dynamics of the host community

Recently, there has been considerable interest in the interaction of ecological and evolutionary dynamics in an attempt to understand them as coupled 'eco-evo' processes. Such eco-evolutionary feedbacks can occur at multiple levels, such as in demographic parameters, community composition, food webs, nutrient cycling and productivity (Hairston et al. 2005, Urban et al. 2008, Pelletier et al. 2009, Hiltunen and Becks 2014, Govaert et al. 2016, Hendry 2016). At the population level, natural selection and population dynamics are closely linked because both are affected by the birth and death of individuals. Thus, if natural selection acts on a trait through survival or reproductive success, it will leave a population dynamical signature. At a larger scale, changes in the genetic composition of a species can affect its fitness dependencies with other species (e.g. through trophic interactions or competition) and hence alter the ecological dynamics of an ecosystem, and vice versa. Given that the gut microbiota is a crucial mediator of host physiology and behavior, and thus of life-history, we here propose that it may be an important piece missing in eco-evolutionary dynamics theory.

Through its effects on reproduction, survival and dispersal, the gut microbiota can affect population dynamics and genetic diversity, and thus play a role in eco-evolutionary feedbacks. Moreover, by mediating interactions between hosts and other organisms, such as parasites, predators or plants in herbivores, gut symbionts can play a direct role in the process of coevolution between these species. So far, most models and laboratory experiments investigating coevolution processes, such as the Red Queen hypothesis, have been based on pairwise-species interactions (Decaestecker et al. 2007, 2013, Salathé et al. 2008, Lively et al. 2014). However, in natural environments a lot of factors, such as complex species interaction networks may constrain coevolution, and should thus be more systematically considered in future research (Koskella and Brockhurst 2014). By combining knowledge of defensive microbe–parasite interactions at the mechanistic level with evolutionary theory, Ford and King (2016) predict how defensive microbes might alter the evolution of host and parasite traits, such as resistance and virulence, which in turn might greatly affect host population dynamics. First, a direct coevolution between defensive microbes and parasites would provide 'real time' control of the infection, whereby evolutionary changes in parasites are met by rapid reciprocal evolution in defensive microbes. Second, given that defensive microbes protect hosts from parasite-induced fitness costs, they could reduce selection for costly immune or behavioural defense mechanisms in the host. Consistent with this, *Trachymyrmex* ant populations harbouring protective antibiotic-producing bacteria exhibit reduced cleaning behaviour (Fernández-Marín et al. 2009). Over evolutionary time, a host may thus become dependent on microbe-mediated protection, a hypothesis that has been invoked to explain the loss of immune genes in pea aphids and honeybees (Gerardo et al. 2010, Kaltenpoth and Engl 2014). Finally, gut symbionts can shape the evolution of parasite virulence, through mechanisms similar to interactions occurring between co-infecting parasites, such as resource competition, interference competition or immune mediation. This principle may be extended to interactions other than host–parasite coevolution, such as plant–insect coevolution, and may be valuable for a more realistic understanding of coevolutionary processes.

Through the horizontal transfer of bacteria, species or populations of the same species can affect the fitness, and thus potentially the evolution, of each other (Feldhaar 2011). Gut symbionts can also act as ecosystem engineers and contribute to modifying the biotic and abiotic environment of their host, potentially affecting other species of the community. For example, by contributing to food digestion, gut symbionts play a major role in the food web and can contribute to the stability of the whole community. If we push this reasoning to its extreme, one could even argue that gut microbes might play a non-negligible role in shaping landscapes. For instance, the African savanna ecosystem, characterized by grasses and small dispersed trees, is controlled by the climate, but also by the dynamics of herbivorous animal populations, which are themselves controlled by predators. Without the appropriate set of gut symbionts, herbivorous species would be unable to consume plants, which would result in very different vegetation types, and savanna might thus not exist, replaced by more arborous vegetation. In the same way, by allowing soil animals to decompose dead organic matter, gut microbes are major players of nutrient recycling. More precisely, the microbiome can change host phenotype and, via an eco-evolutionary loop (Fig. 3), it can also affect the environment via niche diversification and construction. In particular, developmental symbiosis and plasticity – the ability of larval or embryonic organisms to react to environmental input with a change in form, physiology or behavior – has been described as leading to ecosystem engineering, given that such plasticity can provide the phenotypic ranges within which animals can accommodate to environmental challenges such as climate change (Gilbert et al. 2015).

As recently highlighted in a review by Amsellem et al. (2017), the gut microbiota likely plays a role in the process of biological invasions, i.e. when non-indigenous species expand their range in their newly introduced habitat, inducing perturbations in the structure and population dynamics of the recipient community. The microorganisms hosted by alien species can for example facilitate invasion, if they provide a selective advantage for the invasive hosts over native ones (Lymbery et al. 2014, Strauss et al. 2012, Amsellem et al. 2017). This may occur, for instance, when alien species harbor mutualist symbionts that are more efficient than those of native populations, or when they harbor pathogens that are tolerated by the invasive carrier but can affect or kill native competitors in newly colonized habitats (i.e. "spill-over" phenomenon, resulting from the fact that parasites are more virulent in new hosts because of a lack of evolved immunological resistance; Power and Mitchell 2004, Amsellem et al. 2017). Parasite spillover has for example

been shown to occur between commercially produced bumblebees and honeybees and wild bumblebee populations, contributing to the decline of the latter (Fürst et al. 2014, Graystock et al. 2014). Conversely, the lack of appropriate mutualistic symbionts can be a major constraint to the establishment of alien species. The success of exotic invaders may also depend on the microorganisms hosted by native populations (Amsellem et al. 2017). While the horizontal transfer of beneficial mutualistic symbionts from native species can facilitate adaptation of alien species to their new habitat, the acquisition of native parasites may hamper the invasion process. The loss of microorganisms sometimes observed in introduced populations, resulting from either sampling effect (introduced hosts are by chance not infected) or an absence of conditions required for microorganisms growth in the introduction area, can further affect their invasiveness (Amsellem et al. 2017). The loss of pathogens has for example been shown to facilitate invasion in mosquito (Aliabadi and Juliano 2002). In contrast, the loss of mutualists is expected to negatively affect the invasive potential of alien species (Amsellem et al. 2017).

Perspectives for future research on the role of the gut microbiota in ecology and evolution

Shifting from a proximate to an integrative view of host–microbiota interactions

Although studies on gut microbiota are currently booming, many essential details about the reciprocal interactions between host physiology, gut microbiota and environmental factors remain to be discovered. The gut microbiota is often considered as a single entity, even sometimes referred to as a novel "organ" (Guinane and Cotter 2013), a representation that is erroneous and may lead to a certain confusion. The gut microbiota is indeed far more complex, and should rather be considered as an ecological community involving an interacting network of species. The diverse players of this network include microorganisms (e.g. bacteria, yeast, fungi), viruses and bacteriophages, but also host cells (as the physical environment and as resources) and immune factors (Murall et al. 2017). This community is strongly dependent on external (e.g. diet) and host (e.g. genetic background) factors, thus leading to high inter-individual variability (Murall et al. 2017). Given this complexity, studies are often limited to only a subset of players involved in gut microbiota functioning, hence the mechanistic scheme underlying the establishment and the transmission of gut symbionts, as well as their impacts on host physiology, remains incomplete. Deciphering these mechanisms is nevertheless very important to understand the evolutionary dynamics of host–microbiota interactions. There is thus a need for more integrative studies that take all these factors into account, using a biological model amenable to carefully controlled experiments. Excellent resources are available to study such questions in vivo, especially in mice and *Drosophila*, in which germ-free animals can easily be obtained, while the existence of a large number of inbred isogenic lines allows controlling for the host genetic background. In addition, their genome and their immune system are well characterized, making these species favorite models in the study of interactions between host immunity, genotype and microbiota (Hooper et al. 2012). Other species, like the freshwater crustacean *Daphnia*, may offer an interesting alternative to the mouse and *Drosophila* models. Indeed, their high experimental tractability, short life cycle, clonal reproduction and high responsiveness to environmental stressors, combined with the possibility to easily manipulate their gut microbiota and an absence of ethic restrictions, provide a unique opportunity to study the interactions between genotype, innate immune system, environment and microbiota, with a high degree of experimental control (Callens et al. 2016).

To connect microbiota studies with the coevolution of hosts and their symbionts, we think it is also necessary to sharpen our observations of microbiota dynamics. Even though the processes by which microbiota can be horizontally or vertically transmitted from one host to the next are the same for all its constituting microbes, the ability of microbes to infect new hosts may vary (Seedorf et al. 2014). Moreover, different microbes' fitness may benefit differently from the within-host and environmental parts of their life cycles. Caution should be warranted when interpreting the results of host-microbiota coevolution experiments as different means of microbiota transfer and different pace of host life cycle might positively select different microbial taxa. Despite the powerful genomic tools available, our knowledge regarding the functional capacities of gut microorganisms and how their genetic variation influences their ability to colonize the gut remains limited. Recently, Yaung et al. (2015) used an approach termed "Temporal FUnctional Metagenomics sequencing" (TFUMseq) to identify genetic regions that increase microbial fitness in the mammalian intestine, and thus contribute to colonization success. To determine if constitutively expressed genetic loci from *Bacteroides thetaiotaomicron* modulate the ability of *Escherichia coli* to colonize the mammalian intestine, they inoculated germ-free animals with *E. coli* harboring plasmids from a library covering the whole genome of *B. thetaiotaomicron* and tracked the abundance of *B. thetaiotaomicron* genes over time by sequencing DNA samples from fecal pellets at different time points. Applied to different recipient mouse strains, or to already colonized animals, this method may allow disentangling the influence of host organism genome, or of other microbes, on the competitive profile of the targeted bacterial genes.

Estimate the fitness returns from the symbiotic relationship for both hosts and symbionts

To predict the evolutionary consequences of host–microbiota interactions, it is important to determine how far both hosts and symbionts draw a benefit from this relationship. Microbial fitness is still poorly assessed in the literature, which impedes any exploration of the correlations between host and symbiont fitness. Methods do exist to measure and compare microbial fitness between hosts, in space or in time, e.g. by measuring infectivity in time-shift experiments (Koskella 2014). Collecting and freezing microbiota at different points during a host–symbiont coevolutionary experiment, together with reciprocal microbiota inoculations, could help assess local adaptation of symbionts to their host following the general methodology of Red Queen studies (Gandon et al. 2008).

From the host point of view, there is generally a lack of connections between evolutionary and functional questions

on host–microbiota interactions. Although genomic tools provide important information on the proximate mechanisms through which microbiota may affect host fitness, they mostly do not quantify the consequences on fitness itself. Hence, in addition to studying how microbiota functional profile changes in response to the environment and how it affects host physiology, it is necessary to study how such changes affect host life-history traits such as survival or reproduction. Information we have regarding fitness consequences for the host mainly comes from the comparison between germ-free and conventionalized animals, and remains limited and sometimes inconsistent. For example in *Drosophila*, the microbiota can increase lifespan in some studies (Brummel et al. 2004) while other studies demonstrate either negative effects (Petkau et al. 2014, Clark et al. 2015) or no effect (Ren et al. 2007) on lifespan. One possible explanation to such conflicting conclusions is that the composition of the gut microbiome varied between studies, likely due to differences in rearing conditions (Heintz and Mair 2014). Such variation in experimental conditions can have a great influence on the results obtained, thus hampering the determination of fitness consequences of gut microbiota. There is thus a need of standardized microbiota for reproducible experiments, otherwise we may face confusion from variable results attributable to differences between experimental microbiota (Hooper et al. 2012). Moreover, cross-taxon studies investigating the functional consequences of gut microbiota across host phylogeny can provide interesting information on the link between microbiota, host ecology and life history. So far, such studies have focused on vertebrates (Ley et al. 2008, Sullam et al. 2012). There would be much to learn from generalizing these studies to other groups, especially in order to assess the relative importance of diet and habitat on microbiota, and reciprocally of microbiota on host diet and habitat. In the same vein, the link between microbiota and the time since last shift in diet in the focal host species has not been much investigated. This could lead to interesting observations regarding the hypotheses that recent changes in diet could be associated with microbes that are still harmful to the host and/or that such recent changes in diet actually occur because of a shift in microbiota – through host manipulation or changes in ontogeny for instance. Adapting models of trait evolution on phylogenies (Garland et al. 1993, Paradis and Claude 2002, Ives and Godfray 2006, Ives and Garland 2010) to models of gut microbiota associations on host phylogenies, or extending the framework of co-phylogenetic studies (Banks and Paterson 2005, Charleston and Perkins 2006, Conow et al. 2010, Drinkwater and Charleston 2014) to include whole gut microbiota would help understand the link between host–microbe evolutionary contact duration and the effect of the microbe on the host.

Consider more systematically the gut microbiota in the study of host fitness and adaptation

The capacity of animal populations to adapt to new environments has long been considered to rely on nuclear genes. However, evidence is accumulating that some traits that affect the fitness of an organism are not directly encoded in the nuclear genome, but rather in the microbiome. The gut microbiota should thus be considered more systematically in the study of adaptation. This may for example be done by removing gut symbionts with antibiotics, and determine whether local adaptation patterns observed in the field persist. It may also be done by performing experimental evolution, under either germ-free or conventional conditions, to determine whether the evolutionary trajectory will be affected.

Despite its potential key role in driving adaptation to changing environments, the evolutionary history of host–microbiota associations has been poorly investigated so far. One very constraining aspect is the difficulty to find well-preserved fossil records of microbiota, and to study the mechanisms underlying host–microbiota associations (e.g. immune factors) in ancient organisms. Hence, evidence of microbiota evolution is mainly indirect, coming e.g. from the comparison between contemporary populations submitted to different environments. For example, the putative trajectory of human gut microbiota evolution from Paleolithic hunter–gatherer to modern Western societies has recently been traced by comparing the gut microbiota structure of modern populations with different lifestyles (e.g. rural African populations, western communities, etc.; Quercia et al. 2014). However, the analysis of such snapshot spatial patterns in gut microbiota structure does not provide information about the evolutionary processes that have led to the pattern observed in present-day populations. For instance, it is unclear whether geographic variation in human gut microbiota structure results from host genetic evolution, or rather reflects the flexibility of host–microbiota associations. A powerful way of documenting the course of host–microbiota evolution would be to study organisms, like *Daphnia*, that produce temporally stratified dormant propagule banks. By resuscitating past populations in the laboratory and competing isolates against their modern descendants, the function and fitness effects of genes evolving in step with the changing environment can be experimentally inferred (Decaestecker et al. 2007, Orsini et al. 2013). Such resurrection studies can be useful to investigate, e.g. the evolution of the immune system and the consequences for the gut microbiota structure.

Another aspect which is linked to the study of microbiota adaptation is the topology of the microbial interaction network within the gut. Using an analogy with gene network topology, the latter is thought to affect the speed of adaptation to changing environments and maximal fitness in stable ones, with simpler topologies allowing quicker adaptations at the cost of poorer maximal performances (Malcom 2011a, b). In the same vein, the topology of microbial interaction networks should be investigated as a potential driver of adaptation to changing environments. The use of lab-tractable host organisms with short generation times and well-studied innate immune systems would facilitate experimentation to test such ideas and allow determining whether particular immune system components have been lost or are expressed less over evolutionary time.

A gut microbiota perspective on life-history evolution and eco-evolutionary dynamics

Classically, life-history traits, such as age at maturity, the mode of reproduction or dispersal ability, have been considered 'disconnected from the gut', i.e. their evolution has been mostly investigated in the light of population genetics,

or more recently in the light of epigenetics. However, given the mostly horizontal mode of transmission of gut microbiota between individual hosts, host–microbiota coevolution could have unsuspected effects on the evolution of host life-history traits. For instance, the fluctuating epistasis theory of selection for sexual reproduction (Gandon and Otto 2007) could also apply when considering microbial symbionts instead of genes, if offspring microbiota could be regarded as some 'recombined' version of those of its parents. Indeed, different microbial taxa could confer variable fitness to their hosts depending on host adaptation to the microbiota (following the Red Queen explanation of fluctuating epistasis) or depending on the interaction between microbiota and the environment of its host (e.g. diet or climate conditions, thus following the more environmentally based version of fluctuating epistasis). In both cases, this process of host–microbiota coevolution leading to fluctuating effects on host fitness would lead to the same selective pressure acting on the maintenance of sexual reproduction, provided offspring microbiota are related to those of its parents. Transmission of symbionts between parents and offspring, however, does not need to be directly linked to reproduction for this effect to hold. Other life-history traits which are somehow more difficult to explain based on purely vertical transmission of biological information (genes), such as senescence or parity, could also benefit from a gut microbiota perspective.

From a more demographic perspective, host microbiota composition can affect population dynamics and genetic diversity of hosts through effects on reproduction, mortality and dispersal. It is well known that endosymbionts such as *Wolbachia* have important effect on host reproduction, genetic diversity and population dynamics through male-killing, parthenogenesis induction, cytoplasmic incompatibilities, or feminization of genetic males (Werren et al. 2008), which all induce a distortion of the sex ratio and, hence, a decrease in genetic diversity and birth rate. Mildly pathogenic taxa within the gut microbiota could display similar effects, e.g. when they affect behavior and social interactions of the host. Conversely, gut microbiota could also play a positive role on reproduction which could be evinced through experiments on axenic or gnotobiotic hosts. For instance, it has been reported that rifampin-treated termite founders tend to have lower oviposition rates (Rosengaus et al. 2011).

The effects of gut microbiota on life history can have an important impact in eco-evolutionary dynamics, at varying levels, ranging from populations to ecosystems. Future studies investigating eco-evolutionary feedbacks should thus take account of this factor. A great challenge for ecology in the coming decades is to understand the role humans play in eco-evolutionary dynamics. Humans are major selective agents with potential for unprecedented evolutionary consequences for Earth's ecosystems, especially as cities expand rapidly (Alberti 2015). Among the human-induced selective pressures that can affect this dynamics, the massive use of antibiotics and over-hygienic lifestyles (e.g. over-use of sanitizers) may be of particular importance, through their effects on microbial communities.

Gut microbiota and its link to host specialization and speciation

To understand the selective pressures and processes involved in the evolution of symbiotic relationships, e.g. in the gut, and their potential roles in the diversification of their hosts, there is a need for studies of symbiosis diversification at a short time-scale (Vavre and Kremer 2014). To understand the processes driving host–microbiota coevolution, variation in gut microbiota composition, as well as the mechanisms underlying host–microbes interactions (e.g. immunity) within species and between host races or closely related species, should be investigated. Metatranscriptomics and metagenomics provide very useful and powerful tools to study these questions, allowing to investigate the structural and functional profile of gut microbial communities, and facilitating the detection of host and microbial genes involved in local adaptation. It is not yet clear whether the genes revealed by transcriptomics studies in the host are associated with the regulation of the symbiotic compartment. Moreover, the ultimate effect of these genes on host fitness is still to be determined. A possibility would be that such genes select for certain microbiota together with having effects on the life cycle of the host, or could select microbiota which in turn have an effect on its life cycle. Divergence in life cycles between closely related hosts could lead to speciation by inducing reproductive isolation through a mismatch in reproductive timing or through outbreeding depression linked to another part of the life cycles. The role of the microbiota in host race formation (Feldhaar 2011) and speciation (Brucker and Bordenstein 2012) is a process that needs further enquiry. Reciprocal gut microbiota transplant between different host races or closely related species might be a very useful tool to assess the role of gut symbionts in species divergence. It may also be interesting to investigate more deeply the role of gut microbiota in hybrid lethality (or more generally hybrid depression) in species such as *Nasonia*, e.g. by determining if this effect results from a co-adaptation between hosts and symbionts, or from an intrinsic hybrid dysfunction (e.g. immune defects) that leads to the incapacity of dealing with many free-living bacteria (Chandler and Turelli 2014). Evidencing co-adaptation between hosts and symbionts leading to hybrid depression would be an important step because this mechanism parallels the Bateson–Dobzhansky–Muller model of genetic incompatibility at the origin of speciation (Gavrilets 2003), but based on the hologenome rather than the host genome only.

Methodological perspectives on eco-evolutionary studies of gut microbiota

There are at least two main axes for caveats and method development for studies on gut microbiota. The first one is to observe, as is the case in epigenetics studies (Birney et al. 2016), that host–microbiota observational studies relate phenotype with phenotype, and are thus prone to inversion of causality interpretations. In other words, because microbiota can cause trait change in the host or be selected in the host because of some pleiotropic effect linked to the focal trait of the host, it is impossible to conclude on mechanisms linking microbiota to host traits without effective manipulation of the microbiota. Such manipulative designs include gnotobiotic hosts (e.g. obtained through fecal transplants) and hosts

disinfected by antibiotics (with the caveat that such products will probably selectively affect taxa within the microbiome). The second perspective for methods is to treat gut microbiota studies as a community ecology problem, i.e. to adapt methods from community ecology to understand diversity patterns, successions, community assembly of the gut microbiota. Notions from ecology, such as selection, dispersal, or drift, and methods such as looking at diversity patterns to understand microbe coexistence, have begun to be applied to microbiome data (Nemergut et al. 2013). Regarding gut microbiomes among individual hosts as a metacommunity (Leibold et al. 2004) or a meta-ecosystem (Loreau et al. 2003) can indeed bring fruitful parallels. First, understanding microbe diversity within an individual host requires a wider perspective since host-to-host microbe exchanges, akin to dispersal among habitat patches in metacommunity parlance, will undoubtedly affect diversity at both the individual and population scales, with local diversity peaking at intermediate rates of exchange if local host conditions are assumed heterogeneous from the microbe point of view (Mouquet and Loreau 2002). Second, conditions for local microbial community stability can be understood from the point of view of spatial ecology (Coyte et al. 2015), i.e. emphasizing the fact that harsh competition or too much cooperation among microbes might destabilize communities (Allesina and Tang 2012), while spatial structure and exchange of gut microbiota among hosts can stabilize them (Mougi and Kondoh 2016, Gravel et al. 2016). Third, analyzing and partitioning microbial community diversity using methods from ecology, such as separating alpha, beta and gamma components of diversity, and analyzing trait, (phylo) genetic and species diversity at the same time and in connection with variables describing important host information (diet, behavior, etc.) will provide signal regarding community assembly processes, interactions among microbial taxa and selective processes due to the host or its environment (Vellend and Geber 2005, Jost 2007, Villéger et al. 2008, Schleuter et al. 2010, Chao et al. 2012, 2015, Marcon et al. 2014, Whitlock 2014, Gerhold et al. 2015, Laroche et al. 2015, Pavoine 2016). To understand interaction networks among microbes in the gut, data need to be more than snapshot co-occurrences of microbial taxa (as are used in species association networks, e.g. Lima-Mendez et al. 2015), but rather time series (Bohan et al. 2011, Sugihara et al. 2012) or even better replicated pairwise interactions in the lab in conditions similar to the gut, to infer true interactions.

The role of the host microbiome in heritability studies

Variation in a phenotypic trait within a population is traditionally modeled as the sum of genetic and environmental variation, as well as interactions between these effects. Given the impacts of gut symbionts on many host traits, the gut microbiome is expected to be an important contributor to phenotypic variation, and should thus be considered in the study of heritability (i.e. the fraction of phenotypic variability that can be attributed to genetic variation). Gut microbes include both core species (strongly associated to the host and dependent on host genetic background) and non-specific species (modulated by the environment), hence the gut microbiota may act as both a genetic and an environmental factor. The heritability effect of the gut microbiome will most likely be less pronounced than the effects that have been detected for endosymbionts, given the more labile association between the host and the microbiota in comparison with endosymbionts. Nevertheless, given that the core microbiota is partly heritable (either due to vertical transmission, or to interactions with host genotype, e.g. through immune genes), it can affect quantitative genetic variation over multiple generations.

Depending on their transmission routes, gut symbionts may affect heritability in different ways. Vertical transmission of gut symbionts from mothers to offspring (e.g. via symbionts capsules in insects) may lead to an over-estimation of heritability for traits that are affected by these symbionts. Horizontal exchanges of symbionts may also affect heritability, e.g. by increasing resemblance between interacting individuals. Interactions of the gut microbiota with host genotype (e.g. through immunity) likely further reinforce heritability. In the future, when studying variations in a particular phenotypic trait, the structure of the gut microbiota should be ezamined, to determine whether variation in this trait could be explained by variations in the structure of the microbiota (i.e. individuals with similar phenotype have similar gut microbial communities). To determine whether the gut microbiota influences the heritability of this trait, different methods might be envisaged. First, the heritability could be compared between conventional and germ-free animals. Any difference would mean that gut symbionts affect the measurement of heritability. To investigate more deeply the underlying mechanisms, e.g. to determine whether the effect of gut microbiota on heritability is due vertical transmission of symbionts, germ-free juveniles could be colonized with either the microbiota from their mother, or with a different gut microbial inoculum. If heritability is stronger when juveniles are inoculated with the microbiota from their mother, it may indicate that microbiota-mediated effects on heritability occur via the vertical transmission of symbionts.

In future studies, it will be important to disentangle what proportion of the microbiota is really heritable and what proportion is a transient maternally or environmentally induced effect, and to assess whether the core taxa show heritability as if they were vertically transmitted between mother and progeny (which could be mediated through the host immune system) or more like horizontal transmission from the population or the environment (e.g. if a certain bacterial species in the core gut microbiota is genetically more closely related to the same species in the mother than this same species in other nonrelated individuals). There is an urgent need for transgenerational studies investigating microbiome × host genotype interactions (adaptive versus non-adaptive microbiome – host genotype combinations) with respect to host life history and fitness effects, more in particular disentangling the role of the host and the symbiont immune system.

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