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Review

Biological warfare: Microorganisms as drivers of host–parasite interactions

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ABSTRACT

Understanding parasite strategies for evasion, manipulation or exploitation of hosts is crucial for many fields, from ecology to medical sciences. Generally, research has focused on either the host response to parasitic infection, or the parasite virulence mechanisms. More recently, integrated studies of host–parasite interactions have allowed significant advances in theoretical and applied biology. However, these studies still provide a simplistic view of these as mere two-player interactions. Host and parasite are associated with a myriad of microorganisms that could benefit from the improved fitness of their partner. Illustrations of such complex multi-player interactions have emerged recently from studies performed in various taxa. In this conceptual article, we propose how these associated microorganisms may participate in the phenotypic alterations induced by parasites and hence in host–parasite interactions, from an ecological and evolutionary perspective. Host- and parasite-associated microorganisms may participate in the host–parasite interaction by interacting directly or indirectly with the other partner. As a result, parasites may develop (i) the disruptive strategy in which the parasite alters the host microbiota to its advantage, and (ii) the biological weapon strategy where the parasite-associated microorganism contributes to or modulates the parasite's virulence. Some phenotypic alterations induced by parasite may also arise from conflicts of interests between the host or parasite and its associated microorganism. For each situation, we review the literature and propose new directions for future research. Specifically, investigating the role of host- and parasite-associated microorganisms in host–parasite interactions at the individual, local and regional level will lead to a holistic understanding of how the co-evolution of the different partners influences how the other ones respond, both ecologically and evolutionary. The conceptual framework we propose here is important and relevant to understand the proximate basis of parasite strategies, to predict their evolutionary dynamics and potentially to prevent therapeutic failures.

Contents

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1. Introduction: host and parasite-associated microorganisms are key players in the phenotypic alterations induced by parasites

The past decades have seen an explosion of studies characterizing the biology of symbionts, including mutualists, commensals and parasites, and their role in host biology, community structure and ecosystem functioning (Ferrari and Vavre, 2011; Hatcher et al., 2012). It has become widely recognized that symbionts can be responsible for some of the most noticeable variations in the phenotypes of various organisms, as they constitute a low cost source of evolutionary innovation for them (Margulis, 1991). In fact, the short generation time of microorganisms allows faster adaptation to changing environments than variation in host genes. In addition, differences in symbiont metacommunities are observed in different environments. Many examples of ecologically important traits contributed, or impacted, by associated microorganisms have been reported. For instance, numerous microbes manipulate their host's reproduction (Engelstädter and Hurst, 2009); plant adaptations to stress conditions are often dependent on associated microbial communities (Zelicourt et al., 2013); some insect symbionts provide nutrients and/or are responsible for diet specificity (Akman et al., 2002; Shigenobu et al., 2000); other symbionts participate in defense against parasitism (Oliver et al., 2003) or participate in their parasitic host's virulence (Adams et al., 2006), and in some cases they modulate their host's behavior (Heijtz et al., 2011). Thus, the behavior and physiological characteristics of an organism at any given time are the product of its interaction with all its associated microorganisms, and are thus "extended phenotypes" (Dawkins, 1982). The holobiont concept captures this community of interacting organisms including the host and its diverse array of symbionts such as bacteria, viruses, and unicellular eukaryotes (Margulis, 1991). Holobiont evolution is determined by changes in the nuclear and cytoplasmic genomes of the host, but also by changes in the genomes of its associated microorganisms (Herniou et al., 2013; Vavre and Kremer, 2014). These microorganisms form diverse associations with their hosts ranging from mutualism to parasitism, and from obligate to conditional, resulting in extensive and multidimensional variation of the holobiont phenotype on which selection can act (Zilber-Rosenberg and Rosenberg, 2008).

New evidence suggests that we have underestimated the role of symbiotic microorganisms in the co-evolutionary dynamics of hosts and parasites. Here, we use 'parasites' in the classical sense – either macroorganisms (e.g., helminths, arthropods, parasitic plants), including parasitoids, or microorganisms (e.g., protists, viruses, bacteria and fungi) – that induce a reduction in fitness of their host, be it large or small. Because parasites harm their hosts, natural selection often favors host genes that make them resistant or tolerant to parasites. Conversely, parasites are also under selection to overcome this resistance through virulence factors and to optimize host exploitation and transmission. This antagonistic coevolution results in a continuous arms race of defense and counter-defense. However, both the host and its parasite may be associated with microorganisms that can influence their phenotypes and fitness and modulate their coevolution (Dheilly, 2014). Therefore, the evolutionary trajectories of the holobiont-host and the holobiont-parasite may be determined by changes in the genomes of their diverse symbionts. Research on the role of host- and parasite-associated microorganisms is thus urgently needed to achieve a comprehensive and balanced understanding of the ecology and evolution of host–parasite interactions (Dheilly, 2014; Hatcher et al., 2012).

We identified four scenarios, summarized in Fig. 1 where host- and parasite-associated microorganisms participate directly or indirectly in the host–parasite interaction. For the purpose of this article, we focus on the parasite strategies that result from these interactions as summarized in Fig. 2. Host- and parasite-associated microorganisms participate in the phenotypic alteration resulting from parasitism and hence in the development of strategies for evasion, manipulation or exploitation of hosts and their defenses. We discuss how the holobiont perspective could resolve issues pertaining to the evolution of hosts, parasites and their associated microorganisms. This theoretical framework is supported by empirical data from various model species. We review the literature and propose new directions for future research on the neglected role of associated microorganisms.

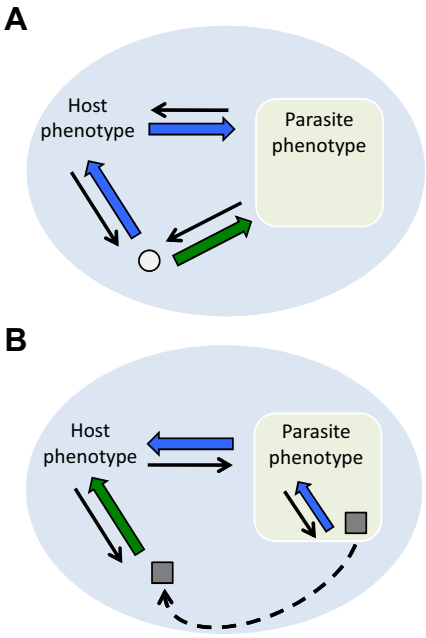

Fig. 1. Role of host- and parasite-associated microorganisms in host–parasite interactions. The three-partner interaction consists in three direct effects between (i) the host and the parasite, (ii) the host and the microorganism and (iii) the parasite and the microorganism. Host-associated microorganisms (A) and parasite-associated microorganisms (B) can directly (green) or indirectly (blue) participate in the host–parasite interactions. Indirect interactions consist of a sequence of two direct effects (Menge, 1995; Moon et al., 2010). The smaller symbols represent the microorganisms; the shape and color indicate whether it is a symbiont of the host (white circle) or parasite (gray square). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. The disruptive strategy

Until now, most studies have focused on the role of host-associated microorganisms in determining the host phenotype. They have revealed remarkable examples of traits that were originally assumed to be the product of host genes but have since been shown to result from the interaction with its associated microorganisms, especially in the context of health and disease. These include phenotypic traits such as resistance to parasites, heat tolerance, diet choice, and development (Clemente et al., 2012; Feldhaar, 2011; McFall-Ngai et al., 2013). In particular, these findings call for a complete re-evaluation of the immune system and its evolution (Dheilly, 2014; McFall-Ngai, 2007). The Host holo-immunome (Dheilly, 2014) includes the host-encoded immune defense systems, whose maturation and efficiency are influenced by the microbiota (indirect interaction Fig. 1A (Buffie and Pamer, 2013; Chung et al., 2012; Schnupf et al., 2013; Weiss et al., 2011), and the microbiome-encoded defense mechanisms that provide the first line of defense against parasites and

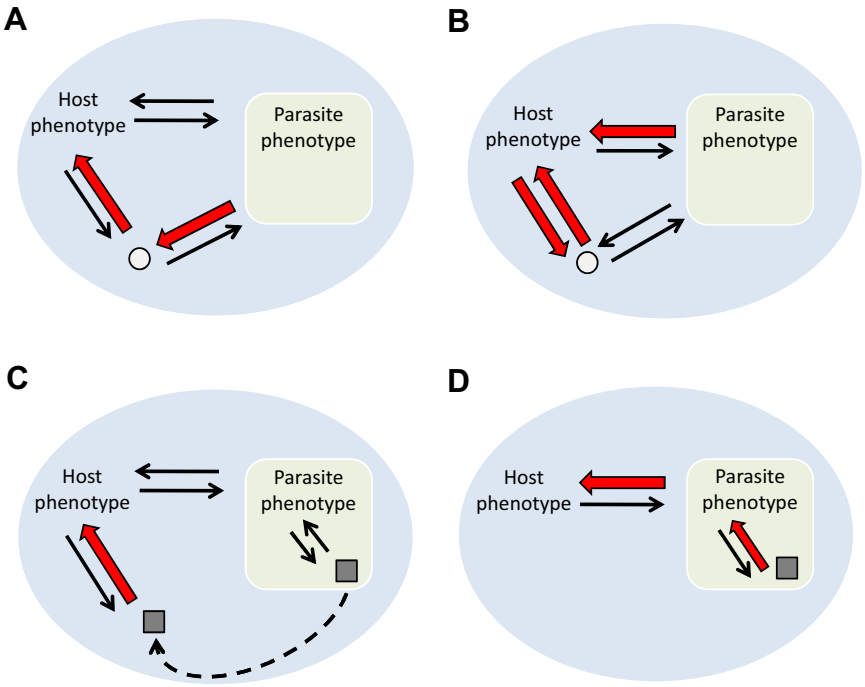

Fig. 2. Role of host- and parasite-associated microorganisms in the phenotypic alterations induced by parasites. New parasite strategies can be envisaged considering the role of host-associated microorganisms in the host–parasite interaction. It includes the disruptive strategy (A and B) where the parasite directly (A) or indirectly (B) alters the host microbiota to its advantage. Parasite-associated microorganisms may participate in the interaction. The Biological weapon strategy (C and D) considers all the situations where the parasite-associated microorganism participates directly (C) or indirectly (D) in the parasite's virulence. The smaller symbols represent the microorganisms; the shape and color indicate whether it is a symbiont of the host (white circle) or parasite (gray square).

pathogens, a process called microbe-mediated refractoriness (Direct interaction Fig. 1A (Barr et al., 2013; Degnan and Moran, 2008; Oliver et al., 2009). Indeed, host-associated microorganisms indirectly participate in immune defense because they are necessary for the maturation, induction and function of a healthy immune system characterized by its ability to respond rapidly and strongly to highly diverse challenges (Buffie and Pamer, 2013; Chung et al., 2012; Schnupf et al., 2013; Weiss et al., 2011), Host-associated microorganisms may also directly participate in defense by producing antibacterial factors, by competing for elements necessary for bacterial growth or by limiting bacterial adhesion to host cells (Buffie and Pamer, 2013; Cirimotich et al., 2011; Oliver et al., 2009, 2005, 2003).

The disruptive strategy (Fig. 2A and B) proposes that the parasite can alter the host microbiota to its advantage. Indeed it is likely that if some alteration of the host-associated microorganism can align with the interests of the parasite (transmission, reproduction, evasion of the immune system. . .), parasites would have been selected to disrupt the interaction between the host and its microorganisms. For instance, induction of diarrhea by gut parasites may be a very effective way to limit competition over habitat and nutrients. The widespread role of host symbionts in defense suggests that they co-evolve with the host–parasite association. Indeed, genotype by genotype interaction was recently demonstrated between the conditional mutualist aphid symbiont, *Hamiltonella defensa* and the parasitic wasps *Lysiphlebus fabarum* by using a uniform host genetic background (Cayetano and Vorburger, 2015). Thus, parasite interactions with host-associated microorganisms could dramatically affect the selective pressures that maintain the interaction between the host and its microbiota. An integrated approach considering interactions between host-associated microorganisms, hosts and parasites now appears compulsory.

The notion that the gut microbiota can affect brain function and behavior is also now widely accepted. Gut microorganisms influence levels of anxiety in mice (Bercik et al., 2011; Foster and McVey Neufeld, 2013) and rats (Gareau et al., 2007), as well as a range of behaviors in other animals, including mate preference (Sharon et al., 2010), scent and olfactory recognition (Archie and Theis, 2011; Lize et al., 2014) and social group living (Matsuura, 2001; Stilling et al., 2014). In most cases, the adaptive value of these effects for the microorganisms is still unknown and requires further studies. It has been argued that the gut microbes can manipulate the behavior of the host to improve their own fitness (Alcock et al., 2014). Regardless, by interfering with the normal functioning of these host-associated microorganisms, the parasite could also manipulate the behavior of its host to improve its transmission. For instance, a parasite with a multi-host complex life cycle could impair the normal digestive system of its intermediate host, resulting in general weakness and increased foraging activity and possibly increased predation risk by the definitive host. Other types of parasites may alter their host microbiota to increase the sexual or social behavior of the host and thus increase their own chances of horizontal transmission. All these theoretical examples fit within the hypothesis of exploitation of host compensatory responses since the parasite does not directly manipulate the behavior of its host but instead it exploits the microbiome response (Dubois et al., 2013). It is likely that if some modifications of the host microbiota alter the behavior of the host and align it with the interests of the parasite, parasites would have been selected to modify the community of microorganisms to their advantage.

It is worth noting that modifying the host microbiome comes down in practice to modifying a community of organisms rather than a single organism. Indeed, subtle variations in the gut microbiota community can have profound effects on the host's susceptibility to parasites (Koch and Schmid-Hempel, 2012). Koch and Schmid-Hempel (2012) transplanted the microbiota between individuals of six bumblebee colonies and compared their resistance to different genotypes of the parasite *Crithidia bombi*. Protection

against the parasite, measured as lower prevalence and strain diversity, was explained mostly by the microbiome rather than by the host genotype. These authors demonstrated that subtle variation in the gut microbiota of bumblebees can account for the geographical mosaic of strain specificity. Therefore, some parasites have been selected to disrupt the host microbial community. Another striking example involves the microsporidian parasite *Paranosema locustae* which relies on acidification of the hindgut of its locust host (*Locusta migratoria manilensis*) and an increased production of reactive oxygen species to modify the host's hindgut microbial community (Shi et al., 2014). *P. locustae* is mutually refractory with hindgut bacteria: the bacteria protect the host against the parasite and the parasite needs to reduce the hindgut bacterial population to insure its development (Shi et al., 2014).

Some complex scenarios may also exist depending on the method developed by the parasite to disturb the host microbiota. The parasite may indirectly alter the host microbiota by interacting with the host itself (Fig. 2B). For example, it can be expected for gut parasites that compete with members of the gut microbiota over habitat and nutrients. Since there is a selective influence of diet on microbiota, it is likely that some parasite would have been selected to manipulate the host diet to either (i) directly modify the environment (pH, food availability...) to their advantage or (ii) indirectly impact the microbial community to eliminate competitors or to alter the host immune system. In fact, all microorganisms in the gastrointestinal tract are under selective pressures to manipulate the host eating behavior to improve their environment. Studies have revealed that obesity can be induced by fecal transplant (Turnbaugh et al., 2006) and may be contagious (Christakis and Fowler, 2007), suggesting that it results from microbes that manipulate host eating behavior (Alcock et al., 2014). Therefore, indirect interactions between parasites and host-associated microorganisms could also explain behavioral modifications that lack apparent purposive design (Poulin, 1995). Indeed, a modification of the host's diet that does not provide protection against the parasite cannot be justified by self medication (de Roode et al., 2013) and remains unexplained. However, such behavioral modification makes much more sense when we consider the effects it may have on the associated microbiota and its potential indirect impact on the immune system. At present, there are no solid empirical examples of parasites indirectly influencing the host's microbiota as a result of their more direct impact on the host, therefore this remains a potential direction for future research.

Manipulative host-associated microorganisms with dual functions in microbe-mediated refractoriness and host behavior may result in conflicting phenotypes. It could explain behavioral modifications of the host that do not seem to match the parasite's interests with respect to transmission or other life cycle requirements (lack of apparent purposive design; see (Poulin, 1995)). For example, in the locust, the hindgut microbiota produces an aggregation pheromone that results in the production of neurotransmitters that initiate and maintain the gregarious behavior of locusts (Dillon et al., 2002). Here, the locust makes use of molecules naturally produced by its hindgut bacteria (Dillon et al., 2002). However, as described above, the parasite *P. locustae* alters the hindgut microbiota, resulting in reduced hindgut bacterial populations and in a solitary behavior of infected locusts (Shi et al., 2014). As discussed by the authors of this study, this solitary behavior does not appear beneficial to the parasite since it is transmitted through cannibalism of infected dead individuals, through fecal contamination and/or vertically from mother to offspring (Shi et al., 2014). Therefore, the resulting phenotype - solitary behavior – is detrimental for parasite transmission, and illustrates a trade-off between the parasite's development and transmission mediated by its manipulation of host-associated microorganisms.

3. The biological weapon strategy

The biological weapon strategy (Fig. 2C and D) covers all situations where symptoms are not caused by the parasite itself or its genes, but by its associated microorganisms and their genomes. The use of another microorganism as a biological weapon is very common in competition (Price et al., 1986). For example, the invasive harlequin ladybeetle *Harmonia axyridis* carries a microsporidia that is lethal for the native ladybird *Coccinella septempunctata* (Vilcinskas et al., 2013). Even though the presence of parasite-associated microorganisms has seldom been envisaged, some studies demonstrate that parasites are also associated with microorganisms that could be used as biological weapons and directly (Fig. 2C) or indirectly (Fig. 2D) contribute to parasite virulence (Adams et al., 2006). It can be expected that this strategy is important in host–parasite interactions because (i) host–parasite interaction is an intimate and long-term interaction, and (ii) in many cases the parasite, or one of its organs, is within the host, which favors the transmission of the biological weapon. An obvious prediction is that the nature and size of parasite symbionts must be strongly dependent on the parasite's size. Bacteria may carry phages; unicellular eukaryotes may carry DNA or RNA viruses, and multicellular parasites may carry RNA or DNA viruses or bacteria.

As a result, the host is being co-infected by the parasite and the parasite's symbionts, the combination of which may interfere with the host's immune response. For example, viruses are harbored by many protozoan parasites including *Trichomonas, Leishmania, Giardia, Plasmodium, Entamoeba, Naegleria, Eimeria,* and *Cryptosporidium* (Wang and Wang, 1991). In most cases, it is unknown whether the associated virus provides an adaptive advantage to its parasitic host. However, the presence of the virus in itself may be sufficient to trigger an antiviral immune response and in some cases to induce an inflammation (Fichorova et al., 2012; Ives et al., 2011). The recognition that parasites may carry associated microorganisms has huge implications for the way scientists develop treatments and cures for parasitic diseases. Indeed, treatment against *Trichomonas vaginalis* is based on the inoculation of the antibiotic metronidazole. Even though the treatment successfully kills the protozoan parasite, it has not effect on the virus it carries, the *T. vaginalis* Virus (TVV) that is released in abundance from the stressed/dying cells, resulting in inflammatory complications (Fichorova et al., 2012). The effect of associated microorganisms on the host immune response may also impact the parasite's fitness. For instance, the *Leishmania* RNA Virus-1 (LRV1) is responsible for an hyper-inflammatory immune response that renders the host more susceptible to infection by *Leishmania* and leads to destructive metastatic lesions (Ives et al., 2011).

When the associated microorganisms' transmission is dependent on the parasitic host's transmission, it can be expected that over evolutionary time the microorganism will be selected to help the parasite avoid the host's immune system or better exploit the host. About 70–80% of *T. vaginalis* carry a dsRNA virus, the *T. vaginalis* Virus (TVV) (Wang and Wang, 1991; Wang et al., 1987; Wendel et al., 2002), that induces various phenotypic changes responsible for an increased virulence of *T. vaginalis* including the upregulation of immunogenic proteins and differential expression of cysteine proteinases involved in cytotoxicity, cytoadherence and evasion of the host immune barrier (Arroyo and Alderete, 1995; Hernandez et al., 2014; Khoshnan and Alderete, 1994; Provenzano et al., 1997; Wang et al., 1987). The parasitic nematode *Phasmarhabditis hermaphrodita* employs a bacteria, *Moraxella osloensis*, to kill its host, the slug *Deroceras reticulatum* before eating the cadaver (Tan and Grewal, 2001). The bacteria lipopolysaccharides display a strong toxicity to molluscs (Tan and Grewal,

2003). Similarly, the plant-pathogenic fungus *Rhizopus* sp. has an endosymbiotic bacterium of the genus *Burkholderia* that produces the toxin rhizoxin and other virulence factors which play key roles in the disease (Lackner et al., 2011; Partida-Martinez and Hertweck, 2005).

Parasite-associated microorganisms could also provide new physiological capabilities that directly help the parasite invade different host species. For instance, entomopathogenic nematodes of the genera *Sterneinema* (family Steirnematidae) and *Heterorhabditis* (family Heterorhabditidae) are associated with enterobacteriacae bacterial symbionts (*Photorhabdus* with *Heterorhabditis* and *Xenorhabdus* with *Sterneinema*) that produce insecticidal toxins that help degrade tissues for the nematode to feed on and antibacterial compounds for sterile cadaver maintenance (Chaston et al., 2011). Intriguingly, the two genera are not phylogenetically related, and have independently co-opted the aid of related bacteria to better exploit their insect hosts. It has been suggested that their surprising evolutionary convergence in life-style and mutualism with enterobacteriaceae has resulted from a transition from parasitism in vertebrates to parasitism in insects (Blaxter and Koutsovoulos, 2014).

Parasite-associated microorganisms may also be involved in the most remarkable parasite-related phenomenon: behavioral manipulation of the host. In *Dinocampus coccinellae*, a parasitic wasp of the family Braconidae, subfamily Helconoid, an Iflavirus (positive strand RNA virus) was found associated with the wasp and is transmitted to the coccinellid host during larval development (Dheilly et al., 2015). The *D. coccinellae* Paralysis virus (DcPV) appears to be involved in the host behavioral manipulation that is characterized by the ladybeetle host protecting the wasp larva from predators (i.e., bodyguard behavior) after the parasitic wasp has emerged from the host. DcPV invades the coccinellid nervous system, which results in host paralysis (Dheilly et al., 2015). The wasp larva then spins its cocoon under the paralyzed ladybeetle, using it as a protective cover, which results in a significant reduction of predation on the developing wasp (Maure et al., 2013). The role of parasite-associated microorganisms should be envisaged in many other species. For example, it seems obvious that the symbiotic *Photorhabdus luminescens* bacteria (Singh et al., 2012) of the entomopathogenic nematode *Heterorhabditis bacteriophora* is responsible for the change in host coloration (luminescence and red pigmentation) that serves as a visual deterrent to avian predators (Fenton et al., 2011). However, it is unknown who between the parasitic nematode *P. hermaphrodita* and its associated bacteria *M. osloensis* (discussed above) is responsible for the spatial behavior manipulation of the slug host, resulting in reduced predation (Pechova and Foltan, 2008). In fact, parasite-associated microorganisms might contribute to the multidimensionality of manipulation (Thomas et al., 2010). Among the suite of host traits that are modified (presumably) by parasites, some may be directly attributable to the microorganism and others to the host. Further studies should compare the manipulative effects of parasites with and without symbionts whenever it is possible. Although new, this observation is not so surprising considering that many microorganisms are themselves manipulators (da Silva and Langoni, 2009; Engelstädter and Hurst, 2009; Hoover et al., 2011; Varaldi et al., 2006) and other microorganisms have a host behavior modification associated with pathogenicity (Le Clec'h et al., 2012). The parasite would simply have to exploit this trait to its advantage. Further research should be undertaken to determine which came first: the manipulative effect of the microorganism or its symbiotic interaction with the parasite.

Therefore, parasite symbionts can provide evolutionary innovations that benefit the parasite and will be strongly positively selected. These symbionts may drive parasite diversification by helping their parasitic host to adapt to new environments and allow niche expansion (host shifts). For eukaryote parasites, the shorter generation time and the rapid genetic variations provided by microbial symbionts can also play an important role in their arms races with hosts. The geographic mosaic theory of coevolution predicts that virulence genes of parasites are locally co-adapted to the most abundant or most heavily-used local hosts (Thompson, 2005). When a parasite-associated microorganism is directly involved in the arms race, we can expect a positive selection of particular beneficial alleles in the microorganism genes. Alternatively, another microorganism may be selected for in a different host. Indeed, a transient microbe that neutrally evolves with the parasite in an environment may be selected for in another environment. The myriad of microorganisms present in immediate environment of the parasite may constitute a pool of genes where beneficial genes may be retrieved from. Therefore, parasite-associated microorganisms can trigger parasite diversification by facilitating niche expansion through local adaptation to host defenses. As suggested by Vavre and Kremer (2014), genes involved in the symbiotic interaction with the parasitic host, such as immune and developmental genes, may also be subject to local adaptation and favor post-zygotic barriers. Thus, parasite-associated microorganisms most probably play a major role in parasite diversification.

The use of parasite-associated microorganisms as biological weapons appears to be a relatively widespread strategy among parasites. The biological weapon strategy would be favored most probably when there is a strong selection pressure for evolutionary innovation on the holobiont parasite. Perhaps, the stronger selection pressure in favor of a biological weapon strategy may be found in parthenogenetic parasites such as the plant parasitic nematodes of the *Xiphinema americanum* group associated with veruccomicrobial symbiont, *Xiphinemobacter* (Vandekerckhove et al., 2000), the nematode associated with maternally inherited bacterial symbionts (Coomans et al., 2000), the parasitic wasp *D. coccinellae* associated with a ssRNA virus (Dheilly et al., 2015), or the protozoan parasite *T. vaginalis* associated with a dsRNA virus (Kulda et al., 1970; Provenzano et al., 1997). Or is it that the presence of microorganisms participating in the virulence strategy alleviates the selection pressure on the parasite to the point that it allows asexual reproduction? We propose that the genetic diversity necessary for the arms race could be provided by associated microorganisms. This consideration opens a new direction for studies that may explain the mere existence of parthenogenetic parasites.

However, parasite-associated microorganisms may alter the phenotype of their parasitic host to their own benefit. This would involve altering the parasite's developmental strategy, transmission route, or mode of host exploitation in ways that promote the associated microorganism's own transmission. The possibility that parasites are manipulated from within by their symbionts has previously been considered only in the case of microorganisms responsible for vector borne diseases (Lefèvre and Thomas, 2008). These microorganisms can alter various features of their parasitic vector, including feeding behavior, immune system, host attraction and defensive behavior to improve their own transmission (Lefèvre and Thomas, 2008). A similar Russian doll effect of microorganisms can be expected in many other biological systems.

As with all symbiotic interactions, a key component of the interaction between the parasite and its associated microorganisms is the transmission of the microorganism to the next generation. The nature of the transmission mode used by a parasite can create conflicts of interests with its associated microorganisms, and thereby strongly influence selection on the microorganism to affect the parasite's phenotype. For example, a mainly vertically transmitted parasite-associated microorganism could benefit from increased opportunities for horizontal transmission. One such example is the case of the *Leptopilina boulardi* filamentous virus

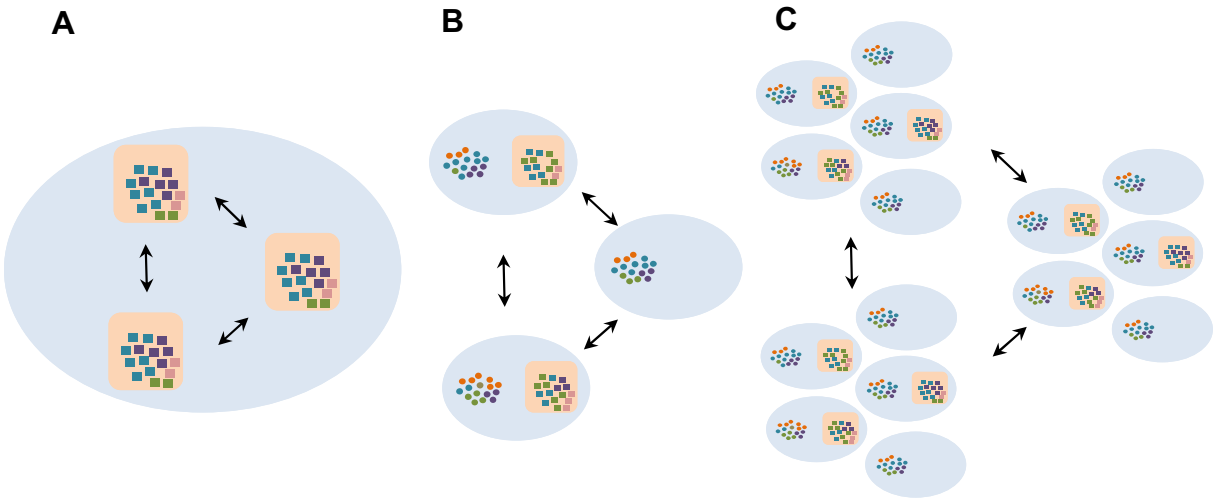

Fig. 3. Representation of host–parasite interactions with their symbiont metacommunities at various spatial scales. (A) Within a host, different parasite individuals house different suites of symbionts that are connected by dispersal of symbionts (represented by arrows). (B) Within a population, host individuals serve as local communities of host and parasite symbionts that are connected by dispersal or transmission from host to host (healthy and parasitized hosts are shown). (C) Host populations serve as patches for symbionts and the suite of symbionts within a population constitutes the local symbiont community. Patches are connected by the migration of healthy and parasitized host individuals.

We propose ecological and evolutionnary research topics that emerge from the consideration of the role of host- and parasite-associated microorganisms in host-parasite interactions and in the evolution of hosts and parasites. The following list, most probably non-exhaustive, is diverse and aimed at a general understanding of the proximate basis of parasite strategies and their evolutionnary dynamics; answering these questions may also reveal new clues toward preventing therapeutic failure.

General questions
-How often are parasites associated with microorganisms?
-Are parasite symbionts related to the host symbionts, or was the parasite-symbiont interaction present before the transition to parasitism?
-How rapid is the symbiont turn-over through selection of new partners from the environment?

Intra host
-How does parasite diversity influence the evolution of host symbionts involved in resistance and what consequences does it have on the host-symbiont co-evolution?
-How can the nature of the interaction between parasite and the host-associated microorganisms influence the nature of the host-symbiont interaction (feedback between mutualism and parasitism)?
-How do parasites influence the acquisition or loss of host symbionts?

Inter host
-How does host dispersal between populations modify host symbiont and parasite symbiont community composition?
-What consequences does it have on host-parasite coevolution?
-How does the level of parasite symbiont gene flow between hosts at the local or regional level affect the host's ability to resist parasitism?

Intra parasite
-How does parasite symbiont transmission mode affect the parasite life cycle?
-How does the parasite's symbionts role in virulence influence the selection pressures to maintain a high genetic diversity within parasites?
-How does heterogeneity (genetic diversity) within a parasite symbiont influence the parasite infection success?

Inter parasite
-Is local or regional parasite diversity or parasite symbiont diversity a good predictor of disease risk?
-What is the role of parasite symbionts in local or regional parasite adaptation to host defense?
-What is the role of parasite symbionts in local or regional variation in parasite virulence?
-What is the role of parasite diversity on local or regional host symbiont community structure?

Box 1. Research topics on the role of host- and parasite-associated microorganisms in host–parasite evolution.

(LbFV) that has a mixed-mode transmission. As a result, the virus dramatically alters the behavior of the parasitic wasp that it infects by making it deposit its eggs in an already parasitized maggot, which increases the virus' chances of horizontal transmission (Patot et al., 2009). The same scenario may take place in other cases involving a parasite-associated microorganism with a mixed-mode transmission. For example, the wasp *D. coccinellae* also deposits its eggs in already parasitized hosts, which seems maladaptive since only one larva ever emerges from the ladybeetle host (Dheilly et al., 2015). However, its associated RNA virus DcPV could benefit from this behavior since it would increase opportunities for horizontal transmission, resulting in a better spread of the virus in the population and a higher frequency of mixed symbiosis with multiple virus genotypes in the parasitic wasp population. Further studies are necessary to investigate this observation.

In some cases, the parasite-associated microorganism may also be capable of horizontal transmission to the host of the parasite in which it resides. This seems to be the case of *Neorickettsia* bacteria associated with trematodes. Several diseases of vertebrates are caused by *Neorickettsia* following horizontal transfer from parasitic trematodes (Gibson et al., 2005; Vaughan et al., 2012). Trematodes have complex life cycles typically involving passage through three host species in a particular order. Many trematodes harbor endosymbiotic *Neorickettsia*, which follow their trematode hosts through their complex life cycles and are transmitted vertically from one trematode generation to the next (Greiman et al., 2014; Tkach et al., 2012; Vaughan et al., 2012). However, since *Neorickettsia* can also pass horizontally to the trematode's vertebrate definitive host and possibly to invertebrate intermediate hosts (Vaughan et al., 2012), the possibility of conflicts of interests

arises. Many trematodes also show substantial intraspecific variability in their developmental or transmission strategies. For instance, in some species that are capable of manipulating the behavior of their intermediate host to increase trophic transmission to the definitive host, some individuals are not manipulative (Thomas et al., 2011). In other species, some individuals can shorten their life cycle by reaching precocious maturity in an intermediate host and skipping the need to go to the definitive host (Lagrue and Poulin, 2007). This variability is often explained as a state-dependent adjustment to external conditions (Thomas et al., 2002). However, with *Neorickettsia* and other symbionts, such as microsporidians (Levron et al., 2005), being found in many trematode species, there exists the possibility that the symbiotic bacteria is directly manipulating the phenotype of its parasitic host. This interesting possibility remains to be tested, and it represents one more situation where parasite-associated microorganisms could indirectly affect the biology and coevolution of the host and parasite.

4. Perspectives

The holobiont definition covers all associated microorganisms: from mutualistic symbiosis to parasitism. Selection could favor the hosts and parasites that best exploit these microorganisms, not only because this meets immediate needs but also because this requires no manipulative effort: the microorganism does the work in the arms race. However, conflicts of interests may arise leading to fluctuating selection of the associated microorganisms and possibly resulting in the gain or loss of symbionts. Indeed, not all associated microorganisms will be beneficial to their host at any time and conflicts of interests will impact the evolution of the interaction, by influencing the net impact of the mutualistic interaction. It is expected that over evolutionary time, the host will be selected to lose the symbiont that makes it more sensitive to parasitism, and the parasite will lose the symbiont that makes it more susceptible to host immune responses. The co-evolution of holobiont hosts and holobiont parasites involves reciprocal changes in which the different partners influence how the other ones respond, both ecologically and evolutionary. This co-evolutionary dynamic could impact the stability of the symbiotic interactions and induce transitions from mutualism to parasitism, and vice versa. Overall, if a host or parasite symbiont is both useful and detrimental, natural selection will most likely favor individuals that ensure the best compromise between satisfying the need to keep the symbiont and minimizing the risk of detrimental consequences. Interestingly, this best compromise may be different in different environmental conditions, in the presence of other parasites, or (for the parasite) in different host species. In addition, transient microbes that may quickly come and go in a given environment may be selected for in another environment. Rapid variations in the symbiotic content may be a way for the host and the parasite to adapt through this changing of partners. This goes well beyond the traditional realm of the Red queen (Van Valen, 1973). Instead, co-evolution within both complex local networks and a geographic mosaic consisting of a diversity of hosts and symbionts can be expected (Fig. 3). Microorganisms of both the host and parasite can play a role in local adaptation, often tested by combining allopatric and sympatric pairs of hosts and parasites to measure infection success, virulence etc. (Kaltz and Shykoff, 1998). Metacommunities of microorganisms vary at different spatial scales (Fig. 3). They can vary between conspecific hosts locally and geographically and can contribute to compatibility between host and parasite. Most interestingly, symbionts can also vary across an individual's lifespan. Therefore, the great intraspecific variability in the phenotypic alterations resulting from parasite infection could be explained by the diversity of host and parasite hologenomes.

5. Conclusions

The general thesis presented here is that in any host–parasite interaction, host- or parasite-associated microorganisms may be involved, producing a complex network of interactions, including feedback on each other. The multidimensional linkages between the host, the parasite and the host- and parasite-associated microorganisms result in a complex web of interconnected organisms, where alteration in any of these components causes a chain of counter responses in the remaining ones. At any given time, the phenotypic outcome of infection is the direct product of natural selection acting on the host genome, the parasite genome and the genomes of associated microorganisms. The new parasite strategies proposed here, grouped under the more general term Holobiont strategy of parasitism, could lead to very different ecological and evolutionary interpretations of host–parasite interactions. Future studies could explore how within-host and within-parasite symbiont communities impact host and parasite coevolution at different spatial scales and with different dispersal or transmission rates in order to reveal new clues to predict high infection rates or resistance (Box 1).

The scenarios we propose will also have implications for how host–parasite interactions are modeled. Indeed, the evidence reported here suggests that no simple case of co-evolution can exist as soon as hosts and parasites are associated with microorganisms. In addition, the shorter generation time and genetic diversity of microorganisms and the potential rapid turnover of the symbiotic content (notably through horizontal transmission) render any system highly dynamic. It may explain the great intra-specific variability of host responses to parasitism. However, in most cases, associated organisms cannot be cultured for identification or experimental purposes, which renders the initial characterization of the community a nontrivial task. Recent molecular analysis techniques will play a critical role in the discovery, sequencing and characterization of the role of symbiont communities, as well as in understanding their diversity at the genetic level within and between species and populations. Only such in depth studies will provide the necessary information to rigorously assess the role of associated organisms in the arms race between hosts and parasites.

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Glossary

Host: An organism that harbors and nourishes another
Symbiont: An organism living together with another one in any type of association including obligate and facultative mutualism, commensalism and parasitism
Mutualist: An organism that lives on or in another organism (usually larger, the host) with both partners benefitting from the association
Commensal: An organism that lives on or in another organism (usually larger, the host) without causing any fitness reduction in the latter
Parasite: An organism that lives on or in another organism (usually larger, the host) at its expense by inducing a reduction in the latter's fitness
Definitive host: A host in which the parasite undergoes sexual reproduction
Intermediate host: In parasites with complex life cycles, it is the host which harbors larval or juvenile stages of the parasite
Microbiota: A collective term for the microbiome or host-associated microorganisms. It includes viruses, bacteria, archae, protozoa, fungi and algae
Community: A group of animals, plants and microbes living together in an ecosystem
Holobiont: A term that encompasses the host and all its associated microorganisms
Hologenome: A term that encompasses the genome of the host and the genome of all its associated microorganisms
Phenotype: The complete set of morphological, developmental, behavioral, physical, biochemical and physiological characteristics of an organism
Extended phenotype: Extension of the phenotype to include all effects that a gene has inside and outside the body, on the environment or on other species
Virulence factors: Refers to the properties (i.e., gene products) that enable a parasite to establish itself on or within a host of a particular species and enhance its potential to cause disease. Virulence factors include all molecules that mediate toxicity, attachment, protection against the immune system and that may contribute to the overall pathogenicity of the parasite
Parthenogenesis: Reproduction without fertilization by gametes of the opposite sex. Thelytokous parthenogenesis results in the production of female progeny and Arrhenotokous parthenogenesis results in the production of male progeny