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The study of host–microbiome (co)evolution across levels of selection

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Microorganismal diversity can be explained in large part by selection imposed from both the abiotic and biotic environments, including—in the case of host-associated microbiomes—interactions with eukaryotes. As such, the diversity of host-associated microbiomes can be usefully studied across a variety of scales: within a single host over time, among host genotypes within a population, between populations and among host species. A plethora of recent studies across these scales and across diverse systems are: (i) exemplifying the importance of the host genetics in shaping microbiome composition; (ii) uncovering the role of the microbiome in shaping key host phenotypes; and (iii) highlighting the dynamic nature of the microbiome. They have also raised a critical question: do these complex associations fit within our existing understanding of evolution and coevolution, or do these often intimate and seemingly cross-generational interactions follow novel evolutionary rules from those previously identified? Herein, we describe the known importance of (co)evolution in host–microbiome systems, placing the existing data within extant frameworks that have been developed over decades of study, and ask whether there are unique properties of host–microbiome systems that require a paradigm shift. By examining when and how selection can act on the host and its microbiome as a unit (termed, the holobiont), we find that the existing conceptual framework, which focuses on individuals, as well as interactions among individuals and groups, is generally well suited for understanding (co)evolutionary change in these intimate assemblages.

This article is part of the theme issue 'The role of the microbiome in host evolution'.

1. Introduction

The evolution of eukaryotic organisms is a story of interactions, ranging from species that engage in intimate and beneficial relations, such as plants and the pollinators that facilitate their reproduction, to predators that rely on the death of myriad prey species for their survival. In all these cases, the evolution of particular species cannot be understood in isolation—the species with which they interact help shape the context in which natural selection acts. This reality has long been appreciated and addressed by evolutionary biologists, with much empirical and theoretical underpinning [1–3]. However, the relatively recent discovery that hosts not only harbour a vast diversity of microbes, but also join forces with these microbes to perform key functions (e.g. [4–6]), has raised questions about whether and when we should consider the host and its associated microbiome as the appropriate unit of selection [7,8]. Are microbial communities living in and on hosts merely species with which the host interacts—something which, as evolutionary biologists, we have confronted before—or is a new conceptual and theoretical framework called for?

Here, we briefly review evidence that hosts and their microbial associates not only influence each other's ecology and evolution, but that *interactions* between hosts and their microbial associates evolve. We discuss how these interactions can be shaped by both deterministic and neutral processes, can

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result in one-sided adaptations that impact patterns of heritability and co-divergence, and ultimately can result in coevolutionary change. Comparisons to other, non-microbial systems are also considered to reinforce the point that these interactions fall within the realm of classic evolutionary thinking. We then discuss the recently introduced concept of the holobiont [7,8] and draw upon theoretical work in this domain to assess under what conditions the union of a host and its associated microbes might usefully be considered a unit of selection. Finally, we compare the limited data that exist for these systems to current model assumptions in order to assess when and if a new paradigm—one that emphasizes evolution of the set of a host *with* its resident microbes—is warranted.

2. The evolution of host–symbiont interactions

The importance of species interactions in shaping organismal diversity, species ranges, community structure and ecosystem function is a long-standing focus in ecology and evolutionary biology. This is due not only to the known impact of species interactions on the evolutionary potential of populations and communities, but also to the possibility that these interactions result in novel functions [9,10]. Like other ecological species interactions, interactions among hosts and their associated microbial communities include hosts being exploited by antagonists such as pathogens, as well as hosts gaining benefits from functions provided by mutualistic bacteria, archaea, fungi and even viruses. Again, like other species interactions [11,12], the outcome of host–microbiome interactions will be critically shaped by context, including host health, the abiotic environment and the composition of the microbial community itself, to the point that a mutualistic interaction in one host type or environment can be an antagonistic interaction in another [13,14]. Moreover, the type of interaction can change over evolutionary time [15,16], and likely shifts in response to local selection pressures and coevolution among the species involved. As such, the outcomes of species interactions are both spatially and temporally heterogeneous, and selection acting on these interactions will be highly context-dependent.

Whether a given species interaction, for example, between plant and pollinator, shapes the ecology and/or evolution of each species involved will depend on both the strength and the specificity of the interaction [17,18]. When these associations are relatively common and contribute strongly to individual organismal fitness, there is likely to be selection on one or both species as a result of the interaction. In the case of hosts and their microbiomes, the degree of specificity of these interactions range from taxa that are transient on their hosts (e.g. [19–21]) to those that are highly specific to, or even obligately associated with, a particular host group (e.g. [22,23]). Moreover, there is temporal variation in these associations across, for example, developmental stages or external environmental changes (e.g. [24]), and spatial variation as a result of factors such as diet or drought (e.g. [25]). Indeed, recent evidence includes cases of extreme dependence of hosts on particular microbiota [26,27] through to a lack of dependence on any specific microbiota [28]. Even in cases of tightly associated mutualisms, there remains the possibility that the mutualism breaks down. For example, a recent study across 10 populations of the native legume *Acmispon strigosus* and its nitrogen-fixing and root-nodulating bacterial symbiont, *Bradyrhizobium* spp., revealed recurrent breakdown of the mutualism, including independent losses of nodulation capacity and nitrogen fixation effectiveness [29]. In the light of this spatial and temporal variation in host–microbiome interactions, there has been a surge in theoretical and empirical efforts to determine when and how these associations shape the evolutionary process [30].

The impacts of the microbiome on host evolution can range from microevolutionary processes, like shaping digestive abilities and thus niche breadth [31], through to macroevolutionary processes, including speciation [32]. In some cases, the microbiome can result in rapid phenotypic change of the hosts that may be particularly important in the face of either unpredictable or fluctuating environments [27,33]. Phenotypic plasticity resulting from species interactions is not unique to the microbiome [34], but given the ubiquity of these interactions, it is quickly becoming the best studied example. One particularly well-explored aspect of such plasticity is microbiome-mediated defence against pests and pathogens. There is evidence from across the plant and animal kingdoms that hosts can acquire defence against pathogens by associating with protective microbiota (e.g. [35,36]). Such rapid acquisition of defence has been predicted to hinder the evolution of host genetic defence [37,38], and lead to host dependence of particular microbial taxa, but see Hrček *et al.* [39]. Such inter-dependencies could reinforce the specificity of the interaction, for example, by reshaping self/non-self recognition within the immune system [40], and lead to host adaptations to recruit particular taxa or functional groups. Of course, evolution resulting from host–microbiome interactions are not one-sided. There is ample evidence that host-associated microbes can and do adapt to their hosts, exemplified by molecular adaptations of both pathogenic and non-pathogenic symbionts to overcome host defences (e.g. [41,42]), and more recently extending to adaptation of whole microbial communities to the host environment [43]. Overall, there is clear potential for reciprocal selection acting between hosts and their microbiota, but the question becomes whether, and at what scales, such selection leads to a (co)evolutionary response.

3. The coevolution of host–symbiont interactions

Coevolution is usefully defined as the evolution of one population in response to selection by another, which then results in reciprocal selection and evolutionary change in the first. It is important to note here that observations of specificity or seemingly tight-knit interactions are not necessarily indicative of coevolution, as this could instead be the result of species sorting [44]. For example, hosts can act as a selective ‘filter’ on the metacommunity of microbial organisms with which they interact based on adaptations that arose as a consequence of other selection pressures, and this could result in patterns of microbiome heritability or co-phylogeny that are not the direct result of host–microbiome coevolution. Similarly, bacteria have adaptations that underpin differential colonization ability that is not necessarily indicative of evolution/adaptation to a specific host, but rather may be a result of microbial community interactions or resource use, and these could generate patterns suggestive of, but not indicative of, coevolution. Moreover, even if the evolution of particular adaptations impacting the interaction are observed, this must be differentiated from one-sided evolution, where, for example, the symbiont adapts to hosts as they diversify rather than driving divergence itself. Finally, if hosts are more likely to encounter/interact with conspecifics than heterospecifics, this could result in higher rates of microbial colonization within—relative to between—host lineages in the absence of any (co)evolutionary processes. In sum, although useful for understanding any interaction, the observation of co-divergence and co-speciation is not sufficient evidence for coevolution [45].

What then is the evidence that hosts and their microbiomes coevolve? This depends in part on whether one sees particular host–symbiont coevolution as evidence for host–microbiome coevolution. Certainly, there is ample evidence for pairwise coevolution between host species and their pathogenic (e.g. [46,47]) and mutualistic (e.g. [48]) symbionts. And this has been extended to cases of diffuse coevolution, where hosts and their associates interact in a metapopulation context (e.g. [49]). But, we would argue, this is not enough to conclude that hosts and the community of microbes with which they associate are subject to coevolution.

One feature that likely sets host–microbiome interactions apart from other well-studied coevolutionary interactions is the possibility of metabolic collaboration, where hosts and their microbial associates rely on one another to complete pathways of amino acid and vitamin biosynthesis. Such close inter-dependence has been explored for many highly specific, and often vertically transmitted host-associated microbes [10]. One of the best studied of these is the interaction between pea aphids and their bacterial symbiont *Buchnera*, the latter of which is known to produce essential amino acids needed by hosts [50]. This long-standing interaction has resulted in substantial gene loss from the symbiont genome, as well as specific host adaptations in the form of bacteriocytes (specialized aphid cells) to house the bacteria and facilitate nutrient exchange. The intimate co-dependence of this relationship suggests that the fitnesses of the two organisms are closely aligned, and indeed, this is true in the sense that symbiont fitness requires vertical transmission during host reproduction. However, there is evidence that *Buchnera* titres can vary significantly among aphid genotypes and that higher titres are correlated with lower host reproductive rates, suggesting that exploitation of the host by *Buchnera* can occur even in this tightly knit system [51]. Moreover, recent transcriptomic work found that aphid gene expression within bacteriocytes varies among host genotypes and is related to bacterial density, and that *Buchnera* gene expression differs across host genotypes, suggesting a 'metabolic tug-of-war' between the host and its symbiont [48].

Similarly, recent work from deep-sea tubeworms uncovered transcriptional profiles from the γ-proteobacteria symbionts that include numerous virulence factors and proteases, suggested to have a role in nutrient acquisition from host cells [52]. These symbionts play a large role in the tubeworms' ability to thrive in vent and seep ecosystems, and are acquired from the environment through a highly specific 'infection' process. However, once inside, these new results suggest exploitation and suppression of host immune responses and highlight the complexity of these interactions at the molecular level. More generally, tight molecular interactions and metabolic collaboration between hosts and their microbiomes might seem to prime these species for gene loss and inter-dependence, but trait loss as a result of species interactions is not unique to host–microbiome interactions. A meta-analysis of compensated trait loss suggests that these events are remarkably taxonomically widespread, and often involve essential functions [53]. As such, whether metabolic collaboration results in evolutionary outcomes unlike those observed in other species interactions (especially those where inter-dependence as a result of trait loss has been observed) has yet to be demonstrated.

An interesting possibility is that hosts coevolve with their microbiome not due to particular species interactions, but rather due to an emergent property of the microbiome in total. Experimental microcosms composed of a five-member bacterial community were found to evolve more rapidly when these members co-occurred, relative to when each was grown in monoculture, and this evolution resulted in higher productivity at the community level [54]. Such responses may result from community-intrinsic properties, i.e. properties of individuals or individual populations that only arise in the context of a community [55], or they might result from selection towards reduced interspecific competition or even facilitation via niche complementarity [56]. Model experimental communities are allowing more detailed exploration of putative community-level properties, revealing how traits like bacterial movement and biofilm formation can be impacted by community interactions [57]. In addition, as discussed above, host phenotypic plasticity may result from variation in microbial interactions. As a consequence, studying one microbial organism without consideration of its community context or predicting host response to a changing climate without consideration of its microbiome is short-sighted. Moreover, separating interaction networks at the scale of microbial versus 'macrobial' organisms is likely to result in incomplete understanding of the system. Recent work from phytoplankton communities found that the host-associated bacteria not only changed host growth rate, but also altered the carrying capacity of host populations [58]. Given the importance of carrying capacity and growth rate in shaping competition and community diversity, this finding emphasizes the great need for better integration of host and microbial networks in understanding species' ecologies, and highlights how host–microbiome interactions can drive eco-evolutionary dynamics.

4. Selection and the holobiont

The tight-knit association between hosts and their microbiomes has suggested, to some, that a new conceptual framework is needed [7,8,59,60]. Underlying this suggestion is consideration of the appropriate unit of selection. In 1970, Lewontin succinctly outlined three principles that inform appropriate units of selection: (i) that phenotypic variation among units exists; (ii) that this variation results in differential fitness (i.e. survival and reproduction); and (iii) that the traits underlying these fitness differences are heritable [61]. Under what conditions might these principles hold true for the union of hosts and their associated microbial communities? In large part, the answer is likely to come down to the third component: heritability [62]. Heritability of individual symbionts or the whole microbiome can result from either vertical transmission of symbionts from parents to offspring or from specific host genetics that differentially 'filter' microbial communities. Importantly, the speed at which selection results in an evolutionary response will depend on the heritability of fitness, and this will necessarily decrease with re-assortment (i.e. recombination in the case of sexual organisms and mixing of bacterial taxa among generations in the case of the holobiont; [63]). Consequently, whether hosts are able to stably transmit or recruit the required microbiota from generation to generation is a critical component of whether selection can efficiently act on these interactions.

One elegant way to test whether selection can indeed act on the holobiont is through experimental evolution/artificial selection. In this way, the assumptions underlying group selection can easily be met and the response to selection can be measured [63]. This approach has proven successful numerous times and across diverse systems, but the utility of these studies for assessing the importance of group selection in nature remains unclear [64]. This is in large part because the experimental approach allows for 'transmission' of the group as a whole across generations, and thus ensures stable interactions that can evolve over time. In these cases, the potential re-assortment of taxa into productive communities is likely to be highly efficient, and adaptations that result in increased fitness in the specific community context can easily spread.

Theoretical exploration of when the holobiont may evolve, although currently rare, is also very useful in determining how relevant this level of selection might be in nature, as the assumptions associated with any predicted outcome are made clear. Simple models incorporating vertical transmission of symbionts reveal that microbial evolution can rapidly lead to phenotypic change within host populations over one to a few generations [65], and allow the impact of such microbiome-mediated traits on host evolution to be explicitly probed [38]. Moreover, the relative importance of transmission mode can be explored, as was done in a recent theoretical study comparing the importance of individual and holobiont-level selection on evolutionary outcomes [66]. Using an agent-based modelling framework, this study examined the outcome of host–microbiome interactions on both the reproductive success of the host and on the microbe's ability to survive within it. The results suggest that either tight vertical transmission or strong host filtering effects are required in order to see an impact of host-level selection on microbial diversity. Intuitively, it seems likely that vertical transmission would reinforce the specificity of a host–symbiont relationship (for example, allowing for the molecular adaptations underpinning host 'filtering' via the immune system), and theory has long upheld the idea that this transmission mode reduces symbiont virulence, instead favouring mutualism [15]. However, the reverse pattern is also theoretically possible, whereby provisions of nutrients or defence can reinforce the interaction and select for mechanisms to increase vertical transmission, as put forward here [67]. This idea is reinforced by multilevel selection models [68], where selection on the interaction can lead to, rather than depend upon, the evolution of transmission modes. Using a community population genetics framework, the impact of species interactions (or 'interspecific epistasis') on fitness can be examined as a higher level of organization upon which selection acts, in addition to selection at lower levels [69]. This approach can be used, for example, to help explain why mutualistic interactions are reinforced (assuming some vertical transmission) while 'cheating' can be self-limiting [68].

Are these assumptions likely to be met in nature? In some cases, perhaps so. For example, recent work suggests that the vertical transmission of microbiota across ramets within clonal plant colonies (which approx. 35% of plants are capable of forming) is likely to allow for multi-generational coupling of plant tissues with their associated microbiomes [70,71]. Similarly, in insects that provision offspring with food prior to eclosion, there exists strong evidence that parents are capable of seeding the substrate with particular microbial communities (e.g. [72,73]). However, for most plants and animals, there is little evidence that the microbiome is vertically transmitted, and in some cases, there is evidence that vertical transmission is highly unlikely or unstable (e.g. [74]).

In cases where only a fraction of the microbiome is observed to be transmitted vertically from one generation to the other (e.g. [75]), a critical empirical and theoretical question becomes whether this re-assortment occurs at random or whether a consistent subset is stably transmitted. In the event that the same subset is either stably transmitted or consistently 'filtered' from the environment by the host, founder effects could impact subsequent colonization and successional dynamics. These priority effects then have the potential to increase patterns of microbiome heritability that are greater than expected based on transmission and host 'filtering' alone. Indeed, there is evidence from both the human gut [76] and the *Arabidopsis* leaf [77] that community composition can be shaped by the presence/absence of particular keystone species. Finally, if de novo recruitment of a microbiome from the environment is required at each generation, a key question becomes whether a new holobiont could ever invade a population from rare, i.e. after arriving by mutation or migration into a new population. Such invasion would require that the new host genotype is able to encounter its optimal assemblage of microbiota and effectively increase representation of these microbial taxa in the environment in a meaningful way while at low frequencies. Current data suggest that the presence/absence of symbionts can indeed limit species ranges [78,79], indicating that hosts unable to recruit the appropriate microbiota in a new environment cannot establish, but also that host–microbiome associations can be maintained during spread into new habitats [80]. To the best of our knowledge, however, no studies to date have specifically documented the emergence and spread of a novel holobiont. Overall, in the light of the apparent transience of many microbiota, the changes in microbiome composition through development, and the lack of heritability for most host-associated symbionts due, for example, to more generalist interactions, there remain many open questions about if and when selection acting on the interaction between the host and its microbiome will result in (co)evolution.

5. Conclusion

The concept of the holobiont, or more specifically the hologenome theory, has become a controversial topic [81–83]. Ultimately, just as group selection theory provides a useful framework in highlighting how population structure can superimpose an influence on individual-level selection, the concept of the holobiont is an important reminder that host growth and survival can impact selection on microbes and vice versa. However, new terms are most usefully added to the lexicon when they bring clarity or forge a conceptual leap that moves the field forward. The concept of the holobiont brings attention away from individual species and towards the group of microbes engaged in interactions with each other and the host in which they interact. As an analogy, this is similar to asking whether one can better understand the evolution of plant pollinator assemblages by obfuscating any consideration of individual species and instead focusing on the growth of pollinator and plant populations on a landscape. Such a change of perspective hides many of the interesting evolutionary dynamics described above, including clear evidence of a tug-of-war between some hosts and their microbial associates that can result in disassociation of the host and symbiont as a result of population-level selection [29]. Even the seemingly unique intimate molecular interactions between host and microbiome can usefully be considered under existing theory on epigenetics [68,84]. Furthermore, there is reason to worry that the introduction of new terminology will further divide the microbial ecology and microbiome sciences away from the rich and well-developed existing theory on species interactions and coevolution, especially for those newly entering the field. As such, we suggest that there is no need for a paradigm shift in how we think about host–microbiome interactions, and that we would do well to retain an emphasis on the behaviour of individuals species that comprise communities while appreciating the significance of these interactions to both host and symbiont ecology and evolution.

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