Review

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Evolution of animal immunity in the light of beneficial symbioses

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Immune system processes serve as the backbone of animal defences against pathogens and thus have evolved under strong selection and coevolutionary dynamics. Most microorganisms that animals encounter, however, are not harmful, and many are actually beneficial. Selection should act on hosts to maintain these associations while preventing exploitation of within-host resources. Here, we consider how several key aspects of beneficial symbiotic associations may shape host immune system evolution. When host immunity is used to regulate symbiont populations, there should be selection to evolve and maintain targeted immune responses that recognize symbionts and suppress but not eliminate symbiont populations. Associating with protective symbionts could relax selection on the maintenance of redundant host-derived immune responses. Alternatively, symbionts could facilitate the evolution of host immune responses if symbiont-conferred protection allows for persistence of host populations that can then adapt. The trajectory of immune system evolution will likely differ based on the type of immunity involved, the symbiont transmission mode and the costs and benefits of immune system function. Overall, the expected influence of beneficial symbiosis on immunity evolution depends on how the host immune system interacts with symbionts, with some interactions leading to constraints while others possibly relax selection on immune system maintenance.

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

1. Introduction

When a kudzu bug is born, something remarkable happens. Emerging from its egg case, it begins to feed. This behaviour seems rather ordinary until you realize what it is feeding upon is not the plant that will be its primary source of food throughout development. Instead, it feeds upon a package left alongside its egg by its mother. This package contains bacteria—bacteria that will be critical for its development and survival [1].

This bacterial gift symbolizes the importance of beneficial bacterial symbiosis for many, if not most, eukaryotic organisms, including ourselves. While our bacterial gifts are not packaged so neatly, we too acquired bacteria from our mothers at birth [2], beginning a symbiotic journey that impacts many aspects of our development and well-being.

Inherently interesting is that these bacteria are often not that different from others that could make us sick. From the original development of germ theory until today, interactions between pathogenic microbes and hosts have been a critical point of study for a diverse array of biological disciplines, from immunology and microbiology to evolution and ecology. Because of this research agenda, in part driven by a desire to protect ourselves and our crops from disease, we have gained fundamental insights into how an array of hosts respond to pathogens. Central to our understanding is host immunity, which consists of a suite of defences that are either constantly vigilant or that are induced upon exposure to microbes. Traditionally, immune systems have been regarded as the mechanism by which organisms defend themselves against foreign threats to the body

[3,4]. However, recognition that most microbes are benign or beneficial has changed the way that the immune system is viewed. Over a decade ago, Margaret McFall-Ngai brought forth the idea that the adaptive immune system may have evolved in order to maintain and manage the vertebrate microbiome, which is often more complex than that of invertebrates due to the greater diversity of microbes that vertebrates tend to harbour [5]. Around the same time, Kitano & Oda [6] introduced the hypothesis that control of a more diverse community of beneficial microbes by the adaptive immune system provides the host with more tools and flexibility to respond to changing environments [6]. Since the publication of these articles, empirical studies have illuminated the role of animal immunity (both innate and adaptive) in association with microbes. The immune system not only protects hosts from germs, but it is also an integral component of the host that affects and is affected by the beneficial microbes that dwell within.

Here, we overview key ways in which beneficial microbes interact either directly or indirectly with animal host immunity. We then consider the evolutionary implications of these processes for host immune system evolution. Many of the potential consequences of beneficial symbioses for host immunity are speculative, untested hypotheses, and we thus attempt to highlight areas where future research could improve our understanding of animal immune system evolution in the light of beneficial symbioses.

2. Overview of animal symbioses

Symbioses are long-term associations between two or more organisms. In the case of microbial symbioses, long-term generally means over the lifetime of the host, the larger of the two organisms in the association. By contrast, symbionts often refer to the smaller of the two organisms, the microbes [7]. In the broadest sense, the fitness consequences of the association can be either detrimental or beneficial for either partner. However, for brevity, we hereafter refer to beneficial microbes as symbionts and interactions between hosts and beneficial microbes as symbioses. We refer to detrimental microbes, which more rarely form long-term relationships (symbioses) with their hosts, as pathogens or parasites. Specifically, we focus on the evolutionary consequences of host–symbiont associations for animal hosts. In most symbioses, less is known about the benefits for the symbiont [8].

Symbioses range from host reliance on a single microbial species to dependence on a complex microbiome of many species [7] and can exhibit substantial variation across host individuals [9–11]. Under either scenario, symbionts can provide a wide range of benefits, including nutritional provisioning, protection from pathogens and predators, and tolerance to environmental stress. All of these benefits open up the opportunity for hosts to occupy otherwise inhospitable niches [12,13]. Because of their influences on fitness, symbioses can have profound impacts on host and symbiont evolution. In some cases, the evolutionary outcome is that host or symbionts (or both) become obligately locked into the partnership, unable to persist without the other [14]. The benefits of such obligate symbioses are typically less ecologically contingent than are the benefits of facultative associations [15], which exhibit less reliance [16], though the benefits of obligate symbiosis can differ across environments as well [17,18]. Thus,

across systems, we see substantial variation in both the composition and consequences of symbiosis.

While beneficial symbioses increase host fitness, they are not without costs. Costs of symbiosis for hosts can be constitutive, stemming from the recruitment of host resources to the symbiont, and may be exacerbated by over-proliferation of symbionts [19] or by symbiont toxin production [20]. Costs can also be induced, for example, when symbiont-conferred defences are deployed [21]. These costs of symbiosis are an important contributor to intraspecific variation in symbiont association and undoubtedly shape the evolution of host phenotypes, including immunity.

3. Overview of animal immunity and other defences

Innate immunity is a common feature of all multicellular organisms. The innate immune system functions by producing responses to foreign bodies recognized as non-self through the detection of conserved microbial antigens [22,23]. Innate immunity in animals is comprised of two arms: cellular immunity and humoral immunity, and is aided by physical barriers, such as the gastrointestinal tract, that restricting entry of microbes into hosts or host cells. Cellular immunity refers to the activation of haemocytes that circulate the body and phagocytize or encapsulate microbes [24,25]. Humoral immunity refers to the recognition of microorganism-associated molecular patterns (MAMPs) by pattern recognition receptors (PRRs), which activate conserved signalling pathways to release antimicrobial peptides (AMPs) and other effectors that kill microorganisms [25]. These responses are historically considered less specific than the antigen-based ‘lock-and-key’ responses of the adaptive immune system, though recent research has highlighted that some of these responses and their associated effectors (e.g. antimicrobial peptides) can be highly specific [26,27].

In addition to innate immunity, vertebrates, but not invertebrates, have an adaptive immune system. Adaptive immunity consists of mechanisms that recognize specific threats and remember previous exposures to specific microbes, thus allowing the host to respond more quickly upon subsequent encounters to these microbes. In jawed vertebrates, the adaptive immune system generates diverse groups of antigen receptors and antibody proteins, which can bind to the surfaces of different microbes and activate subsequent responses from other proteins and cells. Jawless vertebrates have evolved a different adaptive immune system, where the mechanism to recognize microbes involves generation of diverse lymphocyte receptors instead of antibodies [22]. These mechanisms allow vertebrates to maintain an array of complex defences to regulate numerous microorganisms. While invertebrates only have an innate immune system, some species have mechanisms that are akin to memory-like responses or recognition of previously encountered microbes [28].

It is important to note that immune system-based defences, while the focus of this commentary, are not the only forms of defence. Some animals, for example, can medicate themselves, their offspring and/or their relatives, to protect from pathogens and parasites [29]. Others have evolved to avoid exposure to these threats [30].

Investment in immunity (and more broadly in defences) trades off with other components of fitness. More specifically,

Figure 1. Multifaceted links between immunity and symbiosis. Studies of symbiosis and immunity in cereal weevils [36–39], bees [40–45], bobtail squid [46–50] and zebrafish [51–58] have revealed many ways that immunity and symbiosis interact. Other systems central to the study of immunity and symbiosis include tsetse flies, aphids, hydra, mice and humans. Symbols next to each example correspond to their matching interaction type in the bar above. Photo credits: weevil—CSIRO, bee—Charles Sharp, squid—Nick Hobgood, zebrafish—Oregon State University. (Online version in colour.)

investment in immunity uses resources that could otherwise be allocated to other functions, leading to costs associated with maintaining a robust immune system [31–33]. A constantly active immune response can also lead to autoimmune damage [6]. Furthermore, resistance traits may be linked to deleterious mutations or may be negatively pleiotropic, such that they have deleterious impacts on other phenotypes [34,35]. These costs, taken together with the costs of beneficial symbioses, will shape the evolutionary trajectory of animal defence mechanisms.

4. How host immunity interacts with symbiosis

There are three key ways that host immunity and symbiosis interact. First, immune defences may be important in controlling the establishment and regulation of symbiotic associations. Second, symbionts may provide hosts with protection against pathogens, which could complement or ameliorate the need for immune system-based defences against the harmful invaders. Third, symbionts may strongly influence the maturation of components of animal immune systems. These processes

are intertwined and may be happening in the same host (figure 1).

(a) Importance of host immunity in establishing and regulating symbioses

Each generation, symbiosis is established when hosts are colonized by their microbial symbionts. In some systems, the primary mode of symbiont acquisition is through horizontal transmission from the environment. In other systems, symbionts are transmitted vertically from parent (often mother) to offspring. In both cases, once symbioses are established, hosts must regulate symbiont populations by preventing excessive replication and over-utilization of host resources.

The influence of the host immune system on establishment likely depends on symbiont transmission mode (figure 2). The establishment of horizontally transmitted symbioses requires hosts to select beneficial symbionts from potentially large pools of microorganisms. These hosts are likely under strong selection to limit uptake and subsequent damage from pathogens. Many hosts are reliant on horizontal transmission to

Figure 2. Key features that may influence how host immunity evolves in the light of symbiosis. How the evolution of animal immunity evolves in response to symbioses depends in part on how symbionts and immunity interact (figure 1). Furthermore, several features of symbioses and immune systems may influence the evolutionary trajectory of immunity. These include symbiont transmission mode, sequestration and community complexity. (Online version in colour.)

establish critical symbioses each generation and thus are evolving under this selection pressure.

Horizontal transmission is the dominant transmission mode for vertebrate hosts, which often harbour large, diverse microbiomes. Vertebrates have an arguably greater challenge than invertebrate hosts, which typically associate with fewer symbionts, as vertebrates must manage colonization and replication by a large community of microbes, while limiting entry and preventing replication of those that are harmful or unnecessary. For vertebrates, this includes reliance on both innate and adaptive immunity to control microbiome establishment and regulation. By leveraging both innate and adaptive immunity, vertebrates can mount rapid and robust responses to large numbers of microorganisms [59–62]. Specifically, vertebrate hosts use innate immunity to generally distinguish between symbionts and pathogens by recognizing MAMPs. Adaptive immunity then allows hosts to recognize and regulate specific microorganisms, eliminating pathogens and facilitating the establishment of symbionts [59–62]. For example, mice couple innate and adaptive immune responses to discriminate between pathogens and symbionts and to segregate symbionts to the appropriate host tissues [59]. Both pathogens and symbionts can enter the lumen of the digestive tract, where the epithelial tissue is protected by a mucosal layer.

Here, immune mechanisms allow mice to recognize and eliminate pathogens while preserving beneficial symbionts: symbionts that enter the mucus layer are segregated from the host epithelium by antimicrobial proteins of the innate immune system and immunoglobulins of the adaptive immune system. Pathogens that enter are killed by the adaptive immune system. Furthermore, symbionts that happen to cross the mucus layer and enter the host's epithelium are also eliminated by immunoglobulins. Thus, using a combination of innate and adaptive immunity allows vertebrates to preserve interactions with diverse symbionts while mounting targeted and effective defences against pathogens.

Lacking adaptive immunity, many invertebrate hosts reliant on horizontal transmission use a combination of innate immune functions to differentiate between pathogens and symbionts. Common defences include physical barriers and differentiation between pathogenic and symbiotic MAMPs by the host innate immune system. Expression of innate immunity is often compartmentalized, allowing hosts to mount robust immune responses in regions exposed to a wide array of microorganisms and to limit immune expression in regions primarily colonized by symbionts [63]. Compartmentalization is likely important for regulating horizontal transmission because it allows hosts to invest the most energy into screening

microorganisms where they are most vulnerable, while reducing immune investments elsewhere. By coupling multiple strategies, invertebrate hosts can screen a large quantity of microorganisms and limit establishment to specific symbionts based on their expressed traits and the damage that they impose. For example, the Hawaiian bobtail squid *Euprymna scolopes* uses multiple defences, including physical barriers, symbiont-induced morphological changes and innate immunity to screen large quantities of microorganisms and limits colonization to specific strains of its symbiont, *Vibrio fischeri* [46–48,64,65]. Combined, these defences allow the squid to limit establishment in the light organ (the symbiont-storing crypt) to bacteria with the specific characteristics of their symbionts, including symbiotic MAMPs, biofilm formation, bioluminescence and nitric oxide resistance. Similarly, the fruit fly *Drosophila melanogaster* uses physical barriers, morphological responses, and compartmentalized immune expression to eliminate pathogens and to limit establishment in the gut to the relatively few resident symbionts that comprise its gut microbiome [63]. In general, the evolution of complex innate immune responses for establishment and regulation of horizontally transmitted symbioses has been observed across many invertebrate animal taxa [48,63,66–68]. The combination of physical defences and immune functions employed by invertebrate hosts is likely an evolutionary outcome of the strong selective pressures on hosts to limit uptake and damage from pathogens.

In comparison to the above systems in which symbionts are horizontally transmitted, vertical transmission results in hosts passing on relatively fewer symbionts directly to their offspring, allowing for tighter control of symbiont exposure to the offspring. Passaged symbiont populations undergo bottlenecks during transmission and have little opportunity for horizontal gene transfer with environmental microorganisms. These factors allow hosts to tightly control which microorganisms their offspring acquire and limit opportunities for microorganisms to obtain virulence factors that harm their hosts. Parents may facilitate symbiont transmission to their offspring in a variety of ways, for example, by providing offspring with symbiont-enclosed capsules, smearing egg surfaces with microbial symbionts [69], or via transovarial symbiont transmission from the mother to the developing embryo [70,71]. In many systems, this selective transmission is coupled with sequestration of symbionts into specialized organs or cells. Sequestering symbionts allows hosts to limit symbiont replication and further restrict horizontal gene transfer. It also may allow hosts to allocate fewer resources to the immune regulation of symbionts (figure 2). Consistent with this idea, in some systems, systemic immune responses are limited in symbiont-storing tissues. For example, in cereal weevils, many antimicrobial peptide genes are not expressed in bacteriocytes where obligate symbionts are stored but are expressed in other tissues and in response to pathogens [72,73]. More broadly, cereal weevils appear to suppress systemic immunity in order to allow symbionts to persist and to minimize the costs of constant immune activation towards them [36,74]. This process, however, does not preclude immunity from playing a role in regulating vertically transmitted symbiont populations. In cereal weevils, despite the low level of expression of many known immune genes in bacteriocytes, one antimicrobial peptide (ColA) is highly expressed. Knock-down of *colA* leads to over-proliferation of the symbionts and escape of the symbionts into other host tissues [37].

Thus, weevils use targeted immune responses for symbiont regulation but minimize the potential costs of broader immune activation. Ultimately, the evolution of vertical transmission may represent an adaptation that allows hosts to maintain intimate associations with microorganisms, while limiting the costs of immunity.

The benefits provided by symbionts can vary throughout host lifespan. Cereal weevils, for example, require gut symbionts for exoskeleton development, which is complete several weeks following maturation to adulthood [38]. Following the completion of exoskeleton development, adult weevils then eliminate gut symbionts by apoptosing symbiont-bearing cells. The eliminated symbiont components are digested and recycled by the host [75]. This strategy allows weevils to benefit from symbiosis while limiting the energetic costs of symbiont maintenance and regulation after symbionts are no longer needed. For other hosts, symbiont regulation strategies may vary as developmental demands require hosts to invest energy toward functions other than symbiont regulation. Bean bugs (*Riptortus pedestris*), for example, upregulate innate immunity and substantially reduce symbiont numbers prior to moulting [76]. Because moulting is energetically costly and leaves bean bugs vulnerable to injury and infection, upregulating immunity may benefit these hosts both by preventing exploitation and by digesting symbiont cells to provide energy for moulting. Both cases highlight that interactions between immunity and symbiosis can vary over development.

Several of the examples above highlight the critical role that immune systems play in determining whether a symbiosis is mutualistic or pathogenic. As mentioned, in weevils, knock-down of the mRNA associated with a key antimicrobial peptide leads to over-proliferation of their symbionts and symbiont infection in other host tissues, both of which are presumably harmful for the host [37]. This example highlights the complex interplay between immunity and symbiosis, with the need to maintain beneficial symbioses likely shaping the evolution and utilization of immunity, and immunity, in turn, shaping the evolutionary trajectory of symbioses.

(b) Symbiont-conferred protection

In addition to an intrinsic immune system, hosts can harbour symbionts that act as part of their defence against enemies, including pathogens, parasites, parasitoids and predators.

Often protective symbionts and host immunity can work together such that greater protection is exhibited compared to when either is absent [77]. Furthermore, because symbionts can evolve faster than their hosts, they can coevolve with and rapidly adapt to the threat [78], which then selects for the maintenance of protective symbionts. Thus, while hosts are often not obligately dependent on protective symbionts, like with obligate symbionts, hosts may vertically transmit protective symbionts to ensure that they are passed on to the next generation, or may evolve mechanisms to select for protective symbionts from the environment [78].

There is substantial variation in host populations in terms of the degree of association with protective symbionts and in the efficacy of symbiont-conferred protection. In humans, association with protective gut microbes is influenced by diet and by the use of antibiotics [79]. In populations of pea aphids (*Acyrtosiphon pisum*), in the absence of the threat, protective symbiont frequency can decline [80], presumably because of fitness costs of maintaining symbionts for these

insects. Genetic variation in symbionts, in hosts and in pathogens and parasites can alter the efficacy of protection [81–83], and genotype–genotype interactions between hosts and symbionts, and between symbionts and pathogens, can alter the level of protection as well [82,83]. The efficacy of symbiont-conferred protection can also differ across environments (e.g. temperature [84,85]). Overall, the benefits of association with protective symbionts are influenced by both biotic and abiotic factors, which in turn influence selection for symbiont maintenance.

Mechanisms of symbiont-conferred protection vary, with some being more tied to host immune responses than others. Protective symbionts can directly interact with a threat, such as through production of toxins or competition for resources within the host [86]. For example, the gut symbionts of honey and bumble bees express an array of toxin genes that are suggested to play a role in pathogen suppression [87,88]. Symbionts occupying the same space as pathogens or parasites can also form a physical barrier, preventing these enemies from circulating in the host [89]. These mechanisms may limit the need for host immune responses.

Alternatively, symbionts can protect hosts through modulating host immune responses [86]. Studies have shown that symbionts can induce immune responses in a diverse range of animal hosts [40,51,90,91], subsequently preventing pathogen invasion [92,93]. In mosquitoes, for example, *Wolbachia* bacteria increase resistance to a range of viruses by stimulating the hosts' innate immune responses [92]. Similarly, colonization of mice by segmented filamentous bacteria activates expression of T helper cells that subsequently increase host resistance to an intestinal pathogen [94]. Conversely, germ-free mice produce fewer neutrophils and monocytes, which then causes these mice to be more susceptible to pathogens [95,96]. Variation in protective mechanisms, combined with variation in prevalence and efficacy of protection, suggest that symbiont-conferred protection has played an important role in how host immunity has evolved.

While many of the above examples involve host associations with specific, protective microbes, harbouring a compositionally and functionally diverse microbiome can be central to protection in other systems [6,97]. A plethora of studies have demonstrated a correlation between microbiome diversity and resistance to pathogen invasion. For example, in locusts, there is an inverse relationship between gut microbiome richness and bacterial pathogen invasion [98]. In *Hydra*, the resident microbiome of the ectodermal epithelium plays an important role in defence against pathogenic fungal infections, and interactions between commensal members of the microbiome have additive or synergistic effects, such that full fungal resistance is only observed when the entire resident microbiome is left intact [99]. Similarly, in humans, reduced community richness of the pharyngeal microbiome leaves patients vulnerable to respiratory infections [100], and reductions in intestinal microbial diversity increases risk of infection by *Clostridium difficile* [101,102]. In the latter case, transplantation of a healthy microbiome can provide an effective treatment strategy for *C. difficile* infections [101,102]. Species-rich microbiomes are likely to prevent infection through competitive exclusion of invading microorganisms, though they may also be key to modulating the host immune response to a protective state. Overall, a community of beneficial microbes adds further complexity to how protective symbionts can affect the evolution of animal immunity.

(c) Influence of symbiosis on host immune maturation

Some symbionts are necessary for the maturation of the immune system. Maturation is distinct from immune modulation in that it refers to the presence of symbionts during early life stages influencing the development of the host immune system. By contrast, protective symbionts modulate host immunity by stimulating or priming a response when there are pathogens present. In tsetse flies (*Glossina* spp.), for example, mothers vertically transmit obligate *Wigglesworthia glossinidia* bacterial symbionts to their offspring. Larvae that develop without *Wigglesworthia* have a weakened immune system compared to adults with the symbiont, suggesting that the symbiosis may be critical for immune system maturation. Specifically, both cellular-based immune responses (phagocytosis by haemocytes) and immune gene expression are altered [93,103,104]. Furthermore, females lacking *Wigglesworthia* produce larvae that have decreased expression of genes involved in haemocyte differentiation, suggesting that there are transgenerational effects of symbiont association [104]. These phenotypes have important fitness consequences: aposymbiotic flies infected with *Escherichia coli*, which is normally not pathogenic to flies, are overwhelmed by the bacterial infection [49]. Of applied importance, aposymbiotic flies are also less likely to clear trypanosome infections, which they vector to humans. These influences on immune system maturation might have evolved in part because they benefit the symbiont. In bobtail squid, for example, persistent association with their symbiont, *V. fischeri*, leads to reduced haemocyte responses in adults to their symbiont relative to non-symbiotic competitors [49].

Microbial acquisition also influences immune system maturation in animals that form symbioses with a more complex microbiome. Human infants, for example, have a reduced immune repertoire compared to adults (reviewed in [105]). Many aspects of subsequent immune system maturation in humans are dependent upon exposure to MAMPs (reviewed in [106]). Some components of the immune system are influenced by specific microbes, while others are more influenced by exposure to the microbiome as a whole. Exposure of pre-weaning mice to some microbes but not others, for example, increases T-cell proliferation [107]. The gut microbial community as a whole, however, influences the production of neutrophils (reviewed in [108]): as mentioned in the previous section, mice reared germ free have extremely reduced neutrophil numbers. Similarly, early life antibiotic exposure that reduces diversity of the gut microbiome impacts other components of adaptive immunity [106,109,110].

5. Potential evolutionary consequences for host immune systems

Given the many ways in which immunity and symbiosis interact, how symbiosis has and will continue to shape the evolution of host immunity are both complex. Considerations of the evolutionary consequences of symbioses for host evolution must take into account variation across systems in terms of a number of factors (figure 2). Below, we consider, separately, the potential consequences for immune system evolution of the three key ways in which symbiosis and immunity interact. We then highlight approaches that can be taken to address outstanding questions. Finally, we go back

to where it all began, finishing with consideration of the origins of immunity.

(a) Immune evolution in light of regulatory interactions

If host immunity primarily functions to control establishment and regulation of symbiotic microorganisms, then selection on host immunity likely depends on symbiont transmission mode. When symbionts are vertically transmitted from parent to offspring, there may be less role for immunity to shape what symbionts establish in a host than when symbionts are horizontally acquired from a diverse environmental pool. Empirical evidence, especially in humans, indicates environment as a key predictor of vertebrate microbiome composition, suggesting horizontal transmission as the dominant transmission mode in animals with complex microbiomes [111,112]. This acquisition strategy may be selected for because environmentally acquiring a diverse microbiome can be key to fighting infection and can provide resilience in the face of changing or unpredictable environmental conditions, which may be particularly important for long-lived hosts. Adaptive immunity, in turn, is theorized to have evolved to reinforce innate immunity by affording hosts the ability to selectively maintain a broad diversity of symbiotic microorganisms while also preventing exploitation [6]. Correspondingly, the distribution of adaptive immunity and complex microbiomes across animals is consistent with a causal link between the two: the most complex microbiomes tend to be found in vertebrates, including mammals [112–114]. However, there is a challenge in interpreting these distributions. Adaptive immunity has only evolved twice [22], and therefore the extensive list of animals with both adaptive immunity and a complex microbiome may represent a few instances of these traits arising from shared ancestors rather than repeated convergent evolution of adaptive immunity in the face of selection to establish and maintain diverse microbiomes.

Despite this caveat, the two independent origins of adaptive immunity, one in jawless and one in jawed vertebrates, do provide an important opportunity to study how symbiosis has shaped the evolution of two parallel but evolutionary independent adaptive immune systems. We do not yet know how the mechanistic differences between the two systems (one involving antigens, the other involving lymphocyte receptors) alter interactions with the microbiome. Less is known about symbioses of the few extant jawless vertebrates (hagfish and lampreys) compared to the jawed vertebrates, but researchers have begun to examine these organisms' microbiomes and their interactions with immunity, which will allow for eventual comparison of the parallel systems [115–117].

In comparison to vertebrates, invertebrate hosts are typically relatively short-lived and maintain interactions with fewer microbial symbionts. In this case, the memory encoded in adaptive immunity may provide these hosts little advantage if repeated exposure to the same pathogens is less likely [32]. Invertebrate hosts, therefore, may have higher fitness in the absence of the costs of maintaining adaptive immune systems given that there is less benefit of specificity.

Furthermore, unlike in vertebrates, vertical transmission of symbionts has evolved multiple times across the evolutionary history of invertebrates. Vertical transmission may limit the nutritional and metabolic costs of innate immunity by offering tight control over the establishment and regulation of an invertebrate's few symbionts. This tight control can

facilitate selection for beneficial symbionts that are less likely to exploit their hosts, because, through vertical transmission, symbiont fitness depends on host fitness [118]. Vertical transmission also limits opportunities for horizontal gene transfer between microorganisms [119], which decreases the probability that symbionts will acquire virulence factors that allow them to exploit their hosts. Finally, vertically transmitted symbionts are often sequestered into specialized storage cells or tissues, where their proliferation can be controlled through host regulation of nutrient availability. All of these features of vertical transmission allow hosts to limit costs of immune system activation in response to symbiosis. Thus, while pathogens will still select for a functioning immune system, selection on immune system maintenance may be weaker in hosts with vertically transmitted symbioses than in hosts reliant on horizontally transmitted symbioses.

(b) Evolution of immunity in the light of symbiont-conferred protection

Harbouring protective symbionts as an alternative or in addition to host-encoded defences could have implications for the evolution of the immune system. There are, however, few direct tests of evolution of host immunity in the presence and absence of protective symbionts. One key study investigated the evolution of host resistance using *Drosophila melanogaster* with and without *Wolbachia* bacteria that protect the flies against a viral pathogen. After multiple generations of evolution in response to the virus, fly populations with *Wolbachia*, compared to those without *Wolbachia*, had fewer individuals that harboured the fly-encoded resistant allele to the virus. These findings suggest that hosts could become evolutionarily dependent on their symbiont, in part because of relaxed selection on immune function [120].

The consequences for symbiont-conferred protection may be dependent on the mechanism of protection. In the example above, viral protection of the flies is not mediated through the host immune system. If the mechanism of protection is dependent on the symbiont stimulating a host immune response, then selection could act to maintain host immune function. Furthermore, protective symbionts could facilitate the evolution of a more robust immune system if they keep hosts alive long enough for hosts to evolve themselves. For example, symbionts could provide the protection needed for hosts to survive and reproduce when hosts encounter new threats. Over time, host populations may accumulate enough genetic variation to adapt, and removal of the symbiont would not affect host fitness. This phenomenon has not been empirically demonstrated but could potentially be tested through experimental evolution or long-term monitoring of natural populations whose association with protective symbionts varies.

The mechanisms of host defence involved in responding to a pathogen or other threats may influence how protective symbioses alter immune system evolution. Intrinsic host defences may be constitutive or induced. If host defences are constitutive, meaning that they are constantly active, they can result in high metabolic costs. We might, therefore, expect evolution to favour reduction of their use if defensive symbionts can replace these host functions. However, if host defences are induced only upon exposure to threats, then there would be fewer costs accrued across the lifetime of the host and, in turn, there may be evolutionary maintenance of induced host

defences, even in the presence of protective symbionts. Here too, empirical studies are lacking but comparison across systems and modelling of these phenomena would be appropriate steps forward.

Symbiont transmission mode may also influence how protective symbionts alter immune system evolution. Given that the fitness of vertically transmitted symbionts is tied to survival of their hosts, there may be stronger selection on vertically transmitted symbionts to evolve and maintain mechanisms that protect their hosts compared to horizontally transmitted symbionts. While, to our knowledge, there has been no comprehensive investigation of this hypothesis, should it be the case, then there may be a greater likelihood of relaxed selection on the evolutionary maintenance of host immunity in cases of vertically transmitted than horizontally transmitted symbionts.

Overall, in relation to protective symbioses, we hypothesize that the evolutionary trajectory of the host immune system will be influenced by the relative costs and benefits of harbouring protective symbionts and maintaining/using host immunity [121]. These forces, however, also need to be considered within the context of the prevalence and virulence of pathogens. Increased pathogen pervasiveness may select for greater protective symbiont frequency in the host population [80]. If pathogen pressure is high, it may also select for the maintenance and utilization of both host defences and symbionts that confer protection, resulting in a robust immune system in addition to protective symbionts [77,122].

(c) Immune evolution in light of symbiont impacts on immune maturation

In some respects, aspects of immune maturation are similar to protective symbionts that alter specific host immune responses. In the former case, however, symbiont association early in life has wide-sweeping effects on the strength of immune responses for the entire adult life of the organism. One consequence of immune system maturation being dependent on symbiont presence is that dependence could lock hosts into maintaining symbiotic associations. Presumably, this process could in turn provide a fitness advantage for hosts that have mechanisms, such as vertical transmission, to ensure symbiont acquisition. Of course, we cannot presume that all symbioses with vertical transmission are a result of those symbionts being needed for proper host immune function, and more studies are needed on how symbionts influence host immune maturation, and host developmental processes more generally. Given the multifaceted benefits that many symbionts provide, it is hard to evaluate the relative contribution that symbiont-mediated immune maturation, relative to other processes, had in shaping the maintenance of host–symbiont interactions over evolutionary time.

Another interesting evolutionary consideration is why hosts would evolve to rely on symbiont presence for immune system maturation, as it substantially increases the costs of not maintaining the symbiosis. One hypothesis to explain this phenomenon is that, because immune responses are costly, there may be selection to use reliable symbiont signals as an indicator that harmful microbes may now be able to enter [123]. These cues then trigger the mounting of immune responses only at life stages when needed. Another hypothesis is that selection has acted on hosts to maintain symbioses for a number of reasons, and thus there is no cost to being locked into the symbiosis because of immune system maturation, as

the other costs of losing the symbioses substantially outweigh this particular challenge. These hypotheses are not mutually exclusive.

(d) Symbiosis may not always impact immune system evolution

Given the many modes through which symbiosis and immunity interact, and the ubiquity of animal–microbe symbioses, it becomes easy to fall into the trap of envisioning symbioses to be a key force shaping immune system evolution in all systems at all times. However, in some systems, we do not see strong signatures of that interaction, at least in the extant state of the relationship [124]. A lack of such a signature does not preclude the possibility that symbiosis has shaped the evolution of immune mechanisms in a species or its ancestors in the past. However, it does call into question what role symbiosis will play in shaping that species' immune responses going forward. We should consider that there may be a limited role for symbiosis to influence the evolution of host immunity in systems where symbioses evade host immune responses (much like many pathogens do) or in systems in which symbionts are sequestered in specialized tissues, such that sequestration and not immunity is the key form of control (box 1). However, in some systems with these features, such as cereal weevils (figure 1), a limited but highly specialized immune response plays a key role in regulating the symbiosis. Thus, these features do not preclude a role for immunity.

We also need to remember that symbiosis is not for everyone. While association with beneficial microorganisms has shaped the evolution of much of animal life, some species do not maintain steady associations with beneficial microbes [131]. In these animals, the immune system is indeed more likely evolving in response to pathogens, not symbionts.

(e) Approaches to move forward

There are several approaches to studying evolutionary processes that are important to implement as we move forward to gain better insight into the role of symbiosis in shaping host immunity. First, inferences of evolutionary past often benefit from a comparative approach. In this case, comparison of immune system processes in closely related species that have evolved in the presence and absence of symbionts would be ideal. However, given the ubiquity of symbiosis in many animal groups, this approach is often challenging. Continued investigation of animal reliance on symbionts, however, may reveal ideal comparisons. The comparative approach may be easier to implement in regard to the influence of transmission mode on immune system interactions with symbionts. True bug species, for example, vary substantially in terms of symbiont transmission mode [132], providing one possible point of comparison. Second, another approach that can allow for insight into evolutionary trajectory is experimental evolution, through which researchers can set up systems in which some host lineages have symbionts while others do not, and then watch how immune system processes evolve. As mentioned above, this approach has demonstrated that *Drosophila* immune responses towards viruses differ when the flies have evolved with and without protective bacterial symbionts in the presence of the pathogen [120]. Similarly, experimental evolution studies have evaluated the impacts of transmission

Box 1. When the Immune System is Not (As) Involved

While there are complex links between symbiosis and immunity that could fundamentally alter host immune system evolution, it is important to note that both hosts and symbionts may have either ancestral or derived traits that lessen interaction between the host immune system and symbionts. Here are three examples:

Symbioses and Immune System Evasion

For symbionts to succeed in terms of fitness, they have to be able to successfully invade the host, and sometimes its cells, and be transmitted to other hosts. As such, some symbionts are not that different from pathogens in terms of the strategies that they employ when interacting with hosts. Furthermore, some symbionts may have evolved from pathogens themselves, or be closely related to pathogens, such that the mechanisms involved in infecting the host and evading host immunity preceded the symbiosis [125,126]. Additionally, horizontal gene transfer may also facilitate exchange of genes between pathogens and symbionts [127]. One way in which pathogens can avoid detection from circulating host defences is by hiding within host cells or tissues. Likewise, symbionts also possess tools that allow them to enter host cells. For example, type III secretion systems provide bacteria with the ability to inject proteins into host cells, altering cell physiology. These genes are found in free-living bacteria, pathogens and endosymbionts of certain insects, suggesting that some symbionts utilize this system to invade host cells [125,127]. Even symbionts that have very reduced genomes, such as *Buchnera aphidicola*, the obligate symbiont of aphids, are predicted to have retained structures necessary to invade cells and tissues of their hosts [126]. Finally, some insect endosymbionts lack enzymes that synthesize bacterial outer components, which may make it difficult for the host immune system to recognize them [125,128]. There would be little evolutionary consequence of symbiosis for host immunity if a symbiont is essentially invisible to its host.

The Evolution of Other Forms of Regulation

Hosts that horizontally acquire microbial symbionts must rely strongly on physical barriers and cellular recognition to mediate colonization, which may lead to interaction between the symbionts and the immune system. However, hosts that vertically acquire microbial symbionts may maintain greater control over symbionts and experience a reduced risk of exploitation without the need for immune system involvement. Indeed, these hosts often sequester microbial symbionts into specialized cells called bacteriocytes. Sequestration into bacteriocytes limits genetic exchange between symbionts, which may suppress the evolution of exploitative symbionts [119]. Similarly, in mammalian hosts, intestinal bacteria are confined to the gut lumen through mucosal layers that act as barriers. Symbionts able to penetrate the epithelium are eliminated by antimicrobial proteins and phagocytized [129].

Furthermore, transmission of symbionts from mother to offspring can result in population bottlenecks for the symbionts. Over multiple generations, this process can reduce the effective population size of symbiont populations [119]. Reductions in effective populations sizes may leave symbionts vulnerable to the accumulation of deleterious mutations through genetic drift [119]. For many ancient heritable symbioses, genetic drift has resulted in genomic decay, making symbionts irreversibly dependent on their hosts [130]. Therefore, vertical transmission may present an evolutionary pathway by which hosts maintain tight regulation over symbiont colonization and replication that does not require utilization of the host immune system.

mode on symbiont evolution through manipulation to simulate both modes in the same host species [133,134]. Building upon these studies, the next step may be to determine how host immunity is altered when evolving in the presence of horizontal or vertical transmission. Systems where symbionts are environmentally obtained are amenable to such studies, as the symbionts can be cultured outside the host [135,136]. Importantly, experimental evolution could provide the opportunity to study how symbionts and host immunity coevolve. Finally, another approach is to leverage the power of modelling and simulation to shape evolutionary hypotheses. For example, a recently developed model predicts that a weakened immune system can evolve when distinguishing between pathogen and symbiont is difficult, when symbionts provide strong levels of protection and when immune costs are high [137]. All these approaches, taken together with development of additional systems, will play a role as we move forward.

(f) Final considerations

In considering how symbiosis could influence immune system evolution, we have largely avoided discussion of the origins of immune system-based defences. We have touched on

hypothesizes as to how the benefits of maintaining a robust, diverse microbiome could have been a key selective pressure underlying the evolutionary success of adaptive immunity, but what about before that? Did beneficial symbioses play a key selective force underlying the origin of the first defence systems? Would animal immune systems be fundamentally different if animals never associated with beneficial microbes? These questions are hard to test experimentally, but it is important to recognize that symbiosis has been present throughout the evolution of all eukaryotes. Indeed, the origin of eukaryotes started with symbiosis. Thus, all of animal immunity evolved in the light of beneficial symbioses. We may never know the relative contribution of defence versus maintenance in shaping immune systems' origins, but we cannot deny that both have played a role from the very beginning.

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