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Symbiont-mediated immune priming in animals through an evolutionary lens

Kim L. Hoang* and Kayla C. King

Abstract

Protective symbionts can defend hosts from parasites through several mechanisms, from direct interference to modulating host immunity, with subsequent effects on host and parasite fitness. While research on symbiont-mediated immune priming (SMIP) has focused on ecological impacts and agriculturally important organisms, the evolutionary implications of SMIP are less clear. Here, we review recent advances made in elucidating the ecological and molecular mechanisms by which SMIP occurs. We draw on current works to discuss the potential for this phenomenon to drive host, parasite, and symbiont evolution. We also suggest approaches that can be used to address questions regarding the impact of immune priming on host-microbe dynamics and population structures. Finally, due to the transient nature of some symbionts involved in SMIP, we discuss what it means to be a protective symbiont from ecological and evolutionary perspectives and how such interactions can affect long-term persistence of the symbiosis.

INTRODUCTION

Parasites are ubiquitous and can cause substantial harm to host fitness. Animals have thus evolved multiple mechanisms to defend themselves against parasites, such as avoidance, self-medication, and immunity [1–3]. The innate and adaptive immune systems employ a series of cellular and humoral processes to prevent or mitigate damage from infection [3, 4]. The adaptive immune system can provide protection through recognition of previously exposed parasites; such exposure can also prime the innate immune system [5, 6]. Association with microbes is also critical in animal defence against parasites. By providing additional protection, microbial symbionts can buffer hosts against environmental perturbations and allow them to thrive in harsh environments [7, 8]. They can replace functions that the immune system lacks or work together with host immunity to modulate responses to parasites and other stressors.

Protective symbionts are widespread in host species across the tree of life [9]. We define protective symbionts as conditionally beneficial microbes associated with hosts, including bacteria, archaea, fungi, viruses, and members of the microbiome (Box 1). During their colonization, protective symbionts defend hosts through several general mechanisms [9–12]. Symbionts can directly interfere with parasites by producing toxins that reduce parasite fitness [13] or compete with parasites for resources and space within the host [14]. Symbionts can also indirectly interact with parasites by stimulating the immune system to prime hosts for subsequent infection. Such symbionts protect hosts by increasing host fitness upon infection, improving host health, and/or reducing parasite burden.

The molecular underpinnings of symbiont-mediated immune priming (SMIP) vary widely across animal taxa, involving both the innate and adaptive immune systems (Table 1). For example, many invertebrates harbour symbionts that upregulate antimicrobial peptides in hosts, facilitating in elimination of the parasite [5, 15]. Alternatively, members of the vertebrate microbiome modify different arms of innate and adaptive immunity to alter parasite load or reduce harm to hosts [16]. The immune response can be viewed from the perspective of symbionts taking part in indirect competition (i.e. apparent competition) with the parasite [11] or the host immune system recognizing the symbiont as a non-self entity [17]. Furthermore, immune priming is not exclusive to symbionts—parasites, their components, and other abiotic factors also elicit immune protection (Box 2). Similar phenomena involving cross-talk between stress signalling pathways have been detected in plants and insects, where exposure to one stress can provide tolerance to another due to shared stress response pathways [18–20].

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Author affiliations: [1]Department of Zoology, University of Oxford, 11a Mansfield Road, Oxford, OX1 3SZ, UK.
***Correspondence:** Kim L. Hoang, kim.hoang@zoo.ox.ac.uk
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Abbreviations: AMP, antimicrobial peptide; p38 MAPK, p38 mitogen-activated protein kinase; PSA, polysaccharide A; SFB, segmented filamentous bacterium; SMIP, symbiont-mediated immune priming.

Box 1. Symbiont persistence from ecological and evolutionary perspectives

Symbionts are microbes that are beneficial to hosts under certain contexts [86]. However, definitions of symbionts can diverge when examining symbiosis on ecological versus evolutionary timescales. From an ecological perspective, a symbiont has positive effects on host fitness and reside in close proximity or inside the host, with the latter requiring the symbiont to stably colonize the host [87]. While symbionts can proliferate and evolve within hosts, stable colonization is not sufficient for successful passage to the next host generation or transmission between hosts. Conversely, symbionts that can escape hosts and thrive in the external environment gain more exposure to other host individuals, which can facilitate symbiont dispersal and increase symbiont fitness [88, 89]. Colonization within an individual host in the absence of a transmission mechanism does not necessarily guarantee symbiont persistence in the host population. Evolutionary persistence of the symbiosis can thus be impacted. Hosts can also benefit when symbiont fitness is decreased, such as when lysed symbionts provide metabolites necessary for host survival [90]. In this case the symbiont population declines within the lifespan of the host, but is still transmitted across host generations. Finally, while transmission mechanisms in symbionts involved in SMIP are not well-studied, many of these symbionts can proliferate in the environment. This life-history trait may allow for increased exposure to hosts on an evolutionary timescale despite their ephemeral interactions with hosts on an ecological timescale.

Ambiguities also arise because mutualistic symbionts are not always beneficial in all contexts. Costs have been identified in symbionts that have established long-term symbioses (ecologically and evolutionarily) with hosts, from facultative [91, 92] to obligate [93, 94] associations. Symbionts can also shift across the parasite-mutualist continuum, altering host fitness across space and time [86]. As a result, symbionts can vary widely and are more dynamic in terms of how they affect hosts than what may be considered traditional symbionts.

While research on SMIP has focused on foundational processes in symbiosis as well as on applications in agriculture and animal health [12, 21], less is known about the evolutionary drivers and consequences of SMIP. Here, we review recent advances made in elucidating the ecological and molecular mechanisms by which symbiont-mediated immune priming occurs and discuss the potential for this phenomenon to drive host, symbiont, and infectious disease evolution (summarized in Fig. 1). Addressing this gap is critical to expanding our understanding of the evolution of host defenses and parasite virulence, as well as the establishment of symbioses in a microbial world.

Evolution of host-encoded defense in light of symbiont-mediated immune priming

Associating with symbionts can affect the evolution of host immunity. The adaptive immune system has been hypothesized to have arisen partly due to the diet expansion allowed by the jaw, which increased exposure of jawed vertebrates to parasites [22]. Concurrently, adaptive immunity may have allowed hosts to harbour a more diverse microbiome, conferring host adaptations to changing environments and fine-tuning immune responses [22]. By influencing components of the host immune system, SMIP can confer hosts with additional layers of plasticity to respond to parasite infection compared to host-encoded defence alone, and thus can affect host evolution and adaptation [23].

Symbionts that protect hosts through modulating immunity might impact host evolution differently compared to symbionts that directly interact with parasites. Because there are costs associated with immune activation and maintenance [24], symbionts that protect hosts through toxins or competition with parasites may relax selection on host-based defence [24, 25]. Hosts may then divest of their immunity. For example, genomic analysis of pea aphids colonized by defensive symbionts suggests they lack many immune genes found in other insects [26]. One hypothesis is that having reduced immune function may be a consequence of association with a suite of symbionts that protect hosts from biotic and abiotic stresses [27–29], but more evidence is needed to demonstrate that protective symbionts were the cause of immune gene loss. Evolution of reduced immune vigilance may also occur if it is difficult for the immune system to distinguish between beneficial symbionts and parasites (e.g. due to phylogenetic-relatedness or production of similar molecular signatures) [30]. Alternatively, immune functions may be maintained if symbiont costs are too great (e.g. in the absence of the parasite [31]), and the symbiont may decrease in prevalence or be lost altogether.

For SMIP, maintenance of a robust immune system—developed and not lacking immune genes or functions—is necessary because of the direct impact of the immune system on parasites, but we are unaware of any empirical studies on the role of SMIP on the evolution of host immunity [25]. One critical study in *Drosophila* has illuminated the significance of symbiont presence on evolution of host immunity [32]. After only nine generations of host passaging, the authors found that presence of protective *Wolbachia* bacteria reduced the frequency of a host-encoded gene responsible for defence against a viral parasite. This experiment demonstrated that host immune function can be relaxed due to harbouring protective symbionts. Evolution of immunity due to SMIP may also be contingent upon costs associated with host-encoded defence [24]. For example, prolonged overactivity of the immune system due to symbiont exposure may cause a decline in host health, or if immune priming does not impact infection success. The level of costs associated with SMIP may depend on how strongly the response is mounted versus the amount of

Table 1. Examples of symbiont-mediated immune priming in animal hosts

Host	Symbiont	Parasite	Mechanism of immune response	Type of response (and duration if known)	Method of symbiont exposure	Effect on host health or fitness	Effect on parasite fitness	Ref
Honey bee	Gut microbiome	*Escherichia coli* bacteria	Expression of antimicrobial peptides (AMPs)	Response in gut and hemolypmh	Adults fed microbiome right after emergence; sampled after 5 days	Increased host survival	Reduced load	[114]
Honey bee	*Snodgrassella alvi* bacteria (gut symbiont)	*Serratia marcescens* bacteria	Upregulation of Toll pathway leading to increased AMP expression	Non-specific response (similar response to heat-killed *E. coli*)	Adults fed symbiont in drinking water; sampled after 5 days	Increased host survival	Reduced load	[115]
Bean bug	*Burkholderia* bacteria	*E. coli* bacteria *Staphylococcus aureus*	Increased antimicrobial activities and AMP expression	Expression in hemolymph	Second instar nymphs fed bacteria. Infected 3 days after moulting to adult	Increased host survival to both parasites	Reduced load for *E. coli* Fitness not measured for. *S. aureus*	[64]
Weevil	Gut microbiome	*E. coli* bacteria *Serratia marcescens* bacteria	Increased phenoloxidase activity, expression of pathogen recognition receptors, AMPs	Gut and systemic immune response	Conventionally-reared vs. germ-free hosts. Fourth instar larvae challenged with parasite	Increased host survival to *S. marcescens*	Reduced load for *E. coli* Fitness not measured for *S. marcescens*	[116]
Drosophila	*Wolbachia pipientis* bacteria	*Pseudomonas aeruginosa* bacteria	Increased expression of reactive oxygen species and AMPs	Protection from enteric infection, but not from systemic infection	Resident (intracellular) symbiont. Adults were fed parasite	Increased survival during enteric infection	Reduced load in male hosts but not females	[117]
C. elegans	*Pseudomonas mendocina* bacteria	*Pseudomonas aeruginosa* bacteria	Increased expression of p38 MAPK immune pathway	Attenuated parasite also induced response, but not another related *Pseudomonas*	Reared on symbiont throughout larval development, but effects can be seen after 4 h of symbiont exposure	Increased host survival	Reduced load	[35]
C. elegans	*Lactobacillus acidophilus* bacteria (probiotic)	*Enterococcus faecalis* *S. aureus*	Increased expression of p38 MAPK immune pathway and beta catenin signalling pathway	Little effects on other parasites	Young adults on probiotic for 1 day then exposed to parasite. Probiotic cannot colonize host	Increased host survival to both parasites	Reduced load for *E. faecalis* Fitness not measured for *S. aureus*	[73]
Rabbit	*Lactobacillus casei* bacteria (probiotic)	Shiga toxin-producing *E. coli*	Secretion of specific IgA antibodies against parasite	Response in GI tract (adaptive immunity)	Feeding of probiotic occurred twice a day daily until hosts were ten days old. Hosts infected at 3 days old	Reduced damage to intestine	Reduced load	[76]
Mouse	*Bacteroides fragilis* bacteria (human symbiont)	*Helicobacter hepaticus* bacteria	Induces production of anti-inflammatory protein	Requires only a single microbial molecule, polysaccharide A (PSA), to induce response	Symbiont co-colonized with parasite. Hosts remained colonized by both bacteria throughout course of disease	Reduced disease severity	Load unchanged	[118]
Mouse	Gut microbiome	*Streptococcus pneumoniae* *Staphylococcus aureus*	Enhances neutrophil functions through pattern recognition receptor NOD1	Peptidoglycan from microbiome induces response. Systemic response (innate immunity)	Conventionally-reared hosts vs. germ-free hosts	Host fitness not measured (between conventionally-reared and germ-free mice)	Reduce load for both parasites	[46]
Mouse	Gut microbiome	Mouse cytomegalovirus	Poises interferon (signalling proteins) expression, activating natural killer cells for antiviral response	Systemic response (innate immunity)	Conventionally-reared hosts vs. germ-free hosts	Host fitness not measured	Reduced load	[119]
Mouse	Segmented filamentous bacterium (SFB)	*Citrobacter rodentium* bacteria	Induces T helper 17 cells, upregulation of AMPs and inflammation-related genes	(adaptive immunity)	Germ-free hosts fed SFB	Host fitness not measured	Reduced load	[45]
Rat	*Lactobacillus casei* bacteria (probiotic)	*Listeria monocytogenes*	Cell-mediated immunity (e.g., macrophage)	(innate immunity)	Oral administration of viable probiotic daily 3 days before parasite infection	Host fitness not measured	Reduced load	[65]
Mouse	*Lactobacilli* bacteria	*Heligmosomoides polygyrus* helminth	Increased regulatory T cell and T helper 17 responses	Response in the gut	Conventionally-reared hosts fed bacteria in drinking water	Host fitness not measured	Increased load	[120]

Continued

Table 1. Continued

Host	Symbiont	Parasite	Mechanism of immune response	Type of response (and duration if known)	Method of symbiont exposure	Effect on host health or fitness	Effect on parasite fitness	Ref
Bumble bee	Gut microbiome community	*Crithidia bombi* trypanosome gut parasite	Differential expression of immune genes	Variation in response dependent on host genotype	Faecal transplant of resistant vs. susceptible hosts, administered 1–3 days after emergence; sampled 18 h post-transplant	Fitness measured before transplant	Fitness measured before transplant	[121]
Honey bee	*Bifidobacterium spp.* and *Lactobacillus spp.* bacteria (probiotics)	*Paenbacillus larvae larvae* bacteria	Upregulation of antibacterial peptide (abaecin) expression	Non-specific response, may be primarily in hemolymph. Sustained response during development	Added bacteria as needed in diet of larvae	Fitness not measured	Fitness not measured	[122]
Mosquito	Gut microbiome	Vectored *Plasmodium falciparum* protozoa	Hemocyte differentiation	Systemic activation. Persisted throughout lifespan of mosquito	Resident microbiome eliminated via antibiotics	Fitness not measured	Fitness not measured	[123]
Honey bee	*Frischella perrara* bacteria (found in bee gut microbiome worldwide)	No parasite examined	Upregulation of immune genes, AMPs, pattern recognition receptors	Response in region of gut	Adults fed 24 h after emergence (symbiont colonizes host right after emergence in nature); sampled after ten days	Fitness not measured	No parasite examined	[74]

Box 2. What else can prime host immunity?

Beneficial microbes

In addition to symbionts that have established associations with hosts, novel microbes introduced into hosts can prime host immunity. These microbes can have a positive impact on host fitness or be beneficial under specific conditions. For example, when *Wolbachia* native to *Drosophila* flies were introduced into nonnative mosquito hosts, mosquito immune responses were upregulated and reduced dengue viral load, but the introduction also came at a cost to host fitness [38, 39]. Furthermore, some native symbionts do not evoke an immune response [17, 39, 95–97] which may ensure successful colonization by the symbiont, but the lack of immune induction may increase host susceptibility to infection. It is also possible to engineer symbionts able to upregulate host immunity. For example, a native symbiont of honey bees engineered to express double-stranded RNA was able to trigger RNA interference in bees and reduce viral litres [98].

Similarly, probiotics are live microbes aimed at improving host health, usually administered orally. Some probiotics prime host immunity and are derived from the natural microbiome of the host itself [44, 99], while others are isolated from external sources such as diet or different host types [100, 101]. Finally, physical components of symbionts can also evoke an immune response, such that live microbes are not necessary. For example, bacterial components like peptidoglycan or even DNA from microbiome members are enough to upregulate immunity [46, 102].

Parasites

Prior exposure to parasites, including those with attenuated virulence or sub-lethal doses, can prime the host immune system for subsequent infection [33–35]. This priming is sometimes specific, where exposure to a particular parasite species only protects against lethal doses of the same parasite [34], though the evolution of specificity can depend on the parasite species [33]. In some cases, this protection is transgenerational, such that offspring of exposed mothers exhibit increased immune activities and/or survival from infection [21, 103, 104]. Similarly, vaccines, which are composed of specific parasite components, help the immune system recognize and remember a particular parasite so that it can prevent future infections.

Abiotic stressors

In addition to biotic stresses such as parasites, hosts also encounter abiotic stresses in their environment. Consequently, animals have evolved a dynamic suite of defenses that can interweave and overlap. Mechanisms involved in abiotic stress response can share genetic pathways with those involved in parasite response [105–107], a phenomenon well-studied in plants [18, 20]. Consequently, exposure to an abiotic stress can increase host survival to infection by parasites, and vice versa [19, 108–111]. This phenomenon is similar to hormesis, where exposure to low doses of stressors can have beneficial stimulatory effects, a concept which has been explored in biomedical and toxicological fields [112, 113].

protection hosts receive. Similar to symbionts that directly interact with hosts, the influence of SMIP on immune evolution may depend on the degree to which symbionts associate with hosts across generations.

Symbionts involved in SMIP with shared ancestry or molecular signatures to parasites can evoke similar immune responses (Box 2). As such, parallels might be drawn between SMIP and parasites with regards to evolution of immune specificity. A previous study on multiple parasite exposures indicates that red flour beetle hosts (*Tribolium castaneum*) can evolve priming specificity (greater immune response when exposed to the same bacterial parasite, *Bacillus thuringiensis*, during priming and challenge compared to exposure to different parasites) within a short number of generations [33]. Additionally, attenuated parasites have been found to induce immune responses while causing less harm to the host [33–35]. These findings would suggest that a symbiont with similar signatures to a parasite may lead to more specific responses by the host. Moreover, studies of *Spiroplasma* and *Wolbachia* bacteria have found that these symbionts cause a dampened or no immune response in their native *Drosophila* hosts [10, 36–38]. By contrast, *Wolbachia* induces a strong response in novel mosquito hosts, resulting in increased mosquito resistance to dengue viral infection [17, 39]. A possibility for upregulation of immune expression is that *Drosophila* hosts are treating the novel symbiont as a foreign stimulus, akin to parasite infection, which also inadvertently reduces viral litres [38]. These results also suggest that SMIP may be a more recently evolved mechanism of protection compared to direct symbiont-parasite interactions—longer evolutionary history with the host may result in adaptation in host and/or symbiont that dampens the immune response toward the symbiont.

SMIP, like parasite infection, can select for different host defence strategies, namely resistance and tolerance. Whilst the former reduces parasite burden within hosts, the latter response alleviates damages caused by infection without affecting parasite fitness [40, 41]. Because SMIP can affect various aspects of the immune system, tolerance to parasites may more likely evolve if pathways involved in tissue damage repair are activated in the process [42]. However, SMIP-conferred tolerance, and the degree to which this mechanism may underpin natural patterns of host tolerance given constant microbe colonization, remain understudied.

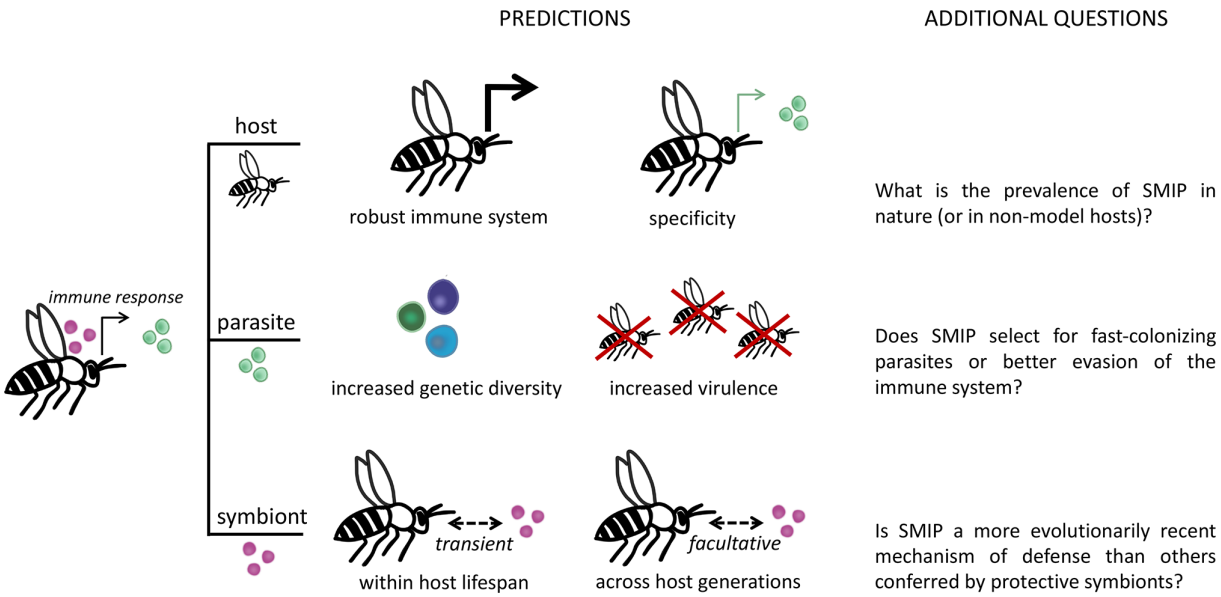

Fig. 1. How might SMIP affect symbiosis? *Hosts*: maintenance or evolution of a robust immune system may occur because a functioning immune system is required for SMIP to work, unlike other mechanisms of symbiont-conferred defence. Similarities between symbiont and parasite may also lead to evolution of more specific responses toward the parasite. *Parasites*: heterogeneity in host populations, whether through symbionts and/or immunity, may select for increased parasite diversity. Increased virulence may also occur due to parasites no longer paying the cost of being too virulent. *Symbionts*: if a short duration of exposure to the symbiont is sufficient to evoke an immune response, persistent interaction with the host may not be necessary, especially if the immune response is still mounted after the symbiont is gone. From an evolutionary perspective, symbionts involved in SMIP may be less likely to be host-dependent or inherited because many are acquired from the environment (Table 1) or are costly to harbour in the absence of threat. Arrow above host represents immune upregulation.

Research on SMIP has largely been focused on a few model or well-studied host systems (Table 1), making them ideal for testing hypotheses on the evolution of host immunity. For example, interactions between bees and their microbiomes make up a significant proportion of SMIP studies in invertebrates. Bees are an attractive model for studying host-microbe interactions due to their relatively complex microbiome compared to other insects, in addition to their agricultural importance [43]. Consequently, much research on the protective effects of symbionts and other host defence mechanisms has been focused on bees (Table 1) [21]. For vertebrates, research using mice has shed light on the effects of gut microbiome and probiotics on expression of the innate and adaptive immune systems [44–46]. The amenability of these hosts to experiments may have contributed to their better characterized immune responses compared to other organisms. Accordingly, these hosts can be used to address questions such as weighing the cost of harbouring defensive symbionts versus the maintenance of a robust immune system [25, 47]. However, model organisms reared in the laboratory are often absent of input from the environment, thus they may be missing members of the microbiome they would encounter in the wild [48, 49]. A better understanding of the prevalence of SMIP in nature might involve a thorough survey of animal hosts in the wild, particularly those with adaptive immunity and those harbouring complex microbiomes. Future research may also help determine whether certain types of microbes are more likely to participate in SMIP than others since previous studies have focused primarily on bacteria (Table 1). SMIP may nevertheless be difficult to detect in the wild. Brief exposure to the symbiont may be adequate to evoke an immune response, which would diminish the chance of documenting the symbiosis in real-time.

Parasite population structure and virulence evolution

Host resistance and tolerance have been predicted to drive parasite evolution [50, 51]. SMIP might similarly impose selection on parasites to respond to the protection conferred by symbionts through immunity. High host genetic variation is important to combat parasites that are constantly evolving [52]. Host populations can vary in the prevalence of protective symbionts—not all hosts in a population will harbour protective symbionts [53–55]. Similarly, both vertebrate and invertebrate hosts can also exhibit variation in defence at the genetic level [54, 56]. Such heterogeneity in host populations, whether through symbionts and/or immunity, may select for increased parasite diversity [57], which in turn may reciprocally maintain variation in host defence strategies. For example, diverse populations of the parasitoid, *Lysiphlebus fabarum*, have been shown to shape the diversity of protective *Hamiltonella defensa* bacteria of black bean aphids [58]. Since SMIP can modulate host immunity, this additional level of plasticity in host defence may facilitate parasite evolution further. Experimental evolution of microbes passaged through hosts have yielded foundational insight into how the host environment can shape patterns and processes of microbial evolution [59–61].

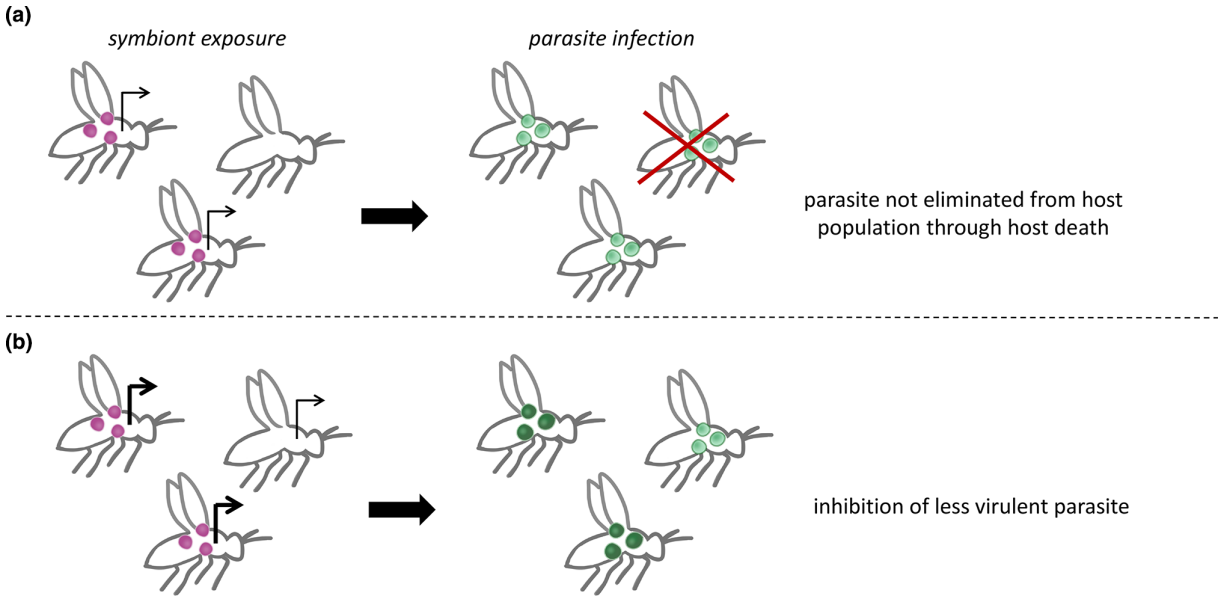

Fig. 2. SMIP may select for increased parasite virulence over time because A) hosts survive when infected by parasite due to SMIP, so virulent parasites are no longer removed from the population through host death; and B) SMIP may elicit a stronger immune response than in the absence of SMIP, which effectively eliminates less virulent parasites and allow more virulent ones to dominate. Symbionts are shown in magenta; parasites in light and dark green. Arrow above host represents immune upregulation.

This approach could be used to passage parasites through hosts with varying levels or sources of defence (e.g. immunocompetent and immunocompromised hosts, with or without symbionts), directly testing the role of host heterogeneity in shaping parasite diversity.

Symbionts involved in SMIP and host-encoded defenses may have different impacts on parasite evolutionary rates. Given their large populations and short-generation times, microbes are likely to evolve more rapidly than hosts and therefore respond to parasite evolution within a short timespan [9]. Faster-evolving symbionts may become better at modulating host immunity (either within the lifespan of the host or across host generations), which in turn may impose greater selection on parasites. Moreover, obtaining symbionts from the environment is also faster than acquisition of new genes (i.e. within one host generation versus multiple host generations). However, it may not be simple for hosts to acquire suitable symbionts from the environment and may require specific mechanisms for selecting and cultivating these symbionts [62, 63]. Finally, early exposure to symbionts may also launch immune responses in advance of parasite colonization, therefore promoting suppression of parasite and be more effective at reducing infection load [35, 64, 65]. Altogether, SMIP may more likely select for fast-colonizing parasites or those better able to evade the immune system.

Despite the protection that symbionts provide hosts at the individual and population level [e.g. 35, 62], SMIP have the potential to be harmful for future host generations. Theories on immune priming by parasites can lend insight into the impact of SMIP on the evolution of parasite virulence. Virulence is predicted to trade-off with transmission, such that high virulence resulting in early host death can prevent transmission [66]. However, when prior parasite exposure prevents host death, reinfecting parasites do not pay the cost of being highly virulent [67]. Additionally, the level of virulence can be positively correlated with the level of protection: a more virulent parasite can elicit stronger immune protection. Thus, hosts previously infected with a more virulent parasite can exhibit the largest reduction in mortality following a subsequent infection. A stronger immune response also prevents less virulent parasites from infecting hosts, thus selecting for higher virulent parasites [67]. Similar predictions have also been made for imperfect vaccines that do not prevent parasite transmission [68, 69]. Protection provided by SMIP may similarly select for increased virulence (Fig. 2). Hosts that survive infection due to SMIP may allow virulent parasites to persist in the population. Furthermore, the stronger immune response mounted by hosts harbouring symbionts would prevent less virulent parasites from successful infection. However, evolution of virulence may be contingent upon increased levels of specificity of the launched response or costs to the parasite [10].

Implications for persistence of the symbiosis

Research in symbiosis has frequently focused on the role of the symbiont on host fitness [70, 71], but the degree to which hosts impact symbiont fitness is not as well-studied [71, 72]. Many symbionts, such as those that are heritable, are solely host-associated

and cannot survive outside the host. Hosts that acquire symbionts from the environment spend some part of their lifespan without their symbionts. Additionally, such associations need not be throughout the entirety of the host's lifespan for the host to obtain benefits. SMIP can occur prior to parasite infection (exposure to the symbiont during infection may not be necessary), and the immune response can be mounted and maintained after only a brief exposure to the symbiont [35, 73, 74]. Therefore, immune-priming symbionts may be different from those that engage in direct competition with parasites, though not necessarily mutually exclusive. Stable establishment within hosts may not be necessary for the symbiosis to exist. Furthermore, since immune activation can be costly to the host [10, 24], SMIP is likely beneficial only when there is a high risk of parasite infection, suggesting that the symbiont prevalence may be low otherwise.

Host-symbiont evolution might be shaped by the duration of symbiont exposure necessary to mount an immune response. While SMIP studies have not directly tested the exposure period necessary to evoke an immune response, most detail how the symbiont was administered (Table 1). The same duration of time for one host species can represent a different proportion of its lifespan compared to another species, making comparisons across host taxa difficult. An immune response can be induced from relatively short symbiont exposures, which are often acquired from the environment (e.g. orally [75]). Thus, there may not be many inherited or obligate SMIP associations, though more studies are needed for SMIP in nature. Furthermore, some hosts may need a continual influx of symbionts to maintain immune upregulation [65, 76]. This requirement suggests that a certain threshold of symbiont density is needed for a response, where the rate of symbiont growth within hosts is not meeting host demands and increased exposure time is necessary for symbiont accumulation.

Because the immune system also functions to regulate symbiont populations [25], it is likely SMIP also targets the symbionts themselves, similar to parasite-induced priming that protects hosts from reinfection. For example, the gut microbiome of *Drosophila* induces a basal level of immune responses in the flies, which helps to prevent over-proliferation of the microbiome [77]. When host-microbe interactions are ephemeral, the definition of a symbiont can become ambiguous (Box 1). We suggest that some microbes involved in SMIP be seen as 'opportunistic symbionts'—transient but have a positive effect on host fitness—which can impact the long-term evolutionary trajectories of hosts despite shorter ecological interactions.

Symbionts involved in SMIP may be vulnerable to host exploitation, particularly hosts that only require short exposures to the symbiont. There is increasing evidence for hosts exploiting symbiont populations for their benefit [78–80]. For example, the cereal weevil 'recycles' their symbiont once they have obtained the necessary nutrients from their bacterial symbiont, *Sodalis pierantonius*, required for development [79]. While we are unaware of any studies that specifically examines exploitation in SMIP, the shorter duration of interaction with hosts may be a cause or consequence of potential asymmetry between partners. The impact of such asymmetry may be that selection for host-associated SMIP microbes is reduced, favouring a less host-dependent lifestyle.

Comparative genomics and phylogenetics of symbionts have yielded insight into evolutionary consequences for host-association. Reduction in genome size and altered evolutionary trajectories are driven by host association [81, 82]. Such approaches may be useful in determining the degree of host dependence and shared evolutionary history with hosts for immune-priming symbionts. For example, comparing the genomic aspects and lifestyle of defensive symbionts differing in protective mechanisms, in addition to the age of these symbioses, will help address whether novel symbionts are more likely to upregulate immunity. Pairing these approaches with empirical studies will shed light on the impact of the immune system and indirect impact of parasites on symbionts.

CONCLUSION

Microbial symbioses have shaped the evolution of animal hosts [83] and of the symbionts themselves [81, 84]. Protective symbionts in particular have had a profound influence on host resistance to infection at the scale of the host individual, populations, and communities [8, 10, 85]. Compared to symbionts that directly compete with parasites [14, 27], symbionts that prime host immunity may be more prevalent amongst new or ephemeral interactions. In these associations, the host has not yet evolved to accommodate the symbiont, or the symbiont has not evolved to evade host defenses. Consequently, the host immune system might recognize the symbiont as an invader and elicit a response accordingly. SMIP may therefore be a first step of a free-living microbe transitioning into a protective, more permanent symbiont. Nonetheless, long-term residents can also modulate immune responses and even be necessary for proper development of the immune system [25]. Immune-priming symbionts thus play important roles in host-microbe evolution across the parasite-mutualist continuum, acting both as an extension and modulator of host defenses.

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K.L.H. and K.C.K., conceptualized the topic. K.L.H., wrote the original draft. K.L.H. and K.C.K. revised, edited, and approved the final version.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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