Trends in Parasitology

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Review

Insects' essential role in understanding and broadening animal medication

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Like humans, animals use plants and other materials as medication against parasites. Recent decades have shown that the study of insects can greatly advance our understanding of medication behaviors. The ease of rearing insects under laboratory conditions has enabled controlled experiments to test critical hypotheses, while their spectrum of reproductive strategies and living arrangements – ranging from solitary to eusocial communities – has revealed that medication behaviors can evolve to maximize inclusive fitness through both direct and indirect fitness benefits. Studying insects has also demonstrated in some cases that medication can act through modulation of the host's innate immune system and microbiome. We highlight outstanding questions, focusing on costs and benefits in the context of inclusive host fitness.

Highlights

Insects have become exciting model organisms for studying animal medication due to their comparative ease of rearing and experimental manipulation, short generation times, and diverse levels of sociality.

Adopting an inclusive fitness framework has revealed the importance of medication for maximizing both direct and indirect fitness.

Historically, medication research focused on chemicals or behaviors directly toxic to parasites. Recent studies have investigated how animal medication modulates the host immune system, revealing limited evidence of a strong role.

Insect studies have led the way in showing that nutrients and toxins can alter animal microbiomes and thereby modulate disease resistance.

A better understanding of the ecological interactions between insects and their natural enemies and environment will help to establish the relative costs and benefits of animal medication.

Broadening the scope of animal medication

Observations of chimpanzees provided some of the first scientific studies demonstrating that animals can medicate themselves (Box 1). They also painted a picture of what animal medicine looks like. That picture consists of a sick, large-brained close relative of humans seeking out a plant that it usually does not eat and then ingesting typically aversive material to kill or remove an intestinal **parasite** (see Glossary), thereby relieving disease symptoms. Studies over the past few decades have shown that this original picture of animal medication needs to be expanded significantly, and many of these studies have involved insects. The goal of this review is to discuss how these insect studies have provided evidence that animals may use medication to protect genetic kin in addition to themselves; that medication can involve immune stimulation and microbiome modulation in addition to direct elimination or killing of parasites; and that fitness **costs** of medication can come in many different forms, from a shortened lifespan to lost feeding opportunities and reduced growth.

From apes to insects

While many advances in animal medication have come from studying chimpanzees and other primates and vertebrates [1–3], insects have come to the forefront as highly exciting and suitable model systems for studying these phenomena [4]. Though chimpanzees often ingest toxic plants to cure themselves, it is now clear that animal medication does not have to be 'self'-medication, but can focus on protecting genetic kin: infected monarch butterflies, for example, lay their eggs on toxic milkweed that reduces parasite infection in their offspring [5] (Figure 1). Chimpanzees are known to use medication in response to being sick (Box 1), a behavior we refer to as **therapeutic** medication. But animals may instead use prophylaxis, by which they prevent future disease instead of curing existing illness: wood ants do this by incorporating antimicrobial resin into their mounds [6]. Instead of ingesting toxins, animals may use macromolecules as medicine: African armyworm caterpillars that suffer from bacterial or viral infections shift their diet choice to consume more protein relative to carbohydrates to make their blood refractory to pathogen growth [7]. Instead of eliminating parasites, animals may relieve disease symptoms without reducing

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Box 1. Animal medication behavior: a brief history

Humans have known for thousands of years that animals use medicine, and have observed and copied animal behaviors to develop their own medicines [79]. Traditional healers in Tanzania developed herbal medicines for bloody diarrhea by observing porcupines and treatment for stomach upset by observing elephants [80]. Native Americans credit bears for much of their medicinal knowledge; the Navajo observed Kodiak bears dig up roots of the plant Oshá (*Ligusticum porteri*) before chewing it and then spitting a mix of root and saliva into their paws before thoroughly rubbing their fur (N. Myhal, MA thesis, University of Kansas, 2017) [81]. These days, one can order Oshá root extract online and use it to treat viral and bacterial infections (N. Myhal, MA thesis, University of Kansas, 2017). Even the discovery of the wonder drug Aspirin was most likely inspired by animal behavior. As bears emerge from their hibernation in spring, they ingest the bark of willow trees to treat their stiff and inflamed limbs. While chemists would ultimately optimize the chemical formula of salicylic acid into a drug with few side effects [82], the original discovery of the compound's pharmacological utility lies with non-human animals.

Because traditional healers have long known about animal medicine, it is fitting that the science of animal medication took off when a scientist teamed up with a traditional healer to study apes. In 1987, Kyoto University primatologist Michael A. Huffman was tracking chimpanzees in Mahale Mountains National Park in Tanzania with Mohamedi Seifu Kalunde, a senior game officer of Tanzania National Parks and traditional healer in the WaTongwe community. As they were studying a small group of chimpanzees, they noticed that a female by the name of Chausiku displayed disease symptoms, including lethargy, lack of appetite, and production of dark urine [83]. Chausiku stopped at a *Vernonia amygdalina* shrub and removed a branch before peeling off the bark, chewing and sucking the pith. Twenty hours later, she had recovered. *V. amygdalina* is routinely used by WaTongwe healers as medicine, and extracts of the plant are toxic to parasitic worms. Moreover, follow-up studies showed that chimpanzees use the plant more during the wet season when intestinal worm infections are common [84], and that ingestion of the plant's bitter pith is associated with reductions in worm burdens [85].

parasite fitness, a phenomenon called **tolerance** [8]. Monarch butterflies, for example, use toxic milkweed not only to reduce infection with protozoan parasites, but also to maintain fitness despite high parasite infection loads [9].

Insect studies are therefore expanding our understanding of what animal medication is. Not only are these animals amenable to highly controlled field and laboratory experimentation, but their varied life histories also allow for dissection of how medication affects self versus kin. We will specifically discuss animal medication in an **inclusive fitness** context; ask how insects use toxins, food, and microbes as medicine; and argue that medication can come in the form of parasite resistance, tolerance, or compensating for costs of immunity (Figure 1). Finally, we discuss current evidence and prospects for future investigation of how medication behavior relates to the host microbiome and immune function.

Animal medication from an inclusive fitness perspective

By definition, being infected with parasites bears costs for the host; however, the nature and scope of those costs are variable [10]. To be considered costly in evolutionary terms, parasitism must impact an organism's inclusive fitness, either via their **direct fitness** (i.e., their ability to directly propagate their genes) or their **indirect fitness** (i.e., their ability to help their kin to propagate their genes) [11,12] (Table 1). In some cases, parasitism can have sublethal effects, such as short-term lethargy; for example, female *Drosophila melanogaster* sleep more when infected with *Drosophila*-C virus [13], which may incur only minor fitness costs. In other cases, parasitism can have severe consequences, as shown by mortality from Nuclear Polyhedrosis Virus (NPV) in larval Lepidoptera [14], honey bee mortality due to American Foulbrood disease [15], or complete castration of freshwater snails by trematodes [16], all of which would have major fitness consequences. The importance of direct versus indirect fitness costs will depend on the animal's level of sociality [17]. Given their reduced levels of interaction with relatives, entirely solitary animals likely have less control over their indirect fitness, although evidence for transgenerational provision of antiparasitic compounds (e.g., [5]) can improve offspring survival even when parents are absent.

By contrast, in eusocial animals, such as ants and eusocial bees, most individuals are nonreproductive and gain all of their fitness indirectly. Their behaviors reflect this, exemplified by kin

avoidance (avoiding direct contact of diseased relatives by healthy individuals to reduce disease transmission) [18], self-sacrifice when parasitized [11], or medicating infected relatives, by recognizing diseased individuals and selectively providing a medicinal substance (**allo-medication**) [19]. Understanding the fitness effects of putative self-, kin-, or allo-medication behaviors, therefore, requires understanding the costs of parasitism to an animal's inclusive fitness, balanced against the costs and benefits of the medication behavior. A better quantification of these costs and benefits would allow us to predict when medication behaviors will evolve. So, how do we determine the benefits and costs of medication behavior?

Benefits of medication

To confer a selective advantage, benefits must ultimately impact inclusive fitness. For **self-medication**, direct fitness benefits could occur via the direct protection of the individual's reproduction or via survival (allowing more time for reproduction or parental care). By contrast, **kin-medication** (including allo-medication and **social-medication**) specifically benefits indirect fitness by protecting the reproduction of relatives (Figure 1). This could take the form of, for example, increased ability to assist members of a colony or other group, or reduction in the risk of transmission of parasites to kin.

Direct fitness benefits should, in principle, be relatively straightforward to measure. **Prophylactic** medication can pre-emptively hinder parasite establishment (e.g., wood ants incorporating resin beads into their colonies [6]), whilst therapeutic medication can treat existing infection directly, reducing parasite load by killing parasites or by inhibiting their growth [7,9], or it can increase tolerance by reducing the negative effects of a given parasite load [20]. Measuring the benefits of medication can, and has, been done in laboratory settings with insects [21,22]. As mentioned earlier, infected armyworm caterpillars (*Spodoptera* spp.) select a high-protein diet, which increases survival relative to those maintained on the 'preferred' diet for healthy caterpillars [7,23]. Indirect fitness benefits, either from self-medication or via kin-medication, can be more challenging to measure as they ultimately require an accounting of the extra offspring produced by relatives attributable to the medication behavior [11,12]. Ideally, indirect fitness benefits should be measured via colony-level survival or reproductive output in eusocial animals, but as far as we know, this has not been done in existing studies [24–26] (Figure 1). In the absence of these data, a proxy for fitness, such as the survival of workers, is often measured.

In contrast to self-medication, kin-medication is likely to deliver predominantly indirect benefits. As mentioned earlier, monarchs have been shown to protect their offspring by selecting high cardenolide milkweeds for egg laying when the adults are infected with the neogregarine *Ophryocystis elektroscirrha* [5]. As adults transmit this parasite to the offspring, adult infection is a good predictor of offspring parasitism, and infected larvae reared on the high-cardenolide plant survived longer as adults than those on the low-cardenolide plant [5]. In eusocial species, the medication of kin is sometimes referred to as 'social immunity' [27,28]. This can involve the transfer of immune components or toxins directly (e.g., metapleural gland or venom secretions in ants [29,30]). Self-produced or collected components are also readily used to protect kin via fumigation of the external environment of the nest (e.g., fecal pellets in termites, resin in ants). Importantly, diet can improve the potency of these secretions, thereby falling under the definition of medication. For example, a tropical ant, *Ectatomma ruidum,* employs kin-medication to directly treat other workers when infected with a fungus [29]. A high-carbohydrate diet improves the antifungal potency of metapleural gland secretions that these ants use to groom nestmates (allo-grooming), improving individual survival, and survival of workers at the colony level, but again, fitness benefits via colony reproduction were not measured [29].

Glossary

Allo-medication: medication behavior, targeted at relatives and nonrelated group members, in which the actor may recognize diseased individuals and selectively feed or apply externally a medicinal substance (e.g., adult to larvae or adult to adult).
Compensatory feeding: shifting nutrient intake to reduce the risk of nutritional loss due to infection, known for self- and kin-medication.
Costs: sublethal or lethal mechanisms or effects reducing the efficiency and fitness of an individual (e.g., physiology, behavior, reproduction). Costs are usually linked to a reduction in energy that, for instance, cannot be invested in reproduction, resulting in reductions of lifetime fitness.
Direct fitness: fitness obtained via an individual's direct reproductive success.
Host resistance: reduction in parasite infection or replication (reducing parasite fitness), which may or may not change the fitness of hosts.
Host tolerance: reduction in the effects of (a given level of) parasitic infection on hosts.
Inclusive fitness: overall fitness obtained through direct and indirect fitness.
Indirect fitness: fitness obtained through increasing the reproductive success of relatives, in addition to the reproductive success without the relative's help.
Kin-medication: medication behavior that benefits offspring or other genetic relatives; special case: trans-generational medication from parental generation to their offspring.
Parasite: here, we use the ecological definition, as 'an organism that lives in or on another organism, causing it harm'. This covers macro-parasites, multicellular organisms such as nematode worms, parasitoid larvae, and micro-parasites, such as viruses, bacteria, fungi, and protozoans.
Prophylactic: occurring prior to infection.
Residual reproductive value (RRV): an organism's expected future reproductive contribution to the population. Low RRV may result in the prioritization of current breeding over future survival.
Self-medication: a form of behavior (consumption or external application) that reduces the negative effects of disease on hosts (for details, see Box 2

Costs of medication

Costs are ultimately measured as reductions in fitness but could be proximately driven by several intrinsic and extrinsic factors (Table 2). Direct fitness costs of medication may be readily assessed if consumption in the absence of a parasite decreases survival [e.g., *Grammia incorrupta* larvae consuming pyrrolizidine alkaloids (PAs) [22]] or reduces reproductive output directly (e.g., delayed egg laying in bumble bees after consuming nectar alkaloids [31]). Despite these clear examples, costs can be hard to quantify, especially in the laboratory. For example, even direct toxicity of medicine may not be apparent in a laboratory setting where temperature, humidity, and nutrient levels are maintained at optimal levels. Additionally, medication could involve time or opportunity costs. For example, prophylactic or therapeutic medication may involve additional time foraging for those medicinal components in the environment, which may be scarce, widely dispersed, or difficult to access. Honey bees fed on low-diversity pollen were more susceptible to *Vairimorpha* (formerly *Nosema*), but agricultural habitats reduce the availability of diverse pollen in the environment [32]. Extra time spent foraging will trade off with the time the insect could allocate to other fitness-enhancing activities. Sublethal effects, such as increased lethargy [13], whilst appearing minor in the laboratory, might reduce the ability to avoid predation, collect resources, or to search for mates, which would incur direct fitness costs. Medicines might reduce the ability to compete for mates, or they might reduce attractiveness by affecting sexual signals, for example, altering pheromone production or cuticular hydrocarbon profiles. For example, larval host plants can alter pheromones in *Heliconius* butterflies [33], and the balance between protein and non-protein energy in the diet can change pheromone production in cockroaches [34].

As with benefits, indirect fitness costs are harder to measure than direct costs. Again, one would need to study colony-level survival and reproduction in the presence and absence of kin-medication behaviors. To our knowledge, no such studies have been completed.

In summary, we would expect medication behavior to evolve when the inclusive fitness benefits of medication use outweigh the inclusive costs, provided those medication behaviors are heritable. Quantifying those costs and benefits in diverse systems remains an outstanding challenge.

Relationship between medication behavior and the host immune system

While it is certain that chemistry plays an outsized role in medication, the exact mechanisms by which primary and secondary metabolites lead to the positive outcomes of medication are unclear [4]. Some benefits of chemical ingestion may be mediated by the immune response, which is sensitive to phytochemicals [35]. For primary metabolites, protein is an essential resource for immunity [14,36,37]. However, excess protein can also limit growth and reproduction in the absence of parasites, making the assessment of parasite context-dependent benefits more challenging. The results are not as straightforward with secondary metabolites, some of which cause a decrease in immunocompetence [35,38], whereas others cause an increase [39,40].

Few studies have specifically investigated the role of secondary metabolites or nutrients in cellular or humoral immune system-mediated resistance to parasites, but those that have offer mixed evidence for the immune system's function. The tiger moth caterpillar (*Grammia incorrupta*) engages in medication behavior with PAs acquired from its host plants [22]. However, caterpillars showed little variation in their immune response when consuming high versus low PAs, demonstrating that the beneficial effects of PAs for surviving a parasite infection were not due to immune system enhancement [41]. Similarly, Adams *et al*. [42] found that the immune response of monarch (*Danaus plexippus*) caterpillars did not vary when reared on five different milkweed species that differed in cardenolide concentrations, suggesting that medication is not mediated by the action of cardenolides on the immune response. Conversely, there are indications that

in the main text). Here, we focus on the effects of environmental compounds in the context of infectious diseases (i.e., parasites), acknowledging that nonchemical means of medication (e.g., fever) and noninfectious diseases exist as well.

Social-medication (kin-medication): medication behavior that benefits individuals at group or colony level.

Therapeutic: occurring following infection.

Xenobiotic: a chemical compound not naturally present in the organism's metabolism.

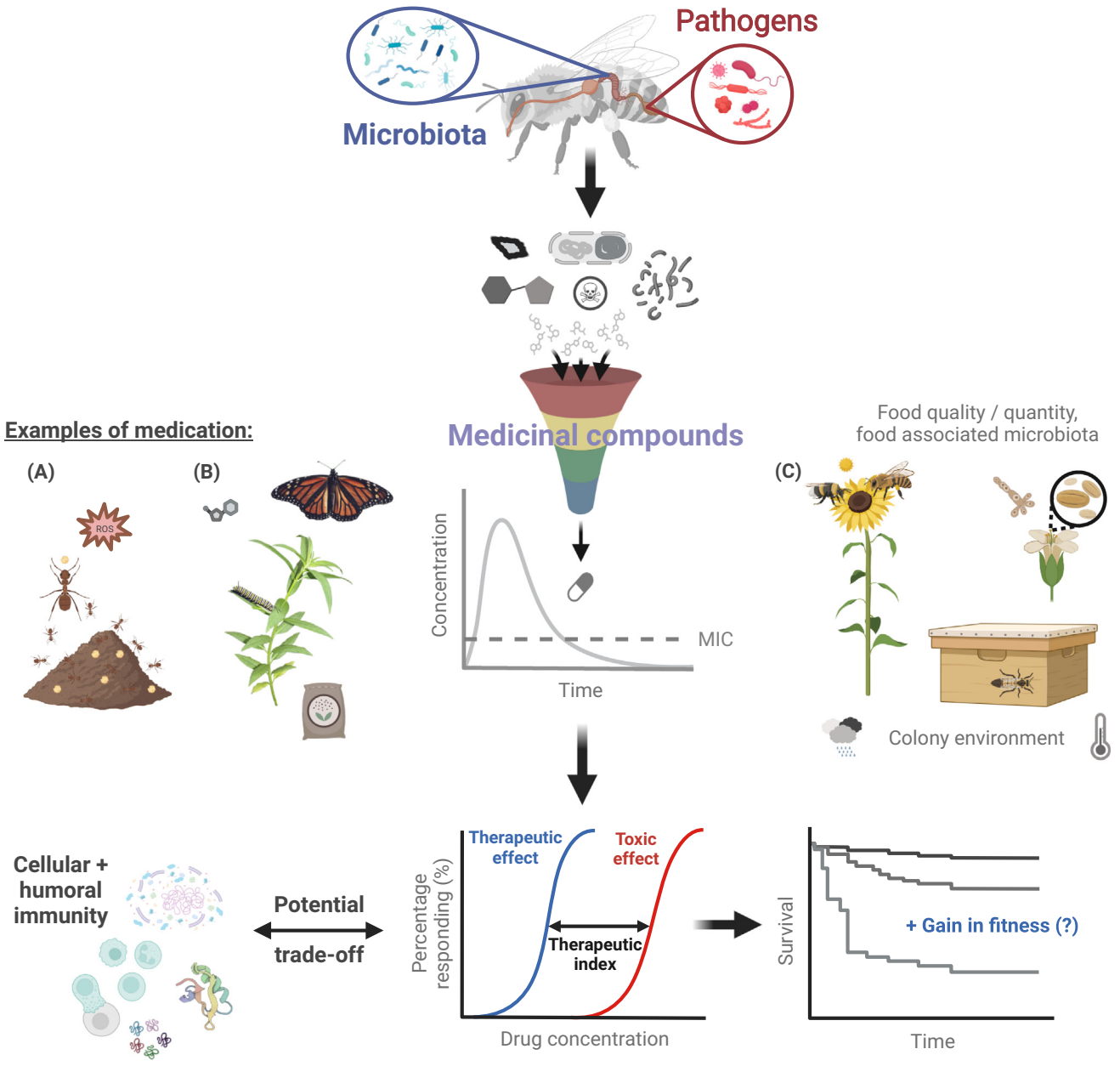

Figure 1. Overview on the potential ways of animal medication. Insects, harboring their symbiotic microbiota, can additionally apply or consume medicinal compounds to fight against parasite or pathogen infections: (A) ants collecting antimicrobial resin buds or consuming reactive oxygen species, (B) monarch butterfly larvae feeding on milkweed host plants with secondary metabolites, or (C) bees collecting health enhancing nectar and pollen. For social insects, the colony environment as well as the food source microbial community are additional factors having impact on host–parasite relationships (C). Food quality and quantity, including associated microbiota, are major drivers for animal medication. The medicinal compounds will have a therapeutic effect on the host organism in cases of therapeutic medication, likely resulting in fitness gains. The host–parasite–medicine interaction mechanisms are in potential trade-off with the insect's cellular and innate immune system regulation, potentially affecting fitness. Figure created using BioRender. Abbreviation: MIC, minimum inhibitory concentration.

phytochemicals such as nicotine, anabasine, and thymol can activate the expression of antimicrobial peptides (AMPs) in honey bees [43]. Also, in honey bees, dietary effects on the immune system depend on the quantity and quality of pollen consumed [44]. Alaux *et al*. (2010) showed

Table 1. Examples of parasite-driven inclusive fitness effects, under the assumption that parasites can reduce host survival or reproduction, either directly or indirectly (i.e., via impacts on kin)

		Impacts on fitness	
		Direct	Indirect
Type of effect on host	Survival	Mortality caused by parasitism – e.g., *Bacillus thuringiensis* infection in larval Lepidoptera [86]	Vertically transmitted parasite reduces fitness of offspring – e.g., *Ophryocystis elektroscirrha*, protozoan infection in *Danaus plexippus*, monarch butterflies [5]
	Reproduction	Reproductive castration – e.g., bumble bee, *Bombus terrestris*, queens infected with *Sphaerularia bombi* become sterile [87]	Social distancing in response to infection reduces ability to provide care for offspring/siblings, e.g., social isolation after infection with *Metarhizium brunneum* in *Lasius niger* ants [18]

that diets including protein increased glucose oxidase (GOX) levels, an enzyme related to social immunity, helping to disinfect brood and food. Dietary quality (same protein amount but diverse pollen types) additionally increased GOX levels [44]. Thus, it cannot be ruled out that phytochemicals in pollen interact with GOX and other immune system effectors to shape **host resistance** to infection.

Even in the more parsimonious scenario in which phytochemicals exert antimicrobial effects independently of the host immune system, ingesting such compounds could spare or complement the host's defenses, activate trade-offs inherent to the host immune system, or have long-term effects on its evolution (Figure 1). For example, the phytochemical callunene from *Calluna vulgaris* induces flagellum loss of the trypanosomatid *Crithidia bombi*, which is essential for infectivity [45]. In the absence of phytochemicals, bumble bee hosts express AMPs upon *C. bombi* infection [46]. Thus, parasite-targeting phytochemicals could result in relaxed selection acting upon immune genes. In the honey bee, collection of plant resin, which contains various phytochemicals, was effective against several bacterial and fungal pathogens [47,48]. These phytochemicals resulted in lowered gene expression levels for the AMP and the immune genes [49], establishing a complementary function that could similarly reduce the importance of the host's innate immune effectors. In the same vein, Tan *et al.* [50] found a small number of immune genes to be downregulated in monarch caterpillars reared on antiparasitic milkweeds with high cardenolide concentrations. This suggests that the antiparasitic effects of medicinal milkweeds were not achieved through strengthened immune system function. Thus, there is evidence for this alternative scenario where immunity is overshadowed by the direct effects of phytochemistry [51].

Table 2. Factors that could affect the fitness benefits or costs of medication include host- or parasite-specific factors, factors intrinsic to the medicine itself, and abiotic and biotic elements of the environment

Host	Parasite	Medicine	Abiotic	Biotic
Condition[a]	Prevalence	Availability	Temperature	Host organism
Life stage	Dose	Composition	pH	Symbionts
Age	Virulence	Efficacy	UV	(Kin-)Behavior
RRV[b]	Reproductive cycle	Toxicity	Humidity	Competitors
Sex	Transmissibility	Ease of utilization	Pesticides	Predators
Sociality		Contaminants	Habitat	

[a]For example, fat/protein stores, physical state (e.g., physical damage caused by wounding, previous infections, reproduction).
[b]**RRV, residual reproductive value**.

Phytochemistry's sparing of the immune system may have further implications for immune system evolution. Immune systems of insects and other invertebrates are characterized by a trade-off between antimicrobial activity and melanization responses [52–54]. Direct antimicrobial action of phytochemicals could defend hosts against parasites without activating these trade-offs or the costs of immune system activation. As a result, medication has the potential to interfere with the evolutionary forces acting on immunity, further promoting and even increasing those existing trade-offs, leading to relaxed selection on parts of the immune system, which has been detected in bees preceding the evolution of sociality [55]. Furthermore, several life-history traits show trade-offs amongst each other, but also with immune responses [56]. Animal medication might interfere with those trade-offs, as indicated by the increased time to egg-laying in queenless bumble bee micro-colonies supplied with dietary anabasine, a nectar alkaloid, which also reduced the intensity of gut infection [31].

Medication and the microbiome

Recent decades have seen a surge of studies on host microbiomes, resulting in a growing interest in the role of microbes in animal immunity to parasites and its relation to medication behavior. Microbiome composition strongly drives insect resistance to infection, particularly with gut pathogens, in some host–parasite systems (Figure 1) [57–59]. While the mechanisms for this in insects are often poorly understood, the resident microbiome can modulate the systemic immune response [60,61] (Table 2) and provide physical and chemical barriers to infection. For example, gut microbiota can produce antimicrobial substances, such as organic acids, due to their primary or secondary metabolism [62]. Beyond the gut, cultivation of symbionts on the skin or in specialized glands can likewise supply hosts and their offspring with antimicrobials [63]. Hence, acquisition and maintenance of a functional microbiome could be considered a form of prophylactic medication that incurs energetic and possibly other costs, including the risk of establishment by microbes that do not benefit the host.

Host-associated microbes can alter the consequences of ingested compounds for infection, but little attention has been given to the role of the tripartite host–microbiome–**xenobiotic** interaction for insect medication. In human medicine, the new field of pharmacomicrobiomics [64,65] recognizes the importance of the individual microbiome in modulating the effect of ingested xenobiotics on host health and may serve as inspiration for further studies in insect medication. Importantly, differences in the microbiota composition among individuals can be responsible for large differences in the medicinal effects of plant compounds [64]. As shown in humans, the gut microbiota metabolizes ingested compounds and thereby directly alters their effect on host health in three major ways: by changing (i) toxicity to the host, (ii) antiparasitic efficacy, and (iii) bioavailability [64–67], each of which is briefly discussed later.

Microbiome-mediated metabolism of ingested compounds could alter the amount of chemicals that can be tolerated by their hosts. Microbial detoxification of potentially harmful plant metabolites is protective against toxins in several insect species [68], including coffee berry borer (*Hypothenemus hampei*) [69], pine beetle (*Hylobius abietis*) [70], and bees (*Apis* spp. and *Bombus* spp.) [71]. However, microbiome-derived changes to ingested plant metabolites could also increase toxicity to the host [72], thereby potentially offsetting the parasite-reducing benefits of consuming these compounds.

Costs and benefits of microbial metabolism of potential medicinal substances for infection can be predicted based on corresponding changes to such substances' antiparasitic properties. Koch *et al.* [73] recently showed that the bumble bee host and the associated gut microbiome can increase or decrease nectar metabolites' antiparasitic activity through changes in glycosylation status. The sesquiterpenoid unedone from strawberry tree (*Arbutus unedo*) nectar loses antiparasitic efficacy against the gut parasite *C. bombi* through host-derived glycosylation in the midgut.

However, efficacy is restored in the presence of the microbiome in the hindgut (the site of *C. bombi* infection) by deglycosylation. Hosts likely face microbiome-mediated trade-offs between prevention of plant secondary metabolite-induced toxicity and retention of the full antiparasitic potential of their diet [73], the relative importance of which could be dictated by parasite pressure and host nutritional niche.

We are unaware of insect studies that demonstrate microbiome-derived changes in the bioavailability of medicinal substances, which could alter the concentrations of ingested compounds to which parasites are exposed. These changes would be significant for parasites that infect the host beyond the environment of the gut lumen, invading the hemocoel, organs, or cells. For medicinal substances to interact directly with these parasites, they must cross barriers such as the gut wall and/or cell membranes [74]. Metabolic activity by the gut microbiome, such as deglycosylation of plant secondary metabolites, as was recently demonstrated by bacteria from honey bees [75], may facilitate this and thereby increase the bioavailability of plant medicinal compounds [66], but experimental verification of this in insects is missing.

Beyond the microbiome's direct effects on the formation and catabolism of infection-modulating compounds, indirect effects that reflect the interaction of the host, microbiome, and medicinal substances could also affect host health via cascading effects on immune activation and host nutrition. For example, endogenous microbial communities can be altered by ingesting plants and associated compounds, favoring taxa associated with immunity [76]. Future research should also consider the microbiome-related costs of medication behavior. For example, ingestion of antimicrobial substances or upregulation of immune pathways [43] could deplete populations of beneficial symbionts as well as pathogenic ones, leaving hosts more susceptible to opportunistic infections (as exemplified by post-antibiotic susceptibility to infection [57]) or vulnerable to nutritional deficiencies or dietary toxins against which symbionts are normally protective [68,69,71]. Alternatively, the microbiome itself could become a liability under conditions of infection if, for example, symbionts attenuate the host immune response, accelerate the catabolism of antiparasitic dietary chemicals, or consume immunity-limiting host resources. The experimental manipulability of the microbiome in diverse insect species enables tests of these hypotheses.

Finally, given the potential for microbiome- as well as parasite-driven changes in behavior [77,78], host-microbiome feedback in the expression of medication behaviors offers another intriguing avenue for future research that explores microbiome-mediated changes in a behavioral context. The cohabitation of, and possible competition between, symbionts and pathogens in the gut, as well as the stake of obligate symbionts in host survival, suggests a selective advantage for beneficial microbes that can elicit antiparasitic behaviors, whereas parasites themselves are likely under selection to suppress them.

Concluding remarks

Insects have moved to the forefront of the study of animal medication. Not only can these animals be maintained in large numbers in laboratory settings, where they can be used for controlled experiments, they also have well-described relationships with parasites, pathogens, microbes, and toxins. Moreover, insect species vary greatly in their levels of sociality, and this variation has allowed scientists to demonstrate that animal medication can involve self or kin. Notably, many studies on insects have conclusively shown that medication can involve nutrients, in addition to toxic secondary chemicals: indeed, many insects alter their diet in response to infection, which may stimulate immunity or change nutrients or other physiological properties that are detrimental to parasites.

Despite the advances in our understanding of animal medication, many questions remain (see Outstanding questions), and we hope to have clarified that insects can provide suitable model

Outstanding questions

What are the best ways to measure or approximate the fitness costs of kin-medication and prophylaxis?

How common is prophylaxis, and how can we benefit from natural variations in parasite pressure to study this form of animal medication?

What is the role of the microbiome in medication concerning beneficial microbes, immune modulation, or physical or chemical interference with pathogens? Do microbiome-mediated effects improve the fitness of the responsible microbes?

What is the genetic basis of animal medication, and how can insects help us establish the genetic architecture of medication?

systems to address these questions (Box 2). As alluded to throughout this review, one outstanding question centers on the relative costs and benefits of medication behaviors. While benefits are often relatively easy to demonstrate at the individual level, the measurement of benefits for kin groups or whole colonies lags behind. Costs have been even harder to quantify, particularly at the colony level, and future studies are needed to determine the ecological costs of medication comprehensively (Box 2). Whether the collection of resin by ants or bees results in fewer opportunities for food collection and whether the greater amount of search time required to locate and consume medicinal substances increases predation risk are two examples of questions that are ripe for study. Ultimately, understanding the relative benefits and costs of medication will also help us better understand under what conditions medication behaviors evolve, which is another outstanding question.

Just as insects have become crucial for studying medication, they have become increasingly essential models for studying microbiome assembly and function. Many species have relatively

Box 2. Guidance for studying animal medication

Studying medication behavior requires the measurement of several criteria [4]. Criteria were first provided by Clayton and Wolfe [81] and then further developed by Singer *et al.* [22] and de Roode and Hunter [4], who suggest that the following five conditions need to be met to conclude that a behavior is a therapeutic medication: (i) the behavior involves the ingestion or external application of a third species or chemical compound; (ii) the behavior must be initiated by parasite infection; (iii) the behavior increases the fitness of the infected individual or its genetic kin; (iv) the behavior is costly to uninfected individuals; and (v) the behavior is relevant in the natural environment of the host. Note that criterion (ii) must be relaxed for prophylactic medication, which protects uninfected animals against future parasite infection. A further behavior is known that may benefit the host via **compensatory feeding** (or compensatory diet choice); for details, see [4].

In addition to addressing these criteria, it is important to consider all factors that may affect host–parasite interaction when designing studies of disease-associated behavior.

Factors to be considered

(i) Medicinal compound of interest: an active compound consumed or used for external application to benefit diseased host individuals or groups or prevent future infections. An alternative might be material from the environment (e.g., rough vegetation) that will be consumed and remove the disease-causing agent from the organism's body due to the morphology of the food item.
(ii) Compound origin: can be either phytochemicals (e.g., nectar, pollen, resin, plant excretions, leaves or other plant material), metabolites or toxins of animal origin (e.g., arthropods), microbial toxins or metabolites (e.g., bacteria, fungi, microbiota of the digestive system), nutritional chemicals (e.g., proteins, lipids, or carbohydrates) or inorganic compounds (including minerals, salts) such as clay.
(iii) Major points in study design: the impact of the natural environment and potential landscape effects (e.g., pH, temperature, humidity, minerals, etc.) of the host–parasite system, the organism life history (e.g., solitary versus social organisms), the nutritional state (e.g., food quality, food quantity, food preferences), and observation time (e.g., short- versus long-term effects).

Criteria to be measured, to characterize real cases of medication and potential problems

(i) Costs for the healthy organism: there might be a trade-off between field versus laboratory assays. Costs may vary across time – studies should consider realistic observation time windows.
(ii) What are realistic costs for the host? Decreased fitness measured via survival or reproduction of host or kin, may be hard to measure – especially in eusocial colonies – and may have to rely on proxies over the shorter term (e.g., growth rates, age at reproduction, ability to secure territories/mates).
(iii) If costs are difficult to determine, it might be more realistic to characterize benefits for the host. For example, if the compound is not toxic or detrimental to the parasite or pathogen, hosts consuming the compound may show increased tolerance towards infections.
(iv) What are the costs for prophylactic versus therapeutic medication? For in-field assays, estimating the costs for prophylactic medication might be more difficult.
(v) How to separate the effects of prophylactic and therapeutic medication if both act in parallel but at different individual stages? Is it mandatory to separate beneficial effects on the host organism into categories (prophylactic versus therapeutic), or might it be enough to describe the medication process with overall benefit?

simple microbiomes, and it is now clear that these microbes can play important roles in shaping medication. However, our understanding of the relevant mechanisms remains in its infancy despite these insights. The extent to which microbes interfere directly with pathogens through physical or chemical means, metabolize phytochemicals, or modulate immune responses remains to be determined. Moreover, little attention has been paid to the potential for insects to seek out specific microbes as a form of medicine per se, let alone to the fitness consequences of host behavior for the microbes involved (Box 2).

As we described, many authors have distinguished between prophylactic and therapeutic (self-) medication. Whereas therapeutic medication is relatively straightforward to study (infected individuals behave differently from uninfected counterparts), it is more challenging to demonstrate prophylaxis, which infected and uninfected animals can display (Box 2). It is often hypothesized that parasite risk drives the evolution of prophylaxis, with greater risk selecting for this behavior. Yet, few studies have tested this idea. Because insects often occur in many different populations that vary in parasite risk, it should be feasible to collect individuals from different populations and correlate their behaviors with levels of parasite risk. As such, we think that insects may ultimately provide us with a better understanding of this outstanding question, and help us elucidate the conditions that drive the evolution of these two crucial behaviors that protect animals from disease.

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Author contributions

S.E. and H.M.G.L. brought together the author team and proposed the initial idea. All authors together developed the focus and content of the manuscript, and all authors contributed equally to the text and edited the final text.

Declaration of interests

The authors declare no competing interests.

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