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Review Article

Heterogeneity in infection outcome: lessons from a bumblebee-trypanosome system

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SUMMARY

Interactions between insect hosts and their parasites are significant because their parasites can also be parasites of humans and of species that we utilize. Host–parasite interactions are complex, even in insects, and there can be heterogeneous outcomes in infection success, load, virulence and transmission, with consequences for the evolution of hosts and their parasites, and also for epidemiology. A comprehension that the triad of host, parasite and environment interact to dictate infection outcome is key for anyone interested in host–parasite research. Studies in model systems used to good effect to characterize insect immunity and infection rarely scrutinize such heterogeneity. Evolutionary ecology studies addressing natural variation offer a window on the causes and consequences of such heterogeneity. A system at the forefront in this area is that of bumblebees and their trypanosome parasite Crithidia. *Placing results and interpretations in a broader context we synthesize the plethora of work on bumblebee immunity and parasite interactions. We describe and discuss the sources of heterogeneity that should also be considered in human-relevant insect–parasite systems, including genotypic variation in both parasites and hosts, the mediating role of the environment, and explore the emerging evidence for microbiota modulating defence against parasites.*

Keywords *arthropod, genetic resistance, innate immunity, trypanosome*

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INTRODUCTION

Genotypic diversity is well known for a number of important insect-vectored human parasites (e.g. 1–3). Often, interest in this diversity is driven by a desire to design efficient vaccines against these parasites, an aim that parasite genetic diversity can hinder (2, 4). However, this diversity is also important on a more fundamental level, determining the interactions between these parasites and their insect hosts, and thus ultimately epidemiology and disease in the human population. The transmitting insects themselves are also genetically diverse (e.g. 5–7). Infections by parasites of insects are not indiscriminate, and infection outcome, whether that is in establishment success, infection load, transmission or virulence will frequently depend on the genotypic identities of both assailant and defender. Moreover, interactions between hosts and parasites take place in ever changing environments. Irrefutably, natural environments vary temporally and spatially, and additionally anthropogenic-mediated environmental changes at local and global scales impact on environments experienced by an untold number of species. How environmental parameters determine interactions between insects and their parasites is only beginning to be fully appreciated. The triad of host and parasite genotypes, and environment, is however ultimately crucial to better understanding infection outcomes and ensuing disease dynamics.

For the most part, the basic scientific studies required to fully grasp to interactive effects of host and parasite genotypes, and environmental variation in infection outcome are difficult to perform in vertebrates due to dimensional and ethical constraints. Invertebrates, and in particular insects, have proven to be excellent model systems for the study of disease for many years (introduced in 8). The attractiveness of insects for this work however goes beyond practicality. In their own right, insects represent an extremely large proportion of the overall

diversity of life on earth (9) and are beneficial components of ecosystems and human societies, performing crucial services such as pollination (10, 11). In contrast, many insects have become major pests in agricultural systems (12). Of utmost importance for human welfare is that insects are hosts to a broad range of parasites that are subsequently vectored to humans or livestock with grave consequences. For example, there were 219 million human cases of mosquito-transmitted malaria in 2010 with 660 000 associated deaths, representing a large disease and economic burden (13). Awareness of heterogeneity in the outcome of infection in insect hosts has value beyond basic science, and it will facilitate our ability to control infectious disease, either to restrain or to eliminate it, or to harness it for purposes such as biological control.

Insects have been studied comparatively for many years because they share important immune system components with humans, notably a large proportion of what is classified as the innate immune system (14). While excellent in terms of describing responses and characterizing insect immune systems, these studies typically overlook or do not attempt to study naturally relevant variation in factors determining the outcome of infections. Much of the research has taken place in molecularly well-characterized laboratory organisms that are ideally suited to broad descriptive studies, but extrapolation to natural populations and scenarios is problematic. It is instead work on the evolutionary ecology of host–parasite interactions using traditionally non-model systems, such as bumblebees and their trypanosome parasites that are focused on here, which have opened our eyes to immune response variation, diversity in the outcomes of infection, and the ecological and evolutionary consequences. Well-designed experimental studies coupled with a strong natural relevance have proved profitable in these systems, even if molecular resources have lagged behind those of more classical laboratory systems.

In this review, we focus on the system of bumblebee immunity and interactions with the trypanosome parasite *Crithidia*. We aim to highlight factors derived from empirical studies that lead to highly context dependent infection outcomes (Figure 1). Genotypic variation plays a role, along with current and past environments. Intriguingly, evidence is mounting that extended defence phenotypes formed by microbes living on or within the host are likely crucial. These are factors that are not only relevant to the bumblebee-trypanosome system but other insect–parasite systems, including those that are relevant to human disease. In fact, the concepts behind the discussion of the heterogeneity of infection outcomes are ones that should be understood by anyone interested in host–parasite research.

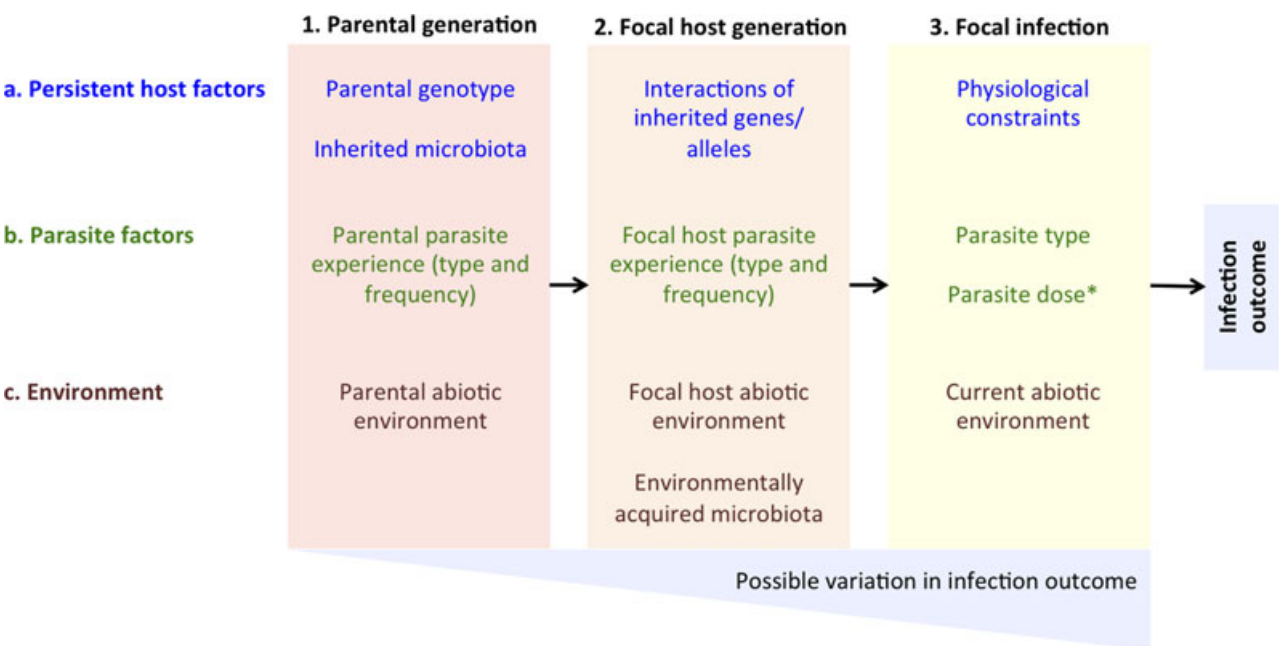

Figure 1 A schematic of factors from different levels (a–c) acting at time points up to and including the focal infection (1–3) that will create heterogeneity in infection outcomes. *Parasite dose is not discussed in this review, nor has it been explicitly studied in the bumblebee-trypanosome system, yet it may play an important role that should be considered.

A KEY POLLINATOR AND ITS PREVALENT PARASITE

Beyond a conceptual study system, the host–parasite interactions between bumblebees and *Crithidia* have to potential to, perhaps unexpectedly, inform about systems where insects vector human parasites. The trypanosomatids, to which *Crithidia* belongs, contain the agents of sleeping sickness, Chagas disease, leishmaniasis and other insect-vectored human and livestock parasites. Thus, the system offers an opportunity to further investigate this important group and its interactions with insects, albeit with some interesting twists. *Crithidia* infecting bumblebees is monoxenous, and therefore aspects elucidated in this system tell us only about the interactions between insects and trypanosomes, without potential artefacts derived from also infecting vertebrates. For example, it appears genetic exchange between trypanosomes is not dependent on the species having a vertebrate-infecting stage (15). Furthermore, unlike many of its companions within the trypanosomatids that infect in the gut but subsequently break through the gut wall, *Crithidia* infects and resides in the gut throughout an infection. Therefore, interactions described in the bumblebee-*Crithidia* system show what may also be relevant for the early stages of infections by other trypanosomes.

Bumblebees, the genus *Bombus*, are widespread primitively eusocial insects with key roles in natural and commercial pollination (16, 17). One of the best-studied bumblebees, especially with regard to host–parasite interactions and immunity, is the common European bumblebee *Bombus terrestris* (Figure 2, a and b). Fertilized queens of this species exit hibernation and set-up colonies, with social life progressing as emerging adult worker offspring provision the colony and help raise their siblings. Colonies can comprise of tens to hundreds of workers representing a single genotypic unit (relatedness of 75% between workers) as mother queens are singly mated to haploid males. In late summer, colonies produce new sexual offspring, which leave the colony and mate. Subsequently, workers, males and the old queen perish, and only freshly mated young queens enter hibernation until the following year, when the cycle repeats.

Crithidia (Figure 2, c) is a flagellate parasite infecting bumblebees and recent molecular work has identified two

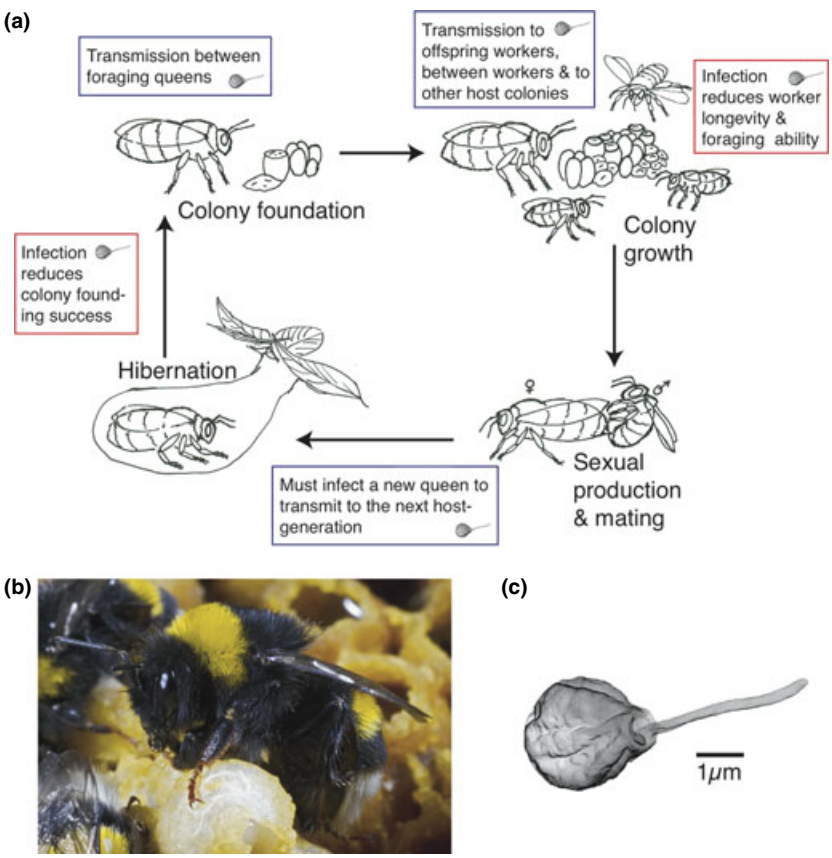

Figure 2 The annual life cycle (a) of the bumblebee *Bombus terrestris* (b) including annotation relating to infection and transmission of its prevalent trypanosome parasite *Crithidia bombi* (c, Scanning electron microscopy image by ETH Zürich, Boris Baer).

different infecting types, classified as the species *Crithidia bombi* (18) and *Crithidia expoeki* (19), with these types also harbouring substantial within species diversity (20). In nature, transmission occurs between host colonies through foraging on contaminated flowers (21), and within colonies through interactions with contaminated food stores or material. Following the uptake of parasite cells by the host, the parasite resides in the gut, replicates and sheds transmissive cells in the faeces. Infections are typically chronic, although exact infection and transmission levels fluctuate through time (22). Strains of these parasites can be stored and cultured *in vitro* in the laboratory, facilitating experimental infections using fixed parasite genetic backgrounds (23).

Crithidia prevalence in wild bumblebee populations varies depending upon the time of year that hosts are sampled. In Central Europe, infection prevalence in queens after hibernation is between 5 and 10%, but these figures rise to over 30% when offspring workers are sampled later on in the same season (24). Based on results when clean host colonies are placed into the field, infection prevalence may even reach 60% (25). The diversity of these infections is high, with almost every typed infection representing a new multilocus genotype. When investigating parasite genotype profiles (microsatellite analysis) across host colony units raised from wild caught queens a high degree of structuring is seen, with infections in different colonies being distinct (20). These distinct infections not only comprise of a single strain, but also multiple infections of more than one strain frequently occur. It has been shown that up to 74% of all wild caught infected individuals carry more than one strain (24). With this in mind, it has recently been demonstrated that genetic exchange occurs between coinfecting strains, and this may in part be responsible for the high diversity observed (15).

Infection by *Crithidia* has a number of fitness relevant consequences for bumblebees, including impaired foraging ability (26, 27), reduced individual survival under harsh conditions (28), and substantially reduced colony founding success and subsequent overall fitness in queens (29). Thus far, all experiments concerning these effects of *Crithidia* on their hosts have used infection cocktails of numerous strains. While multiple infections are common in nature, as highlighted above, infections with individual strains are not entirely absent with between 26 and 47% of infections containing a single strain depending on year and host caste (24). Therefore, an interesting unexplored aspect relating to virulence of this parasite is how parasite genotypes vary in their single infection effects, and which strains drive detrimental impacts on hosts in multiple infections.

RESPONSES OF HOSTS AND LINKS TO THE HETEROGENEITY OF INFECTION OUTCOMES

Insects have a well-adapted and flexible series of systems that help to protect against parasites. Some of these defences exist to either prevent parasite exposure, reduce infection likelihood, or limiting parasite spread (30, 31). This component is typically behavioural, is sometimes referred to as nonimmunological defence and may be especially important in social insects like bumblebees. Honeybees, for instance, vary considerably in their hygienic grooming behaviour that determines the likelihood of colony spread of *Varroa* mites (32–34). Similarly, genetic variation in hygienic behaviour determines the propensity to uncap and destroy infected brood (35–37). Bumblebees infected with parasitic flies spend more time outside of the colony at night, and if infected bees are experimentally given cool night-time temperatures they survive longer than if kept in warm conditions (38). How behavioural defence traits, such as thermal regulation, vary in bumblebees is unknown and has been generally neglected. Yet, given that there is genetic variation in other behavioural traits [e.g. foraging preferences (39)], it is reasonable to expect variation in behavioural traits relevant for parasite protection and infection outcome.

Once exposed to parasites, the traditional insect immune system is engaged, including cellular and humoural responses. Nonself patterns are detected by proteins such as the peptidoglycan receptor proteins (PGRPs) or beta-glucan receptor proteins (BGRPs aka GNBPs) which then initiate the signalling cascades of the Toll, IMD, JAK/STAT, JNK and RNAi-silencing pathways, culminating with the production of a variety of highly active defensive products such as antimicrobial peptides (AMPs), lysozymes, thioester containing proteins (TEPs), lectins, and the prophenol oxidase (PPO) and melanisation response [for a thorough review of insect immunity see (31)]. Some of the genes coding for immunologically important tasks in insects appear to be under selection, such as recognition genes and genes responsible for resistance to viruses (40). It is interesting to note that the rate of evolution of some recognition genes in social insects far outstrips that of model dipterans (41). Given that there seems to be rapid evolution of genes coding for recognition proteins, particularly for social insects, variation at these recognition genes may underlie some variation in immunity in bumblebees. At present, however, very little is known about sequence variation and the role this has in producing different infection outcomes.

Bumblebees, and in particular *B. terrestris*, have been a key system for the study of ecological immunology. Immunity is assumed to be expensive, with costs arising

in different ways: through pleiotropic interactions with other traits (the evolutionary cost of immunity), through the energetic requirements to produce, maintain and use an immune system, and through autoimmune self-harm (outlined in 42). Immune stimulated bumblebee workers die sooner when they are food restricted under laboratory conditions (43), and an artificial challenge results in a small but significant increase in energy consumption (44) suggesting an energetic based cost. Similarly, under field conditions, worker bees restrained from energetically expensive foraging bouts had stronger melanisation responses than those permitted to forage (45). The relationship between nutritional resources, and thus energy, and immunity in this system is complex. Infection of bumblebees by *Crithidia* can be altered by food quality, but not uniformly in one direction, as will be elaborated on later (46). Further, individuals increase their intake of protein-rich pollen after infection, which intuitively would reduce the costs of mounting a costly immune response. Yet, access to pollen can increase *C. bombi* infection load (47). Nutrition affects distinct components of insect host immune responses differently, and high-protein diets reduce the efficacy of some responses (reviewed in 48). Caterpillars of the lepidopteron *Spodoptera littoralis* given a high-protein diet have a strong lysozyme response but reduced PO activity (49). Increased protein in bumblebees could similarly shift immunity away from the optimal response to *Crithidia*, alter investment, at the cost of immunity, into nonimmunological traits, produce a gut environment damaging to protective microbiota (50), or could simply be providing resources for the parasite.

For researchers working with bumblebees, variation among colonies is assumed in almost every conceivable trait. Perhaps as a consequence, these differences are not always discussed in great detail. As mentioned before, colonies of *B. terrestris* represent a single genotypic unit. These host genotypes differ in various immunological responses. Colonies vary in the specificity of their immune response, being susceptible to a smaller or greater number of parasite strains (51), the number of circulating haemocytes (52), and their infection intensity following exposure (22, 53). These differences are also apparent in the commonly observed host–parasite interactions described in the section below on genotype differences. Colonies also vary considerably in the regulation of immune genes. Infection with C*rithidia* induces the expression of a number of immunologically important genes such as the AMPs abaecin, defensin and hymenoptaecin (54), IK2, peroxidase, calcineurin, Tamo, plexin A (55), hemomucin and relish (56). Colonies also differ in their standing expression of immune genes [MyD88, TEP7 (56)] or expression differed on exposure [genotype-by-exposure interaction, defensin, hymenoptaecin (54), hemomucin, relish (56)].

GENOTYPES MATTER FOR THE OUTCOME OF INFECTION

As mentioned above, infections in bumblebee colonies raised from wild queens show distinctness in their parasite genotype profiles (20). While this may in part be explained by differential exposure to distinct parasite strains, the highly structured and genetically diversified populations of *C. bombi* within host-genotypic units are indicative of strong genotypic host–parasite interactions. This is confirmed by experimental infections where parasite exposure is controlled, and a number of genotypically diverse strains are seeded across individuals from different host backgrounds. In these cases, parasite genotypic identity and genotypic units of the host (i.e. individuals from the same colony) interacting to determine the outcome of infection. Some strains are more infective, some hosts are more resistant, but predominantly, the outcome can only be predicted by knowing both host and parasite types, with no parasite being the most infective across all hosts and no host being the most resistant to all parasite strains. These specific host–parasite interactions have been demonstrated repeatedly in the *Bombus*-trypanosome system (51, 57, 58), and there is suggestive evidence that these highly specific interactions could be underpinned by associated specific immune responses (54), as introduced above. Genotypic determinants underlying host resistance to infection are also supported by experiments where different within colony patrilines were created by artificial insemination with these colonies subsequently being exposed in the field to natural circulating parasites (59). Given the biology of these social insects with males dying prior to queen hibernation, the principle contribution of fathers will be genetic, and in this study, it was shown that offspring of different patrilines raised under the same colony environment differed in both the prevalence and intensity of acquired *C. bombi* infections. Across colonies produced by queens each inseminated with sperm from ten different males, genotyping of offspring and measurement of infection showed infection load to range over ten-fold and prevalence to range over 40% between patrilines. Furthermore, using a quantitative trait locus (QTL) approach, it has been shown that infection of bumblebee hosts by *Crithidia* is determined by a network of QTLs with epistatic interactions between them (60). Intracolony phenotypic variation in infection of parasite exposed male offspring was used as the basis for mapping genetic architecture in three natural colonies. In each colony, two to three QTLs were found explaining cumulatively 7–14% of

the phenotypic variation, while epistatic interactions explained more variation in infection outcome. Interestingly, while networks of QTLs were similar in terms of number and complexity, QTL identities and interactions were not shared across colonies. This study showed genetic determinants of host resistance that are likely diverse and varied within natural populations.

Beyond the bumblebee-trypanosome system, evolutionary ecology studies in invertebrate host–parasite systems have shown that specific host genotype–parasite genotype interactions are pervasive (reviewed in 61). It is therefore imperative, where possible, that multiple host and parasite genotypes, representing naturally existing diversity, are incorporated in experiments that strive for natural or broad relevance.

AN EXTENDED DEFENCE PHENOTYPE: HOSTS AS MATRYOSHKA DOLLS

Beyond host and parasite genotypes, it is becoming increasingly clear that there are a number of host-associated microscopic players, each with their own genome, with important roles in determining infection outcome. Presence or absence, and phenotypically relevant strain variation in these microbes between hosts is an additional intimately associated level that may supplement or mask host-genotypic differences.

Excellent examples of how microbes can mediate defence against parasites come from organisms carrying bacterial symbionts (discussed in depth in 30). For example, susceptibility of pea aphids to a parasitoid wasp can almost entirely be determined by the presence of the bacterial symbiont *Hamiltonella defensa* (62). In this study, resistance was found to differ with the symbiont isolate present, and when present the conferred resistance was entirely driven by the symbiont independent of host genotype. This story became somewhat more complex recently, with more nested levels being implicated in infection outcome. The strains of *H. defensa* which are protective are those that carry a particular phage (63). While broad conclusions cannot be drawn across all host genotypes, what these studies tell us is that for certain host and parasite genotype combinations, the role of genotypic variation in the host may be negligible. Rather, hosts can be likened to matryoshka dolls, with the smallest innermost doll being the one that determines the outward phenotype measured in the largest.

In addition to organisms classically regarded as symbionts, the microbiota present on the body of a host at points of exposure and/or infection is likely to play an important role in determining infection outcome. If a microbes evolutionary fitness is tightly tied to that of the organism it dwells on or in, adaptations may well arise in this microbe enhancing host defence or even initiating independent defence responses. Hence, coevolution not only takes place between hosts and parasites, but components of the microbiota will represent intermediate coevolving entities. The tightness of the association between microbiota and host will depend upon frequency of transmission among hosts of microbiota components and the potential to persist in the environment or other species. In scenarios where this is limited, microbiota components may be viewed in a similar way to symbionts, with a single multi-genome organism evolving under uni-directional selection. However, an interesting aspect to be addressed pertains to the fact that the individuals within the microbiota are, in most cases, likely to have dramatically shorter generation times than their carriers. Due to this, a microbiota can evolve during a hosts lifetime, adapting to the prevailing environment, including the encountered parasite community.

Bumblebees have a specific microbiota, especially in the gut (64). That this gut microbiota influences infections by gut-residing (such as *Crithidia*) or gut-invading parasites is by no means inconceivable. Indeed, it has been shown that the presence of a full gut microbiota greatly influences the susceptibility to *Crithidia* infection (50). Reinstating natal gut microbial communities in microbe-free individuals through feeding of faeces from their source colonies reduces subsequent *Crithidia* infection loads by over 80%. The reverse was true for individuals where the gut microbiota was disrupted by antibiotic feeding. Further, an elegant experiment disassociated variation between host genotypes and differences in the gut microbiota that are carried by the bumblebees. Focal transplants of microbiota probes sourced from six colonies among individuals from those colonies in a reciprocal matrix, again through faecal feeding, demonstrated that microbiota origin has the possibility to explain a larger degree of infection variation than the genotype (colony of origin) of the host (65). While sociality on the surface may seem to increase the risk of parasite spread between related individuals, sociality and particularly an intimate overlap between mother and offspring generations, who share the natal nest, may facilitate aspects of defence through transmission of either defensive compounds or protective microbes. Indeed, close coevolving associations with gut microbes bestowing defence against parasites are likely to be facilitated under such scenarios (66, 67).

The work outlined above concerning the importance of the gut microbiota for host resistance to *Crithidia* infection suggests that some of the host-genotype effects and specific host–parasite interactions (e.g. 57) may derive from microbiota differences and not directly from differences in host genotypes. With this apparent importance of

the microbiota emerging, one could ask if host genotype truly matters for the outcome of infection. Yet, it would be naïve to draw such conclusions. As outlined in more detail above, patrilines within the same colony, likely to share microbiota transmitted from the mother, show different infection patterns (59). Moreover, the discovery of QTLs for Crithidia resistance (60) points to a genetic component within the host. However, the microbiota could still act as an intermediate whose constituents are formed by an interaction with the hosts immune system, and the underlying genotype. Subsequently, it could be this distinct host-mediated microbiota that differentially determines the infection outcome.

Exactly, how the microbiota influences the outcome of infection in the bumblebee-trypanosome system, and others, is still an area requiring a great deal of further work. A number of testable alternatives are plausible, each with implications for understanding how the coevolving triangle between hosts, their microbiota and parasites progresses. Many of these alternatives exist in the case of the vertebrate gut microbiota (68). On the one hand, it can be envisaged that bacteria within the microbiota of the gut could exclude gut-infecting parasites, like *Crithidia*, through the persistent occupation of gut niches. This would not require a strong coevolutionary past including the microbiota. Direct interactions between bacterial commensals and invading parasites could also take place, with the microbiota producing antiparasite substances. An additional intriguing possibility is that the microbiota links back to the host itself, essentially acting as a sentry for the hosts gut immune system (69). This could be through either exposure of the host to gut microbes, perhaps as a result of damage to this natural barrier by invading parasites, or through disruption of the gut community thereby producing signals of lost gut homeostasis. These latter possibilities would hint at a much more intricate coevolutionary web with the microbial community evolving defensive roles to protect themselves and their host. More mechanistic details of how microbiota components may influence parasite infection, with a particular focus on vector insects, are found in (70).

It is becoming well established that insect-vector host–parasite systems also have protective bacterial communities. The malaria-vectoring mosquito *Anopheles gambiae* produces an immune response when given their microbial community (71). Importantly, this response contains products that act against the parasite behind human malaria, *Plasmodium falciparum* and the mosquitoes with intact microbial communities carried lower infection loads. This protection, however, comes at a cost. More mosquitoes with their microbial community and infected with *P. falciparum* died within a week than those with only *P. falciparum* infections. Among the community of bacteria within the *A. gambiae* gut, the abundance of *Enterobacteriaceae* seems to be important. Individuals with high titres of this group of bacteria were less likely to be infected with *P. falciparum* (72). In addition to vectorial capacity of *Anopheles* for *Plasmodium* being influenced by gut microbiota, viral resistance in *Aedes* mosquitoes is also modulated by the presence of a natural gut microbiota. Further details of this and further burgeoning work on mosquito-microbiota-parasite interactions can be found in (73). In addition to their native microbiota, exciting new possibilities are emerging to control important diseases, such as dengue (74) and malaria (75), through the establishment of foreign but protective *Wolbachia* symbionts in mosquito populations. While there is no doubt that insect vectors are at the forefront of studies on protective microbiota, additional evolutionary ecology approaches would be beneficial. To date, there is little information of diversity of microbial communities between individuals and variance in their protective roles (but see 76). Reciprocal transplant experiments, such as those carried out in bumblebees (65), would be informative in this regard.

HOST–PARASITE INTERACTIONS IN A CHANGEABLE WORLD

Controlled laboratory experiments, in which environments are kept constant, allow for the elucidation of the contributions of host and parasite components, or as highlighted above the microbiota. However, the natural stage on which host–parasite interactions take place is a variable one. Spatial and temporal environmental variation may have impacts on the threat and also the outcome of infections. The environment may relate to the host and parasite species themselves, or to external biotic or abiotic factors. Social living and adaptations to it have the potential to exacerbate or lessen the spread of parasites (reviewed in 77). Furthermore, density of potentially susceptible hosts will play an important role. In the case of *Crithidia*, transmitted through shared foraging targets, the density of bumblebees per rewarding foraging opportunity may be an important factor determining parasite spread (78). These important considerations for parasite epidemiology should be kept in mind but are beyond the scope of this review, and in this section, we focus on the influence past and present environments have on individual responses and infections.

Previous parasite and immunological experiences

The investment into defence against parasites may be related to previous immunological experiences. When

prior experiences are correlated with the probability of future parasite encounters, immune priming generating an improved future responses will, provided the right weighting of costs and benefits, be beneficial (79). In the bumblebee system, specific (level of bacterial species) and lasting immune priming has been demonstrated (80). Individual workers injected with a clearable dose of bacteria showed an increased probability of survival and bacterial clearance when later faced with a higher bacterial dose homologous to the first in fully reciprocal exposures using three bacteria. Because this demonstration, studies in other invertebrate systems have corroborated the existence of immune priming to a range of natural parasite and artificial challenges (e.g. 81–83), shown higher levels of specificity (82), and focused in on potential immunological mechanisms (81, 84). It is important to note however that the existence of immune priming may be dependent on the particular immune stimuli or parasites used (81, 82).

In addition to an individuals experience mediating future responses through immune priming, the bumblebee has been at the core of demonstrations of transgenerational immune priming (85, 86), the existence of which has again been further demonstrated in other invertebrate systems (e.g. 83, 87, 88). Bumblebee offspring of mothers receiving a benign bacterial immune challenge prior to egg laying show increased levels of induced humoural antibacterial activity (85). Cross-fostering of eggs between mothers further demonstrated that this effect is mediated through a cue transferred before egg laying (86). While not investigated in bumblebees directly, work in other systems has suggested that transgenerational immunity may also show a high degree of specificity to the immune challenges or parasites used (88, 89).

Interestingly, costs associated with immune priming and immune system trade-offs in general may mean that heterogeneous outcomes arise in relation to infection by other parasites than that which is the focus of the immune priming. In fact, studies in the bumblebee-*Crithidia* system have demonstrated such a modulation of infection by a distinct parasite relating to immune priming both within individuals and across generations. A bacterial immune challenge in the body cavity of worker bumblebees increases individual susceptibility to infection by the distinct gut-residing trypanosome, with a greater diversity of parasite strains from an experimental inoculum able to establish, which will subsequently influence the diversity of strains transmitted (90). Across generations, it has also been shown that bacterial-based priming increases susceptibility to *Crithidia* infection (79).

Prior to parasite experience, in an individuals life or that of their mother will shift future infection outcomes due to immune priming. Immune priming will lessen infection in related parasites, while in distinct parasites, it may inflate susceptibility, influencing intensity and diversity of infection and transmission. Empirically determined infection matrices combining priming and infection of parasites concurrently present in the environment are needed to tell us more about how important these influences will be. A greater understanding of the epidemiological consequences of immune priming requires more investment into theoretical studies, but initial work has suggested that it can negatively affect the stability of population dynamics (91) and influence disease dynamics (92).

Further environmental variation influences on host–parasite interactions

Outside of the experienced parasite environment, variation in a number of other factors (e.g. temperature and nutrition) has the potential to create heterogeneous infection outcomes. Sometimes this may be simply mediated by reduced host condition resulting in weakened responses and resistance, which may materialize in greater expression of parasite virulence. For example, the effect of *Crithidia* infection on bumblebee worker longevity is pronounced under food restriction (28). The converse may also result, perhaps counter intuitively, as has been shown through increased *Crithidia* infection in *B. terrestris* given access to nutritional supplementation in the form of pollen (47). Further discussion of how nutrition can influence immunity, and hence, infection outcome has been addressed in the section above on responses.

In addition to broad effects influencing infection outcome without regard for host and parasite genotypes, it is becoming increasingly clear that the genotype-by-genotype interactions, as those outlined earlier, are not static in the face of relevant environmental variation (93, 94). A number of abiotic environmental factors have been shown to interact with host or parasite genotypes in determining the infection outcome in invertebrates (reviewed in 94). Environmental variation will not always result in a transition through the infection landscape, and bumblebee-*Crithidia* interactions may be robust to certain environmental variation, as shown by persistence of basic genotype-by-genotype specificity across experimental treatments, with infection status of adult workers rearing their sibling larvae having no influence on the subsequent resistance to *Crithidia* of those raised siblings (58). However, in another study where the environment studied was naturally relevant variation in food concentration (sugar water), the interactions between bumblebee genotypic units and *Crithidia* strains determining the infection outcome were modulated by the environment (46). Individual infections

of four *Crithidia* strains were carried out in individuals derived from six colonies. These infections were replicated in three environmental treatments with the hosts being maintained on low, medium or high concentrations of sugar water. Responses of infection load and the number of transmitting cells demonstrated a genotype by genotype by environment interaction, with infection outcome only able to be accurately predicted with knowledge of the interacting genotypes and the environmental state. When resistance and infectivity hierarchies are altered (as here), temporal and spatial environmental variation will create a selection mosaic favouring different host and parasite genotypes, and hence increasing the heterogeneity of infection outcomes and potentially overall diversity of host and parasite types. The result of such environmentally mediated modulation for diversity and host–parasite dynamics will depend on the frequency of relevant change. If strong inferences are to be drawn about natural scenarios, the existence of environmental modulation of infection outcomes must be considered. Studies should be designed to investigate how infection loads, virulence, and infection and resistance hierarchies of parasites and hosts are changed by relevant environmental variation. Where possible, a minimum of three environmental treatments should be included so that if main effects of the environment are seen, generalizable directionality can be inferred. If environmental modulation is expected or empirically demonstrated, efforts should be made to ensure sound applicable results for natural systems by replicating relevant environmental parameters as accurately as possible in other experiments.

CONCLUSIONS

The importance of heterogeneity in infection outcome, whatever the cause, has important consequences for disease dynamics in natural populations. Classical models of disease dynamics in natural populations have typically modelled horizontal transmission under a mass action assumption as a linear function of the densities of healthy and infected individuals within a population. However, inclusion of host heterogeneity in susceptibility, based on laboratory and field studies of infection outcomes, has proven to provide models that more accurately fit data on parasite dynamics [e.g. for gypsy moths and viruses (95)]. Host heterogeneity can also influence the evolution of virulence and parasite divergence (96).These models of natural dynamics require knowledge of the factors that contribute to heterogeneity, the distribution of these factors in the field, and their interactions. Carefully designed and relevant laboratory studies together with relevant field experiments, as those discussed and encouraged here, continue to provide crucial parameters for the further development of such models.

While some of the aspects outlined in this review are system specific, valuable general messages can be taken from the research on bumblebee–trypanosome interactions. The highlighted examples in this review, together with work in other systems, show that numerous factors feed into the eventual infection outcome. Referring back to Figure 1, heterogeneity across a landscape of potential outcomes will be determined by host and parasite genotypes, host microbiota, and current or previously experienced environments. The factors involved and the eventual point reached within this landscape will determine the fitness consequences for hosts and parasites, and thus ecological and evolutionary dynamics. It is vital that these factors are taken into account in studies of host–parasite interactions, including those between insect vectors and human-relevant parasites. Where possible, future experiments should continue to: (i) study the existence and influence of natural genetic variation in hosts and their microbiota, and parasites, (ii) consider how the abiotic environment may influence individual infection outcomes, and incorporate naturally relevant environmental variation, and (iii) investigate how historical or concurrent infections with the same or different co-occurring parasites influence infection outcome in host populations. Highly controlled experiments to elucidate mechanisms and general concepts remain crucial; yet, the tremendous context dependency of infection outcomes must be embraced by anyone researching host–parasite interactions.

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