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Vector Immunity and Evolutionary Ecology: The Harmonious Dissonance

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Abstract

Recent scientific breakthroughs have significantly expanded our understanding of arthropod vector immunity. Insights in the laboratory have demonstrated how the immune system provides resistance to infection, and in what manner innate defenses protect against a microbial assault. Less understood, however, is the effect of biotic and abiotic factors on microbial-vector interactions and the impact of the immune system on arthropod populations in nature. Furthermore, the influence of genetic plasticity on the immune response against vector-borne pathogens remains mostly elusive. Herein, we discuss evolutionary forces that shape arthropod vector immunity. We focus on resistance, pathogenicity and tolerance to infection. We posit that novel scientific paradigms should emerge when molecular immunologists and evolutionary ecologists work together.

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An Evolving Outlook for Arthropod Vector Immunity

Have we reached an inflection point in the relative merits of minimizing biological variation? What about the virtues of mechanistic depth over breadth in arthropod vector immunity? Recent research embracing ecological principles in invertebrate immunology have underscored the need to account for natural phenomena, such as multiple microbial exposures, the many trade-offs of arthropod fitness traits, and the role of evolution in shaping close associations between vectors and microbes.

Drosophila melanogaster, hereafter referred to as *Drosophila*, has served as a successful workhorse in the approach to elucidate molecular events in arthropod immunity. Anti-pathogen defenses occur through innate immunity, characterized as a rapid and efficient, but which lacks specificity and memory [1–3]. Cellular and humoral immunity have been historically used to distinguish the two branches of the arthropod immune system. Cellular immunity is carried out mainly by **hemocytes**, which can differentiate into three specialized types: 1) **plasmatocytes** and **granulocytes**, 2) **lamellocytes** and 3) **oenocytoids** [4–6]. Arthropod humoral immunity, lacks antibody-mediated features and is regulated by the **Toll** and the **Immune Deficiency (IMD) pathways** [7–9]. The janus kinase/signal transducer and activator of transcription (**JAK/STAT**) and the RNA interference (**RNAi**) pathways can also protect arthropods from microbial and viral threats [7] (Figure 1).

This view of innate immunity guides many laboratories around the world and defines central paradigms and tenets of arthropod immunology [10–12]. The use of controlled environments provides the benefit of structured variables and assumes that the elimination of statistical noise is the fastest track to distilling insights and tackling human disease [13–16]. caveat to this approach, however, is that repeatable phenotypes obtained from inbred strains in artificial settings may exhibit substantial context-dependence when investigated under more natural conditions [14, 15, 17, 18]. Furthermore, cell signaling mechanisms may not necessarily be conserved among organisms owing to varying selection pressures among arthropod species that differentially shape both genotype and phenotype. Nevertheless, mechanistic insights from model organisms are frequently transposed to evolutionarily distant species (*i.e.*, mice to humans; or *Drosophila* to other arthropods). This approach may lead to incorrect annotation of genes and an apparent lack of protein homologues [16, 19]. Additionally, trying to extrapolate these results for generalized pest management strategies targeting arthropods with different lifestyles has resulted in resurging and emerging pathogens hazardous to human populations despite enormous intellectual and financial investments.

How can we maximize our scholarly and economic efforts to control vector-borne diseases? Can we leverage evolution in our favor to combat public health afflictions? In this *Opinion* article, we argue that, whenever possible, the study of immunology in arthropod vectors should incorporate concepts of evolutionary ecology as part of a variable and dynamic system. We attempt to describe two immunological principles by means of ecology and evolution. We discuss the theory of resistance and tolerance to transmissible pathogens through the topic of contextual pathogenicity and resource allocation in arthropod-borne diseases. Then, we elaborate on the phenomenon of “**trained immunity**” [20–22].

How Evolution Affects Arthropod Vector Immunity

Despite the superficially affable co-existence between arthropod vectors and microbes, immunity is active against pathogens, limiting their transmission. In ticks, phagocytosis, the IMD and the JAK/STAT pathways curb growth of *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, causative agents of Lyme disease and Granulocytic Anaplasmosis [23–27]. In cat fleas (*Ctenocephalides felis*), the IMD pathway suppresses the burden of the murine typhus-causing bacterium, *Rickettsia typhi* [28]. The IMD, Toll, JAK/STAT and the **complement** networks also constrain the survival of mosquito-borne pathogens [29]. If vector immunity can restrain a microbial population, why are pathogens not eliminated? Below, we discuss evolution of arthropod vector immunity in the context of energy and immune evasion.

Arthropod Investment in Immunity

Mounting an immune response is energetically costly and ultimately affects arthropod fitness (Box 1) [30]. In natural settings, where food is scarce, the allocation of resources to microbial resistance in the arthropod vector deprives development, growth, and reproduction. As an example, fruit flies mobilize energy to fuel the proliferation of hemocytes after parasitoid infection, at a cost of insect development [31]. Parasites themselves could exploit resources that fuel immune responses [32], complicating the calculus of energetic investment. Moreover, immune responses can induce collateral damage against tissues (e.g., immunopathology). For instance, the **melanization response** to a parasitoid challenge can inhibit the function of **malpighian tubules** in the mealworm beetle *Tenebrio molitor* leading to larval mortality [33].

Two possible immunological outcomes for arthropods to respond to infection are: (1) resistance, which focuses on the eradication of the pathogen; and (2) disease tolerance, which decreases the impact of infection on host health or fitness [34, 35]. For example, flies that carry a mutation in the JAK/STAT regulator G9a are more likely to survive *Drosophila* Virus infection despite a negligible impact on viral titers [36], indicating an increase in tolerance. Tolerance [37], which describes the dose response curve of health versus microbial density [34, 35], is predicted to exert different effects on the dynamics of vector-pathogen interactions relative to resistance, which generally curtails the probability of transmission (Figure 2). This transmission phenomenon has been observed in chickens administered a vaccine that increases symptom tolerance but not resistance against Marek's disease virus. So far, however, the impact of tolerance variation on disease transmission has not been tested in arthropods. Mathematical models suggest that investment in tolerance over resistance is less likely to lead to oscillations in the frequency of negative genetic traits in the arthropod and the microbe, favoring instead the fixation of tolerant alleles in populations [38, 39].

Theory on the evolution of resistance and tolerance assumes that microbes contribute to pathogenesis, and that vectors have adapted to minimize that pathology. We can point out aspects of microbial life within a vector that are likely to fulfill this first obligation.

Plasmodium sp. **ookinetes** damage the mosquito gut as they pass into the **hemolymph**, while *Leishmania sp.* parasites obstruct the sand fly gut to promote regurgitation [40].

Nonetheless, the vast majority of reports about vector fitness and microbial infection repeatedly only show a modest effect of parasite infection on vector survival and reproduction [41].

Contextual Pathogenicity in Arthropod Vector-Microbial Interactions

Are arthropod vectors tolerant? Pathogens need to complete their life cycle, but not necessarily within the same host. A hallmark of vector-borne diseases is that these processes are distributed among different species. The cost of infection to the fitness of each host will be determined by the unique mechanisms through which pathogens exploit resources and are transmitted. For example, *Plasmodium berghei* parasites within the *Anopheles gambiae* mosquito exploit host energetic resources as blood stage parasites, but their hijacking of vector lipids is timed in such a way to avoid competition with mosquito reproduction [42].

Unlike pathogens that exploit adult arthropod vectors, *B. burgdorferi* is intimately associated with the entire ontogeny of the tick, requiring survival through multiple life stages. Thus, the cumulative effects of even modest resource exploitation are hypothesized to have a bigger impact on tick fitness and survival. In line with this are reports that show improved fitness in microbial-infected ticks to the apparent benefit of the arthropod during lower temperatures [43–46]. Such conditions would favor high vector tolerance towards tick-borne pathogens. This feature is abetted by the absence of immunogenic molecules common in the membrane architecture of Gram-negative bacteria, such as lipopolysaccharide (LPS) and **DAP-type peptidoglycans**, which microbes transmitted by the black-legged deer tick, *Ixodes scapularis*, lack [47–50]. Factor C, found in horseshoe crabs and other chelicerate arthropods such as ticks, recognizes LPS and sets off a coagulation cascade resulting in clot formation that prevents proliferation of bacteria [51]. DAP-type peptidoglycans are the canonical agonists of the IMD pathway, which culminates in the secretion of bactericidal antimicrobial peptides (AMPs) (Figure 1) [52]. Therefore, the lack of immunogenic molecules might benefit the microbe and the arthropod by suppressing the costly investment in immunity, but this warrants further investigation.

The needs of a microbial pathogen at a certain life stage may not require exploitation that results in virulence. This may be particularly true for pathogens that undergo mixed transmission modes [53], such as *Babesia* spp. and *Rickettsia* spp. (causative agents of Babesiosis, Rocky Mountain Spotted Fever and other Spotted Fever Group Rickettsioses). [54]. These pathogens are transmitted vertically in tick populations from mother to offspring through **oocytes**, and, thus, a cost of virulence to arthropod reproduction would reduce transmission. These life history considerations contribute to the problem of contextual pathogenicity, where pathogens that we think of as virulent to humans, may not be detrimental to arthropod vectors. Consequently, genetic diversity and the ecology of vector-borne microbes make contextual pathogenicity a central tenet in the study of vector-borne diseases (Figure 3).

Trained Immunity in Mammals: The Molecular Immunology View

“Trained immunity” is a phenomenon that describes memory conferred by innate immune defenses [21, 55–61]. The mechanisms orchestrating trained immunity remain largely

undefined, but it is deemed to be regulated by metabolic as well as epigenetic changes [56, 60, 62] (Figure 4).

Cellular function directly correlates with metabolism [63, 64]. Mammalian inflammatory macrophages (also known as M1 in the literature) are largely dependent on glycolysis and exhibit a disruption in the tricarboxylic acid (TCA) cycle and an impairment of oxidative phosphorylation (OxPhos) after exposure to microbe-associated molecular patterns [65, 66]. In contrast, anti-inflammatory macrophages (M2), (or macrophages alternatively activated by IL-4 (10–20 ng/ml) for 24 hours in rodent models) have been shown to be reliant on OxPhos and show increased β -oxidation relative to inactivated macrophages [67–69]. Increased glucose metabolism is often seen during immune cell activation and is presumed to be important for quickly generating energy in the form of adenosine triphosphate (ATP) [65, 66]. Although glycolysis is less efficient than OxPhos, it can be upregulated to rapidly produce ATP [70]. This shift from OxPhos to glycolysis associated with trained immunity can persist for days in the absence of stimuli and ultimately lead to enhanced protective responses upon secondary challenge [56, 62] (Figure 4).

Epigenetic modifications are non-permanent changes in gene expression. Mechanistically, these changes may take several forms, such as alterations of the **heterochromatin** architecture, post-translational modifications or by directly ‘marking’ nucleic acids [21, 56, 71]. Posttranslational modifications to histones, particularly on lysine residues within the unstructured tail region that protrudes from a nucleosome, control gene expression. These “epigenetic marks” regulate accessibility of the DNA wrapped around the histone octamer to block or make a region accessible for gene expression [72]. Trimethylation and acetylation events on histone 3 (H3) at conserved residues lysine 4 (H3K4me3) and lysine 27 (H3K27ac) have been associated with trained immunity in both plants (*i.e.*, *Arabidopsis thaliana* challenged with the plant pathogen, *Pseudomonas syringae* pv. *Maculicola*, or a synthetic analogue of the plant hormone salicylic acid) and mammals (*e.g.*, human-derived macrophages, trained with β -glucan and challenged with LPS) [21, 56, 71]. Fluxing metabolites can also directly control gene expression through epigenetic modifications. For example, increased lactate from a glycolytic shift can suppress histone deacetylase (HDAC) in a human colorectal cancer cell line (HCT116) [73]. In addition, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), an enzyme involved in glycolysis, can suppress translation of the pro-inflammatory transcript coding for interferon- γ (IFN γ), in a murine-derived, activated T cell line (EL4)[74]. In an OxPhos state, GAPDH is not active and can bind the 3' untranslated region of IFN γ -encoding RNA (*ifng*) in CD4⁺ T helper 1 cells, thereby, suppressing translation of this proinflammatory cytokine [74, 75]. From these studies, a mechanistic relationship has emerged that links, in certain instances, epigenetic shifts and metabolic fluxes with a trained immune phenotype.

Arthropod Trained Immunity: The Evolutionary Ecology View

While mammalian studies have illuminated many of the elements coordinating trained immunity, the molecular mechanisms involved in arthropod trained immunity remain a frontier of investigation. Theory predicts that parasite exposure frequency, rather than host longevity, should influence the evolution of memory relative to constitutive resistance [76,

77]. Evidence from invertebrates suggests that previous exposure to a microbe can enhance resistance and survival upon subsequent exposure [26, 55, 78–81]. This phenomenon is termed “**immune priming**” in ecological immunology [26, 55, 78–81] and it is functionally analogous to “trained immunity” [20–22] in mammals. There are four important criteria to consider when evaluating trained immunity in arthropods: 1) duration of memory (within and across generations), 2) degree of specificity, 3) dynamics of inducibility and 4) mechanism of activation [82]. Duration of primed immunity against bacteria has been described within and between generations as a benefit to post-infection survival in honeybees [83], bumblebees [84, 85], tenebrionid beetles [79, 86–88] and lepidopteran insects, such as the meal moth *Plodia interpunctella* exposed to baculoviruses [89]. There is evidence for specificity in arthropod trained immunity. For example, the flour beetle *Tribolium castaneum* is better protected from disease-induced mortality upon infection with the bacterium *Bacillus thuringiensis* when first received a sub-lethal challenge with the same, but not a different strain of bacterium [90, 91]. Specificity studies, however, are frequently confounded by microbial virulence or co-evolutionary histories between the vector and the microbe.

The dynamics of inducibility in arthropod immune priming could take two forms: A) a first response that recovers to near baseline levels, followed by an enhanced second response, as seen in traditional adaptive immunity; or B) a first response that remains elevated, giving a head start to a secondary immune response [80]. Trained immunity in mammalian monocytes contains hallmarks of both scenarios. Epigenetic changes in macrophages after primary challenge can create distinct response dynamics upon secondary stimulation. However, metabolic reprogramming can persist through the observed life of a trained cell [20–22]. One might speculate that in arthropods, a trained response that relies only on hemocyte differentiation would qualify as a head start, while epigenetic reprogramming would provide a clear opportunity for a biphasic immune response.

Trained immune phenotypes are observed in several invertebrate vectors. *I. scapularis* is protected against bacterial colonization when primed with infection derived lipids, which hinges on the non-canonical IMD pathway [26]. *A. gambiae* mosquitoes colonized by *Plasmodium* instigate an upregulation of eicosanoid signaling components that prime cellular responses of naïve mosquitoes upon hemolymph transfer [92]. Similarly, in *Drosophila*, priming with the heat-killed pathogen *Pseudomonas aeruginosa* or *Streptococcus pneumoniae* induces a protective response against infection-induced mortality that involves the IMD and Toll pathways, and phagocytosis [78, 93]. In the snail vector for Schistosomiasis, *Biomphalaria glabrata*, immune priming against *Schistosoma* parasites causes a shift from a cellular response involving **encapsulation**, to a humoral response involving the circulating immune factor **biomphalysin** [94]. In tsetse flies, immune stimulation with *Escherichia coli* bacteria prior to challenge with a trypanosome-infected blood meal blocks parasite colonization [95].

Arthropod vectors reared in the laboratory tend to be immunologically naïve or even artificially **gnotobiotic**, relative to their wild cohorts. Wild arthropods do not receive such consideration, and it is probable that the constant barrage of microbes from the environment shapes immunological maturity, training of the innate immune system, and phenotypic

susceptibility to a focal microbe [96, 97]. The presence of **symbionts** may also induce trained immunity that provides cross-protection against other microbial species. For instance, *Aedes aegypti* mosquitoes colonized by the endosymbiotic bacterium *Wolbachia pipiensis* are more resistant to a variety of pathogenic bacteria [98], based on bacterial density and host survival after infection. Finally, immune priming may alter the native community of microbes in the midgut (microbiota), which could have indirect effects on how a vector responds to subsequent challenges. For example, in blood-fed *A. aegypti* mosquitoes, immune-mediated protection against Sinbis virus (SINV) may depend on the microbiota, which first activates the IMD pathway and modulates viral dynamics [99–102].

Broader evidence is available for a cellular-based immunity that contributes to a training phenomenon in arthropods [78, 79, 83, 103]. Flour beetles trans-generationally primed against bacteria exhibit a shift in gene expression of the TCA cycle and glycolysis relative to unprimed beetles, echoing metabolic shifts observed in trained mammalian macrophages [104]. Whether epigenetic changes [56] also accompany priming responses in arthropods remains elusive, although mealworm beetles (*T. molitor*) appear to undergo reduced RNA cytosine methylation after infection with the bacterium *Micrococcus lysodeikticus* [105]. Investigating the molecular control of trained immunity in arthropods will facilitate our understanding of how vectors control transmissible microbes.

Multiple variables need to be considered when studying the dynamics of trained immunity, resistance and tolerance in arthropod vectors and there is much to be gained with an improved dialogue between evolutionary ecologists and molecular immunologists. What happens after primary exposure to different microbes? How does the transcriptional, epigenetic, and metabolomic landscape change over time within and across generations? How specific is specific? Do arthropods prime effectively against microbes with which they have co-evolved? Does the environment and arthropod developmental stages influence the efficacy of trained immunity [106, 107]? Is it always in the best interest to invest in trained immunity [108, 109]? A better understanding of the underlying mechanisms of trained immunity may shed light onto the blurred line between innate and adaptive immunity.

Concluding Remarks

Modern scientific approaches have contributed a swath of information in laboratory-reared, genetically inbred organisms for arthropod vector immunity. However, the outcome of this knowledge does not always translate into the complex interaction between a microbe and an arthropod vector in nature (Box 2). Moving forward, if one wants to successfully implement strategies to manage vector-borne diseases, the ecology and evolution of arthropod immunity must be considered (see Outstanding Questions).

Investigating the role of immune mechanisms on arthropod phenotypes should move beyond survival against a high dose of an artificially introduced pathogen [35]. As a starting point, scientists should reflect on the frequency and diversity of pathogen exposure that a vector is exposed over the course of its life, including its native microbiota (see Outstanding Questions). Whenever possible, laboratory and fieldwork should be combined to quantify the temporal dynamics of arthropod immune responses and fitness phenotypes [110].

Moreover, it should be kept in mind that stimuli such as LPS, peptidoglycan, or heat-killed bacteria, which are typically intended to isolate the costs of immunity (Box 1), may not adequately capture immunological costs or tolerance phenotypes during chronic infections. These unknown-unknowns could have a crucial role in shaping the deployment and evolution of the immune system.

In this article, we offered the opinion that characterizing the molecular mechanisms of tolerance, resistance and trained immunity would fundamentally advance the field of vector immunology. Such an undertaking is complicated by the relative lack of resources available for non-model organisms. However, transposing information across evolutionarily distant species must be cautiously evaluated in the context of both the vector and the microbe (see Outstanding Questions). With the advent of new gene targeting techniques and the decreasing cost of large-scale sequencing, studies that directly focus on the arthropod vector are becoming more feasible. Thus, we assert that studying immunological networks in non-model organisms will give a more authentic view of biological processes occurring between microbes and vectors. When molecular immunologists and evolutionary ecologists work together, novel concepts can be uncovered. These emerging principles should be used with the intent to control and/or mitigate the impact of vector-borne diseases.

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Glossary

Adaptive Immunity

immunity characterized by the presence of lymphocytes, major histocompatibility complex and recombinant activating gene-dependent antigen receptors

Arthropod Cellular Immunity

immune response carried out mainly by hemocytes in arthropods

Arthropod Humoral Immunity

immune response mediated by components present in the fluids, which in arthropods lacks *antibody*-mediated features

Biomphalysin

pore-forming toxin involved in *Biophalaria glabrata* immunity

Complement

a series of proteins that react with one another to promote opsonization, phagocytosis and melanization of pathogens

Contextual Pathogenicity

term used in microbiology to explain microbial virulence in one organism, but avirulence in another due to biotic and abiotic factors

DAP-type peptidoglycan

Diaminopimelic acid (DAP)-type peptidoglycan; found in the periplasmic space of most Gram-negative bacteria

Ecological Immunology

sub-field of immunology which considers the causes and consequences of natural variation in immune function in host populations

Encapsulation

immune defense in arthropods involving the formation of a capsule-like envelope around invading objects

Epigenetic Modifications

modifications that change DNA and/or chromatin accessibility; thereby, regulating the profile in gene expression

Euchromatin

form of chromatin that is loosely packed and transcriptionally active

Evolutionary Ecology

sub-discipline of ecology where the evolutionary history of a given species and its interaction among biotic and abiotic factors are considered

Factor C

LPS-sensitive serine protease proenzyme. Component of the clotting cascade in chelicerate arthropods

Gnotobiotic

an environment where all microorganisms are defined; can refer to an organism with a defined or limited microbiota

Granulocytes

strongly adhesive cells involved in degranulation, encapsulation and phagocytosis

Hemocytes

Blood cell within the hemolymph of invertebrates that carries out immune functions

Hemolymph

blood equivalent in invertebrates; found in the hemocoel

Heterochromatin

fundamental structure of eukaryotic chromosomes consisting of nucleosomes with DNA wrapped around them. Nucleosomes are considered the basic packing unit of DNA and are made up of four histone dimers: H2A, H2B, H3 and H4. It is densely packed and generally considered transcriptionally inactive

IMD pathway

humoral immune pathway that responds to the presence of Gram negative bacteria; induces the secretion of antimicrobial peptides

Immune priming

term used in ecological immunology to describe previous microbial exposure that enhances resistance and survival upon subsequent challenges. Functionally analogous to “trained immunity”

Innate Immunity

immune response characterized by the presence of receptors that are encoded by intact genes inherited through the germline. Historically, believed to not have a memory or specificity component

Innate Immune Memory

Term used in the mammalian immunity literature where innate immune cells show increased response during pathogen re-exposure

JAK/STAT pathway

Janus kinase/signal transducer and activator of transcription; immune pathway triggered by the endogenous cytokine, Unpaired (Upd); involved in the arthropod antiviral response

Lamellocytes

large, flattened cells involved in encapsulation of foreign objects

Malpighian tubules

excretory system of insects involved in osmoregulation and removal of nitrogenous waste

Melanization response

process that is catalyzed by phenoloxidase; results in the deposition of melanin and the production of toxic molecules

Metabolic Reprogramming

change in metabolic function by a cell, tissue or an organism; often related to some form of plastic response to biotic and/or abiotic forces

Oenocytoids

large blood cell involved in the release of prophenoloxidase

Oocyte

immature egg

Ookinete

motile zygotic *Plasmodium* parasite that traverses mosquito gut lining

Plasmatocytes

macrophage-like cells involved in phagocytosis and encapsulation; major constituent of the hemolymph

Resistance

measure of an arthropod vector to reduce microbial infection or numbers of microbes

Resource allocation

distribution of stored energy to physiological and reproductive functions within an organism

RNAi

antiviral immune pathway that responds to double-stranded RNA from viruses

Symbiont

an organism living in very closely associated with another; typically, a mutually beneficial relationship

Trained immunity

immunological memory conferred by innate immune processes

Tolerance

the slope or reaction norm of the relationship between microbial burden and health or fitness of the organism

Toll pathway

humoral immune pathway triggered by Gram positive bacteria and fungi that leads to the production and secretion of antimicrobial peptides

Transgenerational Immune Priming

The transfer of primed protection from a microbe-exposed parent to his or her offspring that results in improved resistance upon infection with the same microbe

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Box 1:**Immune signaling in an ecological context.**

What does an optimal system look like? The answer is, “it depends.” Mounting an immune response is balanced against the costs of rogue-self (*e.g.*, cancer) and pathology induced by microbes over an evolutionary time. We categorize the major types and modifiers of immune costs:

Energetic costs:

A variety of resources is necessary to fuel the rapid creation of properly folded proteins and cellular structures associated with cellular and humoral immunity. These resources are generally mobilized from energetic stores, where they would otherwise be allocated to development and reproduction.

Immunopathological costs:

Immune responses that kill non-self can also induce collateral damage to the arthropod’s own tissues, reducing their functionality or requiring the expenditure of energy for self-repairing.

Multiple front costs:

Immune responses that are effective at controlling one type of microbe could facilitate the colonization of others. This phenomenon can occur either directly, as seen with antibody-dependent enhancement of disparate dengue virus serotypes, or indirectly, by stimulating negative feedback on other arms of the immune system [111].

Life stage:

Arthropods may vector a disease only in one stage of their life or throughout their ontogeny. However, all life stages are exposed to microbes. Exposure rates and relative investment in immune responses will be defined by the ecological context. This is illustrated by the aquatic larval and terrestrial adult stages of the mosquito, and by differences in the ability of different life stages to utilize immune pathways [110].

Environment:

Temperature, humidity, seasonality, and associated environmental factors shape the growth and other life history features in both the arthropod and the microbe.

Box 2:**Apples and oranges: model organisms out of context.**

Organisms that reproduce quickly and have available a wide range of genetic tools are unique. They can be used to gather evidence and study biological processes in a short period of time. Yet, the diversity of immunological processes may be overlooked or become lost when concepts derived from these systems are strictly applied to non-model organisms. For instance, most microbes used to challenge the *Drosophila* immune system have not co-evolved and are not capable of being transmitted by this organism. This is in stark contrast to studies done in arthropod vectors where microbes are transmitted, but do not typically kill these species. Thus, differences in immune networks are being increasingly recognized between *Drosophila* and other arthropods. Model organisms will remain critical for obtaining novel mechanistic insights in medicine. However, more attention should be given to the type of immune response mounted (*i.e.*, resistance versus tolerance) and the ecology and life cycle of the arthropod being analyzed. The establishment of generalized principles in immunology with just a few model organisms is a recent phenomenon in science, centered on the development of genetic manipulation. However, given the relative lack in understanding of how tolerance, resistance and immune deployment affects vector competence, it would be wise consider the context of the experimental design in *Drosophila*. It would also be prudent to contemplate the genetic diversity, biotic and abiotic factors that affect the immune response in arthropod vectors outside laboratory settings

Outstanding Questions

Do arthropod vectors show different dynamics of inducible immunity after infection in natural versus laboratory settings? To what extent do abiotic factors (*e.g.*, temperature) relative to biotic factors (*e.g.*, microbes: pathogenic and commensal) contribute to the observed variation?

How does the extent of co-evolutionary history between vectors and pathogens affect tolerance and resistance to microbial infection?

Are arthropod vectors unusually tolerant of microbial infection relative to arthropods with complex life cycles that do not vector human pathogens?

In what way does intermittent versus prolonged feeding influence immune response in hematophagous arthropods? Does intermittent and/or prolonged feeding influence vector competence?

To what extent are cell signaling mechanisms conserved among traditional model organisms and evolutionarily distant species?

Does natural variation in metabolic traits and resource allocation among arthropods predict microbe killing rates during infection?

Are the mechanisms of “trained immunity” observed in mammals evolutionarily conserved in arthropod vectors? To what extent does each fulfill the criteria of longevity, specificity, and biphasic inducibility typical of traditional adaptive immune responses?

Figure 1. Arthropod immune signaling pathways.

The Immune Deficiency (IMD) pathway responds to DAP-type peptidoglycan (DAP-PGN) from mostly Gram-negative bacteria. DAP-type PGNs are recognized by transmembrane peptidoglycan-recognition proteins (PGRPs), leading to the translocation of the nuclear factor (NF)- κ B-like factor, Relish, to the nucleus and upregulation of antimicrobial peptides (AMPs) [52]. AMPs are small cationic, amphipathic peptides that kill invading microbes by disrupting the pathogen membrane [6]. The Toll pathway responds to lysine-type peptidoglycan from Gram-positive bacteria and β -glucans from fungi. Recognition leads to a proteolytic cascade that cleaves the endogenous cytokine-like protein, Spätzle (Sptz). Spätzle binds to the Toll receptor, causing the translocation of NF- κ B-like factor Dorsal (or Dif) to the nucleus and the expression of AMPs [9]. The janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway responds to the endogenous cytokine unpaired (Upd) that is secreted by hemocytes in response to infection. Binding of unpaired to the Dome receptor leads to dimerization and translocation of STAT to the nucleus and production of AMPs [112]. The RNA interference (RNAi) pathway recognizes double-stranded RNA (dsRNA) derived from invading viruses. dsRNA is cleaved by Dicer and then loaded onto the RNA-induced silencing complex (RISC). The small interfering (siRNA)-RISC complex induces an anti-viral state by binding to complementary RNAs through base-pairing, causing cleavage and degradation of viral RNA [113, 114]. Excellent reviews discuss these mechanistic processes elsewhere [7, 9, 112, 114, 115].

Figure 2. Resistance and tolerance to infection in arthropod vectors.

Two vectors from populations that differ in their tolerance to microbes will have the same fitness, proxied by survival and reproduction, at different microbe densities. Thus, the less tolerant mosquito (orange) needs to invest more in resistance to maintain that level of fitness relative to the more tolerant vector (blue). Higher tolerance can facilitate greater transmission through allowing more microbes to persist for longer, and/or muting the effects of pathogenicity on host survival, lengthening the transmission period. Circles highlight the placement, in microbe density by fitness space, of the two vectors pictured above.

Figure 3. Contextual pathogenicity in arthropod-borne pathogens.

When microbes transmitted by arthropods cause disease in humans, they are often referred to as pathogens. Biotic and abiotic factors determine whether the microbe is virulent to arthropods. For instance, *Anaplasma phagocytophilum* and *Borrelia burgdorferi* likely play an important role in tick immune education and fitness [43–46]. Conversely, the tick bite favors a more pathogenic role upon microbial transmission because of the different genetic landscape and the host immune status. These considerations underwrite contextual pathogenicity, where microbes that humans often consider virulent, may not be harmful to arthropod vectors.

Figure 4. Molecular mechanisms of trained immunity.

Innate immunity can induce immunological memory through mechanisms that are distinct from adaptive processes. Training the immune response results in a shift from oxidative phosphorylation (OxPhos) to glycolysis to rapidly produce energy in the form of adenosine triphosphate (ATP). This metabolic shift may remain active for several days within the cell after the stimulus has been removed or cleared. Metabolic shifts induce epigenetic changes to alter expression of targeted genes. Common epigenetic marks associated with trained immunity are trimethylation (me3) and acetylation (ac) events on lysine 4 and 27 of histone 3 [21, 60, 71, 116]