The within-host ecology of insects and their parasites: integrating experiments and mathematical models

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Running Title: Integrating experiments and models

Abstract

The within-host ecology of hosts and their microbes involves complex feedbacks between the host immune system, energetic resources, and microbial growth and virulence, which in turn affect the probability of transmission to new hosts. This complexity can be challenging to address with experiments alone, and mathematical models have traditionally played an essential role in disentangling these processes, making new predictions, and bridging gaps across biological scales. Insect hosts serve as uniquely powerful systems for the integration of experiments and theory in disease biology. In this review, we highlight recent studies in fruit flies, moths, beetles and other invertebrates that have inspired important mathematical models, and present open questions arising from recent modeling efforts that are ripe for testing in insects.

Introduction

Insects provide tractable systems to test hypotheses on host-parasite interactions, immunity, and disease at multiple ecological and evolutionary scales. The benefits of testing theories on insects stem from their short generation time, ease of keeping and breeding as well as their relatively small body and genome sizes. For a vast range of species, we have in-depth knowledge of their biology, including their immune systems and life history [1]. These insights have given us tools to understand and even sometimes manipulate insect population and disease dynamics in the spheres of agriculture, health, and ecosystem services [2, 3].

However, insects are different from vertebrates – and especially mammals – in almost every aspect of life. Insects can be eusocial, and many undergo a dramatic transformation of morphology during metamorphosis. They are ectothermic, relying on conditions in the external environment for developmental and metabolic processes. Though they lack the classical adaptive immune system found in vertebrates, they benefit from a robust and multi-faceted innate immune system [4] capable of generating an alternate form of immune memory [5, 6]. These immune systems face a vast diversity of parasites, many of which use obligate-killer and vertical transmission modes not commonly observed in mammalian parasites [7]. As a result, mathematical models of infection dynamics designed with vertebrates in mind rarely capture the salient biological processes of insect systems. Fortunately, the unique tractability of insect systems allows continuous iteration between experimental and theoretical approaches to understand host-parasite interactions at multiple levels of biological organization.

In this review, we discuss some case studies of within-insect host dynamics, infection outcomes, and transmission that have singularly benefited (or are ripe to benefit) from the integration of experiments and theory. While work in disease vectors has long adopted this approach, the associated literature is reviewed elsewhere (e.g. [8, 9]), and so here we highlight other systems relevant to evolutionary ecology and infection biology.

Within-host dynamics

Scientists have long recognized that the magnitude of an immune response can spell the difference between infected host survival or death, as supported by innumerable experiments in insect strains sporting immune gene mutations [1]. These experiments have also suggested that many infections in insects are a race against time between host control of microbial proliferation and the microbial production of virulence factors that suppress the immune system and pathologically destroy host tissue (**Fig. 1**) [10].

A recent study in *Drosophila melanogaster* added quantitative teeth to these observations by demonstrating that, regardless of pathogenic bacterial strain, microbe loads bifurcate over the acute infection phase into predictable trajectories of high load (and host death) or low-level chronic infection [11] (e.g. **Fig. 1**). The authors proposed that variation in the relative frequency of these outcomes could be described mathematically and predicted by a parameter describing the time until onset of immunological control of the infection [11]. The subtle manipulation of this parameter is not trivial from an experimental perspective, as it would require small quantitative changes in immunological signaling rather than the blunter loss-of-function

phenotypes accomplished by genetics or RNAi methods. Moreover, there is no guarantee that any given gene target would ultimately alter the rate of immunological induction in the early phase of infection (e.g. **Fig. 1** parameter "*c*") rather than, for example, the magnitude of effector production when the host is already destined for death.

In cases like these, mechanistic mathematical models can help to sort through competing hypotheses and identify targets for experimentation, as exemplified by a new modeling study that explains within-host bifurcation trajectories and the persistence of chronic infection in insect hosts [12]. The modeling results suggest that mutual negative feedbacks between the immune response (e.g. antimicrobial peptide (AMP) production) and the production of immunosuppressive molecules (e.g. proteases) by microbes are required to explain the alternative trajectories. The results demonstrate that small differences in initial conditions, including constitutive immunity and bacterial dose, are sufficient to produce bifurcation in infection outcomes, as is modest variation in parameters describing the kinetics of inducible immunity. The model further predicts that the observed chronic infections are only possible if the protease production trades off with microbial growth rates.

Many of the model predictions are experimentally tractable and ripe for testing. For example, the modeling results suggest that bistability is only possible if the host responds immunologically to bacterial levels substantially below the lethal load. Previous experiments in insects have already demonstrated variation in immunological sensitivity to microbe density [13-16], providing evidence that this might be an ecologically relevant variable in nature. This prediction could be directly tested using a microbial dose-response approach, or by targeting particular nodes in immunological pathways predicted by more complex mechanistic models (including an dynamic model of IMD pathway signaling presented in the supplement to [12]) to alter sensitivity specifically. Finally, if the bifurcation dynamics do depend on negative feedbacks from the microbe on host immunity, then experimental manipulation or ablation of specific microbial proteases should alter the within-host trajectories in predictable ways.

While the crucial first few hours of host-pathogen interactions likely determines dynamics of particularly virulent acute infections, the availability of energetic resources plays an important role in directing within-host trajectories of many other insect host-symbiont associations [17, 18]. There have been several modeling approaches aimed at capturing the within-host ecology of immunity and parasites, often employing a dynamic energy-budget framework to connect host resource acquisition, allocation to different life history traits, and exploitation by parasites [19]. An open question, however, is whether parasites and the immune system are actually competing for the same resources or using different ones, and how the competition mode influences the role of resource acquisition on infection outcomes. To explore this question, Cressler et al. [20] built a set of alternative models using ordinary differential equations to allow immunity and parasites to directly compete for the same resource pool, allow immunity or pathogens priority over resources, or force them to draw from independent energy reserves. The results suggest that, as resource acquisition increases, pathogen load will increase under a direct competition scenario but decrease if the immune system gets priority over resources. These predictions were complemented by a literature search of empirical studies that

connect resource acquisition to parasite load. The results suggested that vertebrate animals tend to have lower loads as food increases, favoring the immune priority model, while an increase in food acquisition by invertebrates was most frequently associated with an increase in parasite load [20].

Are insect immune systems more likely than vertebrates' to directly compete with parasites for the same energetic resources? This is actually a difficult question to answer because it requires knowledge of the metabolic needs of different arms of the immune system and the particular parasites under investigation. Recent experimental studies are starting to resolve these issues, allowing us to inch closer to connecting dynamic energy budget-style models with empirical observations. For example, one study in *D. melanogaster* identified a metabolic switch, initiated by parasitoid infection, that goads the fat body into mobilizing the carbohydrate trehalose to fuel the cellular immune response [21]. Turning off this switch rendered the host more susceptible to parasitoid infection, suggesting that in this scenario at least, the immune response enjoys priority over competition for trehalose. Future work that quantifies the metabolic needs of hosts and microbes, for example by manipulating specific metabolites within the host or *in vitro*, would allow direct testing of model predictions.

A central challenge still facing our understanding of within-host dynamics in insects, from both theoretical and empirical perspectives, is temporal structure. Models of within-host dynamics frequently assume that there are constitutive and inducible components to immunity, with the latter generated only after recognition of infection [22, 23]. We know from experiments that the inducible response, potentially to minimize energetic and immunopathological costs [24], is not just turned on all at once, but layered over time and in response to variable cues like microbe density and damage [13]. In bumblebees (*Bombus terrestris*) and mealworm beetles (*Tenebrio molitor*), for example, activated phenoloxidase appears to be depleted within minutes of an immune challenge and not subsequently upregulated, while AMPs are induced gradually but steadily after infection [25, 26]. When juxtaposed with microbial temporal structure, including colonization bottlenecks [27], density-dependent quorum-sensing [28], and other forms of plasticity, the relative importance of a particular immune effector for infection outcomes can change rapidly. We recommend that future models of immune system dynamics and evolution account for the constraints, costs and benefits associated with the temporal structure and regulation of multiple immune effectors.

Connecting within-host to between-host dynamics

Since Anderson and May demonstrated with a simple model that baculoviruses could plausibly act as key regulators of moth population cycles [29], a large body of work integrating experiments and theory has embraced insect systems to answer fundamental questions in disease ecology. Currently, one of the most intriguing research avenues in the field is to understand how differences among individuals, for example due to immunological variation, infection history, sex, or life stage, scale up to influence disease transmission and prevalence in host populations. Given the tractability of their generation times, population sizes, and ease of manipulation relative to vertebrates, insects provide ideal systems for conducting carefully controlled experiments that integrate within- and between-host processes.

Moths and their baculoviruses remain central to this effort. Empirical work in the gypsy moth (*Lymantria dispar*) and the Indian meal moth (*Plodia interpunctella*) has demonstrated that host susceptibility and transmission potential both depend on the larval instar of the host [30, 31], reinforcing the need for subsequent mathematical models of insect disease dynamics to account for life stage [32]. A recent study in the gypsy moth employed stochastic modeling of empirical data to demonstrate that both inter-host variation and viral demographic stochasticity can explain observed variation in infection outcomes [27], emphasizing that deterministic models alone may not be sufficient to capture relevant dynamics. Studies in these systems have also integrated experiments and theory at multiple spatial scales to demonstrate that the behavior of moth individuals can influence disease dynamics through structuring contact networks [33], and that local networks cannot always explain disease dynamics at larger scales [34]. These insects are likely to remain powerful model systems for connecting disease dynamics across biological levels of organization due to their tractability, natural history, and tradition of attracting researchers interested in the marriage of theory and experiments.

The naturally occurring symbionts of traditional model insects have also lately inspired integrative studies connecting within- and between-host dynamics. For example, recent work using *Drosophila C* virus in *D. melanogaster* took advantage of its enviable genetic resources to demonstrate variation in susceptibility and viral shedding dynamics of hosts from different genetic backgrounds and sexes [35, 36]. Stochastic individual-based models inspired by this data suggest that individual heterogeneity can substantially alter the subsequent dynamics of outbreaks in theoretical fruit fly populations [35]. Moreover, insect hosts are commonly infected with more than one type of parasite at a time, and the within-host interactions among parasites, including facilitation or antagonistic competition, can alter infection outcomes for both hosts and parasites. Inspired by parasite experiments in flour beetle model systems (*Tribolium* spp.) [37-39], a recent modeling study [40] bridged ecological scales to explore the impact of within-host interactions among parasite species (e.g. bacteria and protozoa) on disease transmission and competition dynamics among beetle host species. The results suggest that asymmetrical mortality rates among coinfected host species could alter the transmission of each parasite, flipping competitive exclusion outcomes from one host species to the other [40]. Finally, while water fleas (*Daphnia* spp.) are not insects, the extensive body of integrative empirical and theoretical studies in this system have enriched our understanding of concepts – e.g. susceptibility [41, 42], parasite transmission modes [43], and multiple infections [44, 45] – directly relevant to connecting within- and between-host dynamics in insects.

Conclusions

While we have focused here on the ecology of host-parasite interactions, insects and other invertebrates have also served as powerful models for the evolution (e.g. [46, 47]) and coevolution (e.g. [48-51]) of hosts and symbionts. Insects offer unique and alluring advantages to both modelers and experimentalists, including a simpler (though still not simple) and tractable innate immune system, an intriguing distribution of symbiont transmission modes not seen in vertebrates, and ecological breadth and diversity abetted by metamorphosis. Their importance as agricultural pests and pollinators, disease vectors, and contributors to ecosystem services adds

instant applied relevance to basic research on insect disease ecology. As we attempt to scale within-host dynamics from controlled laboratory conditions to multi-host, multi-parasite natural systems, the tight integration of theory and experiments will be essential to make sense of complex interactions and outcomes at different levels of biological organization.

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Figure 1: Mathematical models help formalize empirical observations of within-host processes to explain observed variance in infection trajectories and transmission. The parameters associated with variation in infection outcomes among hosts (solid = recovered, dashed = infection-induced mortality, red = microbes, blue = immunity) can be expressed mathematically. For example, rates of microbial growth (*a*), immune-induced microbial death (*b*), microbe-independent (*c*) and microbe-dependent (*d*) inducible immune induction, microbial suppression of immunity (*e*) and negative feedback on immunity (*f*) exert individual and combined effects on the slope and magnitude of microbial and immunological dynamics (as suggested by color-coded letter placement on trajectories over time), although many of these parameters affect multiple aspects of the within-host dynamics and are not mutually exclusive. Initial conditions, including levels of constitutive immunity and microbial dose (as represented by the intercept at time = 0), can also affect outcomes by altering the dynamics of host-microbe interactions early in infection. The connection between microbe load, infection outcome, and transmission rate depends on microbial transmission mode, as obligate killer transmission (common for insect pathogens) requires host death induced by high microbe loads, while host death would stymy the transmission of directly transmitted microbes.