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Seven challenges in modeling pathogen dynamics within-host and across scales

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ABSTRACT

The population dynamics of infectious disease is a mature field in terms of theory and to some extent, application. However for microparasites, the theory and application of models of the dynamics within a single infected host is still an open field. Further, connecting across the scales – from cellular to host level, to population level – has potential to vastly improve our understanding of pathogen dynamics and evolution. Here, we highlight seven challenges in the following areas: transmission bottlenecks, heterogeneity within host, dynamic fitness landscapes within hosts, making use of next-generation sequencing data, capturing superinfection and when and how to model more than two scales.

Introduction

Driven by new data sources and new questions, modelers are increasingly trying to link within-host dynamics to population-level dynamics using cross-scale models. This is a time-honored tradition in macroparasite models, where the link between within-host parasite abundance and transmissibility cannot be ignored, but it is much less well-developed for microparasites. Here we outline some of the current and future challenges in within-host and cross-scale modeling of microparasites. We are focusing in particular on the within-host questions that may inform cross-scale dynamics.

1. New models and new data to elucidate the processes underlying transmission probabilities and bottlenecks

Transmission is the defining characteristic of infectious diseases, and is the fundamental point of contact between within-host and population-scale models. Despite considerable attention in recent years, there are major outstanding challenges in linking within-host dynamics to probabilities of transmission, and understanding how transmission events seed the dynamics in a newly infected host.

Focusing first on the donor host, how does infectiousness (interpreted as probability of infection given a contact) depend on pathogen load? This relationship is crucial to linking scales but as yet little understood. The experimental literature gives some insight from dose–response experiments with particular pathogens, but often an insufficient context is given for generalization, combined with routes of exposure that do not represent what happens naturally (e.g., virus injected under the skin rather than intranasal exposure for respiratory pathogens) limit utility. Experimental transmission studies in quasi-natural settings such

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as contact transmission of mammalian influenza offer clear potential to enrich our understanding, if appropriate measures of viral load are taken (Imai et al., 2012; Murcia et al., 2010). Valuable insights have been gained from studies of HIV-1 in discordant couples (Gray et al., 2001), but it is not clear that these can be applied to acute infections or different transmission routes. Overall, it would be desirable to identify classes of functional relationships – and to understand when and why these relationships apply. This requires consideration of how pathogen load in the sampled body site links to pathogen excretion by the relevant route(s), and then how a given excreted load relates to the probability of establishing a new infection.

Next, focusing on the recipient host, initial infection is an invasion process across particular cell and tissue types, depending on the pathogen. The pathogen population is seeded by a given dose, route of transmission and period over which exposure occurs. Here, stochastic and spatial invasion models may offer insight into important mechanisms of establishment, and could be used to make inference from available data. Detailed spatial knowledge of infection initiation *in vivo* may be firmly out of reach for most systems, but insights may be gained from *in vitro* experiments combined with stochastic spatial models (Howat et al., 2006). An example of a basic question is whether the infectious particles in a given dose operate independently from one another, such that the effect of dose is easily calculated from probabilistic considerations, or whether some interactions arise through cooperativity, local saturation of immune response or target cells, or other mechanisms (Wood et al., 2014; Zwart et al., 2009). Another example: invasion models could help in understanding the basis for the phylogenetically derived result that most HIV-1 infections are founded by a single virus (Keele et al., 2008). Is this because infection events are very rare or because one of many infecting lineages wins out in early competition?

All of these processes converge to determine the probability of infection and the number and diversity of pathogen particles transferred to the newly infected host, i.e., the transmission bottleneck. The transmission bottleneck is vital to coupling within-host models to between-host models, which will be particularly important when considering pathogen evolutionary dynamics.

Mathematical and computational models may also play a role in designing experiments to explore bottlenecks. Infection experiments both *in vitro* and *in vivo* are often challenging and the number of replicates may be limited, so it can be extremely valuable to use models in advance to help plan the most informative approaches. For example, in transmission experiments involving infection with isogenic tagged pathogens (Coward et al., 2008), models can make use of preliminary data and assumptions to suggest the range of doses, mix of strains and sampling times to gain the most information on the size of the bottleneck.

2. Heterogeneity within a single host

Many within-host models treat the host as a single population of target cells without any structure, as if we are well-mixed uniform cell cultures. This is obviously not the case in practice, and heterogeneities in cell type, cell demography, immune response, and the spatial structure of the host will all play important roles in shaping the dynamics of infection. Like population ecology, while the homogeneous models come first and reveal many key ideas, more detailed models are less tractable but for some phenomena may prove essential in understanding the full dynamics.

We know from population dynamics that spatial structure can fundamentally change the range of possible system behaviors (Bolker and Grenfell, 1995). For a within-host model, space can be anything from host-scale, e.g., a metapopulation where sites are different organs, down to fine cell-to-cell transmission, e.g., a lattice of epithelial cells. For example in influenza A in mammals, infection dynamics may involve small distinct foci of infection in the respiratory epithelium (Saenz et al., 2010). Broad-brush non-spatial compartmental models may be successful in capturing general dynamics when the biological readouts for comparison are themselves broad, such as antibody levels in the blood (Handel et al., 2010). However the spatial organization of the respiratory system must be modeled to understand the selective pressures on the virus from tissue tropism and hence, crucially, transmission rates between hosts (Reperant et al., 2012). Study of chronic viruses from HIV-1 (Sanjuán et al., 2004) to Plum pox virus (Jridi et al., 2006) has revealed population genetic structure among different tissue compartments; there are many open questions regarding how such structure influences transmission and evolutionary dynamics, across pathogen and host types.

While the impact of the pathogen on the host cell population is usually considered, the effect of the host cell demographics on the pathogen dynamics are often overlooked, again in common with population dynamics. This may be as cellular dynamics (other than those driven directly by infection) are assumed to be irrelevant on the timescales considered, or too hard to capture in terms of mathematical tractability or lack of suitable parameterization, or even the lack of knowledge of a plausible form of the dynamics. However again there are known examples where details of target cell demography shapes pathogen dynamics. For example, the recruitment rate of red blood cells is a crucial driver of malaria parasite abundance (Metcalf et al., 2011). For chronic viral infections, the probability of *de novo* drug resistance depends on the interplay between appearance of new resistant mutants and time needed for host cell regrowth (Alexander and Bonhoeffer, 2012).

The issue of how much detail of the immune system to include is more specific to current within-host models of infection. Any attempt to construct a comprehensive model of all known varieties of immune cells, secreted proteins and signaling molecules, as well as the immunodynamics of target cells, will be certainly doomed: the models will be intractable, unparameterisable and almost certainly will misrepresent some immunological subtleties. Conversely, entirely neglecting any form of adaptive or innate immunity (as is often done in within-host models) may be appropriate for some questions and applications, but often the core of within-host infection kinetics is found in the dynamic interplay between the pathogen and the immune system. This is most apparent in chronic infections such as HIV (Novak and May, 1991) but even in acute infections, ignoring any form of dynamic immunity can give misleading results, such as infections only being resolved by total target cell depletion (Saenz et al., 2010). When can the immune system be modeled in a simple way such as target cells being able to move into a protected antiviral state, and when do we need to grapple with fuller detail of immune dynamics? Are there generalities here, perhaps according to timescale, pathogen type, or something else?

3. Dynamic fitness landscapes

A 'fitness landscape' is essentially a mapping from a pathogen's genotype, to its reproductive phenotype. Here, 'fitness' can be seen either in replication terms (within-host) or as a transmission fitness (at the population level). Fitness landscapes are important drivers of natural selection: an understanding of how and why they come about would therefore play an important role in understanding the forces shaping pathogen evolution. These ideas are closely linked with issues described in challenge 2, above, but over the course of infection the landscape changes, hence dynamic fitness landscapes.

Current models of within-host evolution typically operate on a genotype space, adopting simplified scenarios for the corresponding fitness: for example, 'hill-climbing' versus 'fitness

valley' scenarios. However, there remains a need to address important cases where fitness landscapes change over time: a key example being evolution for immune escape. For HIV, replication fitness landscapes change on timescales shorter than the infectious period of the virus (da Silva et al., 2010). Meanwhile, the evolution of human influenza is clearly shaped by population immunity, but there are indications that the immunity built up over a host's lifetime is more complex than is often assumed (Lessler et al., 2012).

Modeling these landscapes in their full mechanistic detail would arguably be neither feasible nor helpful, given the prohibitively high dimensionality of the immunological, virological and genetic spaces involved (Kouyos et al., 2012). Therefore, a key challenge will be to narrow this space to more manageable proportions (e.g., as suggested in Bedford et al., 2012). Could transmission fitness landscapes ever be studied in a laboratory setting? The ability to do so would generate a wealth of biological data for understanding these landscapes, e.g., under different conditions of immunity. Achieving better correlates of transmission (see challenge 1, above) could pave the way for such experimental approaches in future.

4. Interfacing models with deep-sequencing data

Empirical studies have demonstrated for both acute (Murcia et al., 2010; Hughes et al., 2012) and chronic infections (hepatitis C, HIV, SIV), that there is massive genetic variation of viral pathogens within infected hosts. Recent evidence shows that this is true for *Plasmodium* as well (Manske et al., 2012). In some cases this genetic diversity is transmitted, and in other cases it is not, so the bottleneck width is itself not constant. Also interesting is the speed at which the most common variant, or the consensus pathogen variant can establish itself within- and then between-hosts. For HIV, these processes may take days or weeks, whereas very limited data for mammalian influenza suggest that this can happen in 24 h (Murcia et al., 2010). The implications of this for scaling from within host, to between host, to global phylogenies remain essentially unexplored.

Quantitative methods have been established for epidemic level, or global level phylogenetic data, typically based around consensus sequence of pathogens, and some methods also exist for the contact tracing analysis of well sampled epidemics, but there seem to be no well established methods for analysis of pathogen deep sequence data that use the richness of deep sequence data to allow inference about the epidemic and evolutionary characteristics of the transmission process in question. This should be a massive frontier for within-host modeling, since in principle the data provide a new and higher-resolution window into within-host and indeed between-host dynamics. But how should we judge which apparent signals can be trusted (McKinley et al., 2011)? What are robust signals that can be extracted? How should these hugely high-dimensional data be represented in models? What are the generalities here? Further deep sequencing data will surely challenge our understanding as we see new patterns which do not fit our current theory.

5. When and how to model superinfection

Superinfection is defined as the introduction to a host, after an infection that has triggered some immune response, of a second (heterologous) strain (Smith et al., 2005). Ignoring superinfection greatly simplifies model analysis as it allows a modeling framework based on the concept of time-since-infection, which allows the application of the well-developed theory of next-generation matrices to the study of endemic equilibria (Lythgoe et al., 2013) and epidemic final size.

It remains unclear, however, when neglecting superinfection is a valid approximation and what its implications are. Superinfection is known to occur in HIV infections, where it could potentially increase viral load and hasten progression to AIDS (Korenromp et al., 2009), although biological mechanistic explanations and reliable quantification are still lacking. High entomological inoculation rates typical of malaria suggest opportunities for multiple infections, which can affect within-host strain competition and alter dynamics and outcome. In the case of TB, there is an ongoing debate about relative importance of reinfection *versus* reactivation, but perhaps modeling will help disentangle these processes (Gomes et al., 2012).

In addition, even in contexts where superinfection is shown to be vital to understanding the observed dynamics, the challenge would remain of how to infer superinfection rates from obtainable data. The growing body of sequence data (both across hosts and in deep sequencing within a single host) may open the possibility of inferring recombination and reassortment rates, though elucidating how they connect to superinfection rates still remains an open challenge.

6. When to use more than two scales

While the bottleneck of transmission naturally distinguishes within- and between-host dynamics, it must be recognized that both of these scales involve further nested levels of organization, e.g., from cell to tissue to host, or from household to city to country (see other papers in this issue).

Multi-scale models at the population level are common, mostly because of the clear impact of spatial heterogeneity in human and animal population on disease spread (see Ball et al., 2015) and because data can be collected relatively easily. However, these models generally ignore the within-host components.

On the other hand, within-host multi-scale models are less common mostly because so little has been measured regarding between-cell or organ infection rates *in vivo*. However, microbiologists and virologists have well-resolved understanding of many key processes at cellular scales, such as genome replication or virion assembly, and if we hope to develop more mechanistic models of how genetic diversity is generated then we will need to consider molecular interactions. There is a need to synthesize these processes (e.g., modeling the cell cycle of a virus) to understand whether our simpler representations are accurate enough (Loverdo et al., 2012). To do this, there is an important need for highly resolved empirical data about the dynamics of cellular-scale processes.

Some models involving more than two scales exist (Metzger et al., 2011), but this area is quite underdeveloped. A key challenge, in common to any other case of dramatic increase in model complexity, is to understand when the inclusion of another scale is relevant in gaining more insight or is motivated by a sufficient increase in model accuracy. The answer to such a question is of course dependent on the pathogen under consideration, the data available, and the particular question at hand.

7. Approaches to linking processes across scales

In general we cannot (or do not wish to) model multi-scale processes in full mechanistic detail, and even simulating such models becomes computationally intractable. Can we come up with ways of extracting the essence of lower-scale models so that they can be embedded into higher-scale models efficiently (Mideo et al., 2008)? When are these approaches safe to use, in that they give representative results?

One approach that has yielded success is separation of timescales, where essentially separate models may be used for different scales. For example, embedding a Markov chain model at the within-host level in a stochastic branching process for

between-host transmission can help capture evolutionary dynamics of pathogens (Park et al., 2013): here the separation of timescales is that the fate of each new mutation is determined before the next mutation arises. Another example of a within- and between-host dual-scale model is the application to immune waning and vaccination in measles (Heffernan and Keeling, 2009): here the smaller scale model is simplified to make the dual-scale model tractable.

The challenge is to develop better methods for incorporating two or more scales into a single framework. We might look to population-level epidemiology, for example the approach of embedding a full solution for a single season's influenza epidemic into a multi-annual model for population dynamics and viral evolution (Boni et al., 2004; Andreasen and Sasaki, 2006). Or we may be able to adapt approaches from other areas of biological modeling, for example creating a "look-up" directory for the small scale interactions between small swimming organisms to computationally model a large population (Ishikawa et al., 2006). There may well be other approaches from completely different scientific areas, particularly those which also have an extensive history of mathematical modeling and also share the need to work with multiple scales, for example climate and meteorological dynamics. Cross-seeding across areas should yield valuable new directions for multi-scale modeling of within-host pathogen dynamics.

Summary

Clearly it is possible to incorporate more scales and finer detail regarding transmission bottlenecks, geography, cell types, immunodynamics, and demographics within host. The large open challenge is to do so in a way that is (a) shaped by empirical data, (b) consistent with current biological knowledge, (c) mathematically and/or computationally tractable, and (d) appropriate in complexity for the questions under investigation.

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