

Linking within- and between-host dynamics in the evolutionary epidemiology of infectious diseases

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Nested models (also called embedded models) explicitly link dynamical processes that occur at different scales. Recently there has been considerable interest in linking within- and between-host levels of disease dynamics in the study of pathogen evolution. Here we review the extent to which these nested models have increased our understanding of pathogen evolution. We suggest that, although such models have been useful for determining the nature of tradeoffs between epidemiological parameters and for evaluating the consequences of conflicting selection pressures at different scales, the vast majority of previous results could likely have been obtained without the use of nested models *per se*. Nevertheless, these models have proven very useful through their highlighting of the importance of within-host disease dynamics on pathogen evolution.

Introduction

Biological processes occur at several nested levels of organization. Biological communities are composed of interacting species, and these species are composed of interacting individuals with differing phenotypes. The phenotype of each of these individuals is affected by the dynamics of their component physiological systems, and these systems are affected by smaller-scale biological processes from the cellular down to the molecular level. One of the major challenges in evolutionary biology is to understand the evolutionary causes and consequences of these levels of organization [1].

The importance of nested levels of biological organization is particularly apparent in the evolutionary epidemiology of infectious diseases. Pathogens can have demographically significant effects at the level of the host population, as a result of their transmission among individuals (see Ref. [2] for a review of the importance of metapopulation structure in disease dynamics). At the same time, pathogen transmission is intimately tied to the expression of disease in infected individuals, and this disease expression arises from the within-host dynamics of pathogen replication and its interaction with host defense mechanisms. One important goal of evolutionary epidemiology is to understand how such nested processes affect the epidemiological and evolutionary dynamics of host–parasite interactions.

In this review, we discuss recent theoretical research on so-called nested dynamical models in the evolutionary epidemiology of infectious diseases. Our main objective is to evaluate whether or not the nesting of models *per se* has been important in developing our understanding of pathogen evolution. As will be seen, our answer is a qualified 'yes.' We conclude by pointing to some interesting open questions for which this modeling approach is likely to be useful.

What are nested dynamical models of pathogen evolution?

The focus of this review is on linking between- and within-host dynamics in the evolutionary biology of infectious diseases. Between-host models of infectious diseases follow the dynamics of disease spread at the level of the host population by tracking the number of susceptible and infected individuals of different types [3,4]. These models have been widely used to study the evolution of pathogens by allowing for multiple pathogen strains to circulate in the population, and then determining which strains are best able to persist.

Models for the evolutionary dynamics of infection at the within-host level have also been developed, and these typically track the dynamics of the pathogen density of different strains as well as the state of host defense mechanisms (e.g. density of lymphocytes) over the course of an infection. These within-host models of pathogen evolution

Glossary

Acute infections: infections that last for a short time; they typically are cleared by immune responses.

Between-host selection: selection on pathogens that arises by differential transmission success between hosts.

Case mortality: the probability of an infected individual dying from the disease.

Chronic infections: infections that persist indefinitely; they are typically not cleared by immune responses.

Life history: refers to an organism's age-specific survivorship and fecundity and, more generally, all the traits that determine the two.

Morbidity: symptoms of disease and disability due to damage done to a host by an infecting pathogen.

Nested or embedded model: a model that explicitly links the relationships between processes at different levels of biological organization. For infectious disease modeling, this generally involves linking between-host processes to within-host processes.

Recovery rate: rate at which the host recovers from infection.

Virulence: the host fitness reduction resulting from infection by a pathogen; this is often equated with disease-induced mortality rate.

Within-host selection: selection on pathogens that arises through differential reproductive success within an infected individual.

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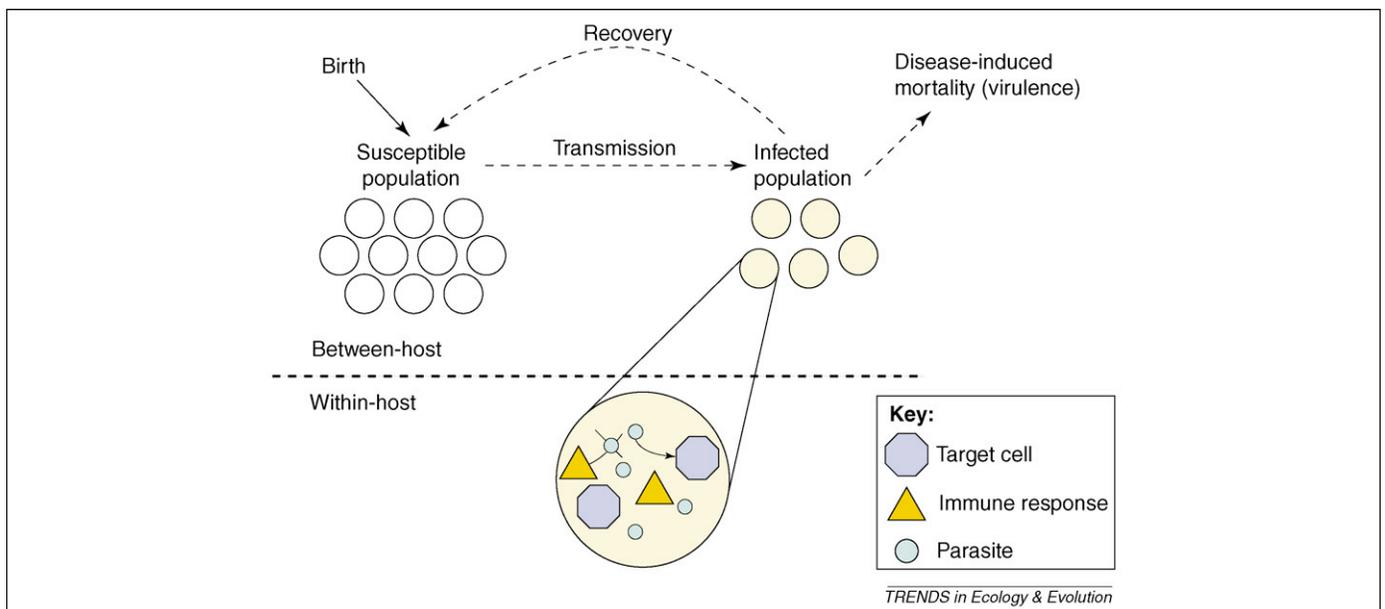


Figure 1. Schematic of a nested model. A basic nested model is constructed by defining a between-host model, in this case a simple susceptible-infected epidemiological framework, and linking parameters of that model to within-host dynamics. Some of the potentially important elements and interactions of a within-host model are highlighted.

have been used, in particular, to study rapidly mutating viruses such as HIV or hepatitis C, and to understand how within-host dynamics affect antigenic escape [5], viral diversity [6,7] and fitness evolution [8].

More recently, there have been several efforts directed toward nesting models of within-host dynamics into models of between-host dynamics when studying pathogen evolution (Figure 1; Box 1). These are what we refer to as ‘nested models’ in this review. Notice that this definition excludes models of multilevel selection for pathogen evolution in which there is no explicit dynamical model at the within-host level (e.g. models based on superinfection or

coinfection frameworks, e.g. [9–11], as well as kin selection models of pathogen evolution, e.g. [12]). We recognize that this distinction is somewhat ambiguous (e.g. models of superinfection implicitly rely on some form of within-host model), but it is the explicit nature of the within-host dynamics that sets recent work apart from other approaches.

It is also useful to distinguish two ways in which nested dynamical models might contribute to our understanding of pathogen evolution. The first, and most significant, would be if such models provided insights that were not possible without the explicit nesting of within- and be-

Box 1. Nested models

Here we outline a simplified example of how many studies construct nested models of within- and between-host dynamics. To account for changes in transmission, virulence and recovery rates during an infection, the between-host model must first explicitly track the age of each infection. If $S(t)$ is the density of susceptible hosts at time t and $I(a, t)$ is the density of hosts at time t that were infected at time $t - a$ (i.e. a is the ‘age of infection’), a simple susceptible-infected (SI) model is [3,54,55]

$$\begin{aligned} \frac{dS(t)}{dt} &= \theta - \mu S(t) - S(t) \int_0^{\infty} \beta(a) I(a, t) da \\ \frac{\partial I(a, t)}{\partial t} &= -\frac{\partial I(a, t)}{\partial a} - \alpha(a) I(a, t) \end{aligned} \quad [1]$$

and

$$I(0, t) = S(t) \int_0^{\infty} \beta(a) I(a, t) da,$$

where θ is a constant input rate of susceptibles, β is the transmission rate, μ is the constant host mortality rate and α is the loss rate of infected individuals through natural death, parasite-induced death (i.e. virulence) and recovery. Transmission, virulence and recovery are all assumed to vary with the age of the infection (a).

In addition to the between-host model (Equation 1), a within-host model can be constructed to follow the changes in parasite density $x(a)$, the state of the immune response $y(a)$ and any resource consumed by parasites $r(a)$ (e.g. red blood cells) as a function of the ‘age of the infection’ a . In the simplest cases, this gives a

dynamical model of the form

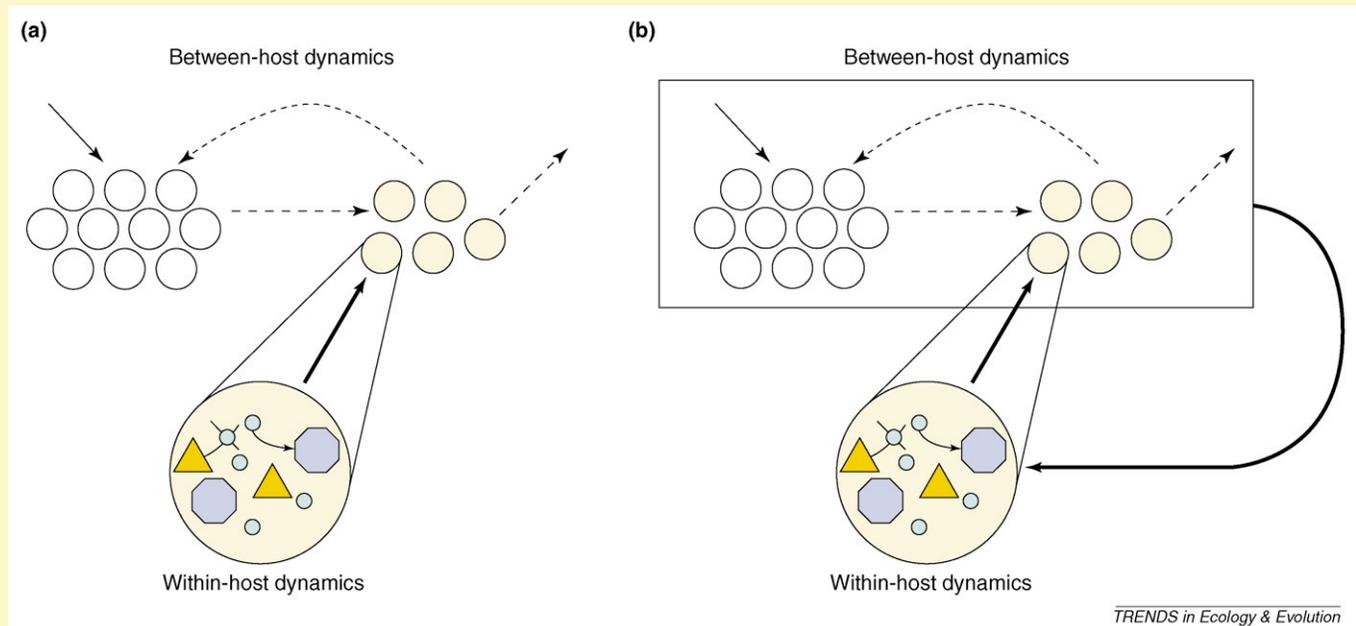
$$\begin{aligned} dx/da &= f(x, y, r, a) \\ dy/da &= g(x, y, r, a) \\ dr/da &= h(x, y, r, a) \end{aligned} \quad [2]$$

The within-host model (Equation 2) is then embedded or nested into the between-host model (Equation 1) by specifying a functional relationship between the transmission rate and mortality rate at infection age ‘ a ’ (i.e. $\beta(a)$ and $\alpha(a)$) and the within-host variables x , y and r [15–17,21,22]. This model can allow for evolution by introducing multiple pathogen strains by, for example, keeping track of the number of hosts infected with each strain (i.e. $I_j(a, t)$, where j denotes strain) and by extending the within-host model (Equation 2) to keep track of different strains as well [38,40,41]. In a similar fashion, other complexities such as differences in inoculum size or immune system states among hosts can, in principle, be incorporated by expanding the number of kinds of hosts in the model (Equation 1), each with their own form of within-host dynamics in the model (Equation 2).

Finally, some analyses rewrite the system in Equation 1 with a different notation, expressing the dynamics in terms of mean values of $\beta(a)$ and $\alpha(a)$ over the stable infection age distribution [18,32], but the underlying model is the same [46]. Some treatments further simplify the analysis, however, by assuming that the within-host dynamics (Equation 2) are fast relative to the between-host dynamics (Equation 1). As such, the within-host model is then assumed to remain at equilibrium, allowing the between-host parameters $\beta(a)$ and $\alpha(a)$ to be treated as constant during an infection [19,20].

Box 2. Inessential and essential nested models

To illustrate the distinction between the inessential and essential nesting of models, consider the following simple examples in Figure 1.



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Figure 1. Schematics of inessential and essential nested models. **(a)** Inessential. Within-host dynamics influence between-host processes but not vice versa. For example, suppose that parasite replication within a host determines the rate of transmission to new hosts as well as the rate of disease-induced mortality. Also, suppose that the availability of target cells and the immune cell dynamics determine the rate of host recovery. In this case, the interaction between levels of biological organization in this nested model occurs only in one direction – from the within-host to the between-host level. Using a nested model in this case allows for the relationships between within- and between-host processes to be defined mechanistically. Nevertheless, from the standpoint of the between-host dynamics, these relationships could just as well have been captured phenomenologically by choosing appropriate mathematical descriptions for transmission, virulence and recovery rate as a function of infection age, without any explicit reference to within-host dynamics. **(b)** Essential. A model in which nesting is essential is one whereby there is reciprocal feedback between levels of organization. Consider a disease that is transmitted via environmental spores. The within-host dynamics will determine transmission, recovery and mortality rates, and thereby will affect the between-host dynamics, including the number of infected individuals. This, in turn, will affect the number of infective spores in the environment, which might then alter inoculum size. If inoculum size influences the progression of disease within a host [48], this would then lead to a feedback from the between-host dynamics back down to the within-host dynamics. Nesting of models is therefore essential to capture this reciprocal, dynamical feedback.

tween-host processes. In particular, perhaps some aspects of pathogen evolution cannot be understood without considering a reciprocal feedback between processes occurring at different levels of biological organization. The second, and more modest, possibility would be if nested models simply refined our understanding of pathogen evolution by providing an explicit link between the within-host dynamics of a pathogen's replication and its spread at the between-host population level. In this case, the explicit nesting of models is, in a sense, inessential, as there is no reciprocal feedback between the within- and between-host dynamics (Box 2).

What have nested models of infectious disease taught us?

The importance of nesting models of within-host dynamics into epidemiological frameworks for between-host processes was recognized over a decade ago [13] (Figure 1). Since then, much has been done in this area and many interesting insights have been gained.

Interdependence of parameters

One important insight gained from nested models is that epidemiological parameters such as disease-induced mortality (virulence), recovery rate and transmission rate are often interrelated in subtle and complex ways. The majority of current theory for virulence evolution assumes

that there exists a tradeoff between virulence and transmission rate across pathogen genotypes, and builds such constraints into models for the between-host dynamics in a phenomenological way (see e.g. Refs [11,12,14,15]).

Recent work has developed an explicit description of the within-host dynamics of pathogen replication and the defensive response of the host, and nested this into the traditional between-host models of pathogen evolution (Box 1). This approach provides an explicit link between the mechanism of pathogen replication (e.g. the host cells or resources used by the pathogen to replicate, and mechanisms by which the host attempts to rid itself of infection) and the virulence and transmission rate of the pathogen at the level of the host population. Thus, the nature of any tradeoffs between virulence, recovery rate and/or transmission rate can be directly linked to mechanistic hypotheses of within-host dynamics. Indeed, it has been demonstrated that such tradeoffs can emerge through a variety of interactions between pathogens and both host immune responses [16–19] and host resources [20], leading to the evolution of intermediate levels of virulence (e.g. [15,16,21]).

Although the above examples have deepened our understanding of pathogen evolution, the nesting of models *per se* in these examples is inessential. The within-host dynamical model provides an explicit description of how the mechanisms of pathogen replication and host defense

give rise to transmission rates, recovery rates and virulence, and these feed into the between-host dynamical model so as to affect the epidemiological dynamics of disease at the level of the host population. There is not, however, any reciprocal feedback from the between-host dynamics back down to the within-host dynamics in these studies (Box 2). In this sense, the nesting of models is inessential – the within-host model could be analyzed in isolation from the between-host model, and any important insights drawn from it then simply incorporated into the between-host model in a phenomenological way.

That none of the above-mentioned studies have nested models in an essential way is not meant to be a major criticism, however, as much has still been learned from these. For example, they have demonstrated that the mechanism through which a pathogen causes harm to its host, and how it is transmitted from one host to another, are important determinants of virulence [20,22–24]. Studies have also shown that the optimal virulence is strongly dependent on within-host parameter values, potentially providing an explanation for why transmission–virulence tradeoffs have been difficult to demonstrate empirically [19].

Nested models have also demonstrated that the common practice of treating recovery rate as an independent parameter in between-host models of pathogen evolution is typically not justifiable. A primary focus of between-host models has been on determining how the recovery rate affects the evolution of virulence and transmission [12,25–29]. A similar analysis of recovery rate has also been used to study the effects of vaccination on virulence evolution (enhanced recovery rate is one way in which vaccines might act) [30]. A vaccine is unlikely to simply increase recovery rate, however, and instead it might decrease the time until a significant immune response occurs or increase the abundance of relevant immune effector molecules. Whereas this might increase recovery rate, it will also change the within-host dynamics in a way that can simultaneously alter transmission rate, virulence and even any tradeoff between the two [31,32]. Therefore, even though the nested nature of these recent studies is inessential, they have nevertheless refined our understanding of how differences in the mode of vaccine action can result in differences in pathogen evolution.

Conflicting selection

Pathogen fitness is determined by processes that occur both within and between hosts. Within a host, pathogens might compete directly for host resources, as well as indirectly owing to the presence of a common immune response. The strain that is most successful at this within-host competition, however, need not be the one that is best able to transmit among hosts. The ultimate evolutionary outcome will depend on how these potentially conflicting patterns of selection at the within- and between-host levels are resolved [11,12,33].

The idea of conflicting natural selection at the within- and between-host levels is broadly supported by a body of experimental work. Serial passage of parasites often results in increased parasite virulence, a phenomenon that can be explained by artificial transmission regimes largely

removing the constraints imposed on virulence at the between-host level (see Ref. [34] for a review) [35]. Similarly, in diverse *Plasmodium chabaudi* (rodent malaria) infections in mice, more virulent clones (i.e. those that induce greater red blood cell or weight loss) will competitively suppress less virulent competitors [36] and make up a greater proportion of the total parasite burden within a host [37].

Nested dynamical models can be useful for elucidating the consequences of these different levels of selection. Such models have been used to show that the shape of the tradeoff between virulence and transmission, mediated by within-host mechanisms, determines whether selection acting on pathogen growth rate pushes this trait to different optima at the within- and between-host levels (i.e. whether selection is in conflict between levels; [20]). Extending nested models to the case of multiple infections has also allowed for the exploration of how selection in response to both within-host competition and between-host transmission is resolved [38,39] and the conditions under which one level of selection dominates [40]. Further, these models have shown that multiple infections can lead to evolutionary diversification in the pathogen population, with one strain specializing in infecting susceptible hosts and another, more virulent, strain specializing in coinfecting already infected hosts [40,41].

As before, virtually all of the above examples represent cases of the inessential nesting of models. Although studying the effects of selection at different scales requires some implicit model of dynamics at each scale, this need not be accomplished through an explicit mathematical tracking of within-host dynamics. For instance, implicit within-host modeling is sufficient to show that adding multiple infections can favor more virulent strains [9–11], even without a tradeoff between transmission and virulence [42]. As far as we are aware, there are only two studies for which nesting was essential: one used a nested model to explore conflicting levels of selection [38] and the other studied multiple infections more generally [41]. In these studies, the within-host dynamics of pathogen replication influence the between-host epidemiological dynamics, just as in many of the other nested models mentioned earlier. In addition, however, because the between-host epidemiological dynamics affect the relative frequency of different pathogen strains, they also affect the composition of the inoculum entering newly infected hosts [38] or the order of arrival of coinfecting strains [41]. These, in turn, influence the within-host dynamics, resulting in a reciprocal feedback between levels of biological organization that cannot be captured without an explicitly nested model (Box 2).

The reciprocal feedback in Ref. [38] leads to the interesting new insight that the balance between conflicting selection at the within- versus between-host level can be shifted depending upon the timing of infection. In particular, strains that are ultimately destined to be outcompeted within a host might nevertheless reach a very high frequency within hosts early in an infection when target cells are abundant (see Ref. [43]). As a result, if there is a premium placed on early transmission during an infection, then the effects of within-host selection will be mitigated and a strain that is less competitive within hosts can

dominate these early transmission events. Early transmission will be important when future transmission opportunities are likely to be limited by host death, or when there is an abundance of susceptible hosts in the population. Of course, the depletion of susceptible hosts at the population level will eventually feed back, making the longer-term competitive success of strains within an infection more evolutionarily important. Interestingly, there is some empirical evidence suggesting that competitive abilities of different strains do, in fact, change during the course of an infection [37].

Future prospects and challenges

We have suggested that, out of all previous studies that use nested models for studying pathogen evolution, in only two of these has this nesting *per se* been essential. What does this imply about the utility of nested models, and how should such models be used in future research? While we do not claim to have the definitive answers to these questions, we close by offering a few possibilities.

Although the insights gained from most existing nested models might just as well have been obtained without such explicit nesting, we nevertheless believe that these models have been useful because they have emphasized the importance of the timing of events during an infection (i.e. the disease life history; Figure 2) on the epidemiological and evolutionary dynamics of pathogens. Indeed, these studies have highlighted an important, and still unanswered, question in evolutionary epidemiology – what factors govern the evolution of disease life histories? A great deal of theory has been devoted to understanding virulence evolution, but surprisingly little has been done to examine the evolution of other important aspects of disease. Parasite transmission rate, parasite-induced mortality (virulence) and infection recovery rate are the three most important epidemiological quantities related to disease [3]. Each of these typically changes quite dramatically over the course of an infection, yet very little theory has been devoted to understanding the evolution of the temporal patterns of these

traits. Why, for example, do some diseases, like measles, have substantial overlap in the timing of mortality and transmission during infection, whereas in other diseases, such as HIV, these are temporally separated (Figure 2)? Furthermore, what factors are most important in driving evolutionary change in these patterns within any given pathogen species?

Nested models can be used to address such questions about the evolution of disease life history. Strain-specific differences in the replication rate of a pathogen, and how a pathogen interacts with host defense mechanisms and host resources, will give rise to strain-specific differences in the temporal patterns of transmission, virulence and recovery during an infection. These effects are explicitly captured by within-host models which, when nested in a between-host model, will allow for predictions to be made about the evolution of disease life history at the host population level. Such an approach will provide a more holistic theory of parasite evolution, with virulence being treated simply as one of several important aspects of disease life history. Furthermore, it opens the door to making realistic predictions about the evolution of other measures of parasite-induced harm, such as morbidity and case mortality, which typically result from a complex interaction between parasite-induced harm and host defense mechanisms [17,19,29,44–47]. Nested models also allow for predictions of whether a disease is expected to evolve a chronic or an acute life history, rather than assuming one of the two as in most current theory.

There are also other interesting open questions in evolutionary epidemiology for which nested models will be useful. For example, the nesting of models will be essential for studying the evolution of diseases in which the inoculum size affects the progression of the disease [48], because this will likely lead to a reciprocal feedback between within- and between-host dynamics (Box 2). In such cases, either the prevalence of the disease at the host population level or the specific within-host details of the ‘donor’ at the time of transmission (e.g. pathogen density) will affect the dynamics of the ‘recipient’ host.

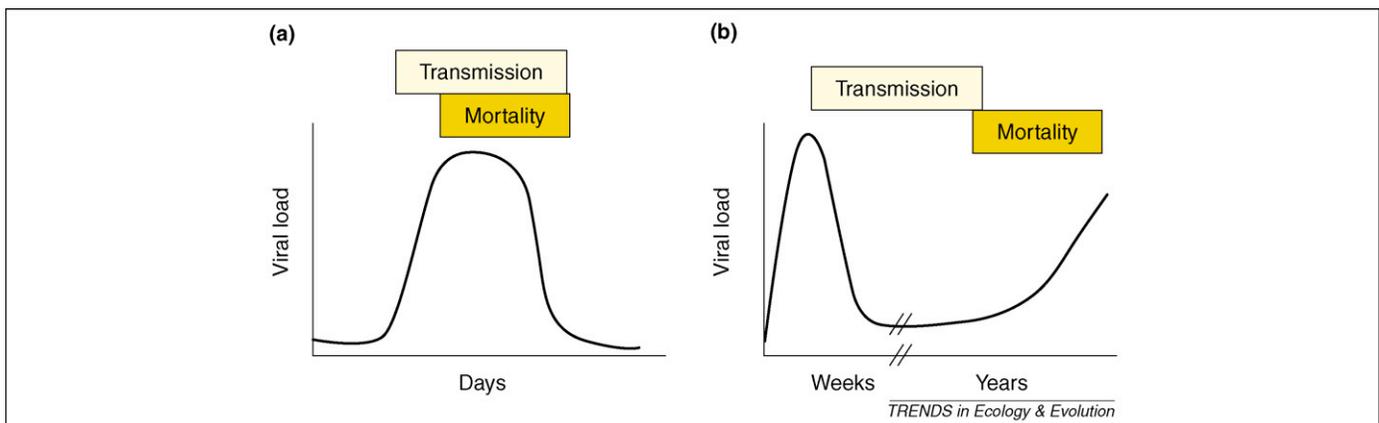


Figure 2. An illustration of disease life history. The term ‘life history’ refers to the age-specific pattern of survival and reproduction of an organism. If we view an infection as the ‘organism’ in question, then the life history of a disease is its age-of-infection-specific pattern of transmission, virulence and recovery [53]. Parasites with different disease life histories therefore have very different clinical profiles. Solid lines show the within-host pathogen dynamics, and the colored boxes indicate the approximate timing and duration of transmission and disease-associated mortality (recovery rate is ignored for simplicity). (a) A disease such as measles where transmission begins with the onset of symptoms and, although the host’s immune response is usually sufficient to rapidly clear the infection, complications can lead to parasite-induced mortality. (b) A disease such as HIV where the timing of transmission and mortality are largely nonoverlapping. Transmission can occur throughout the infection, with the highest risk of transmission associated with high viral loads (e.g. during the initial acute phase shortly after infection). Mortality associated with HIV generally occurs much later in the infection. From the perspective of a host, HIV is a chronic infection, characterized by a long asymptomatic period followed by rapid progression toward AIDS.

Previous results have focused on the fact that the composition of an inoculum can alter the pattern of disease progression as well [38]. This might be particularly relevant for diseases such as HIV for which the viral diversity in the early stages of infection has been shown to affect the speed of disease progression [49]. Genetic diversity in an infection is determined by a repeated cycle of within-host (e.g. immune pressure, replication and diversification) and between-host processes (e.g. transmission bottlenecks), with the outcome of selection at each level feeding into the other. Capturing all of these processes and their interactions in a nested model could help to answer important open questions about the dominance and pattern of disease progression of particular HIV subtypes (see Ref. [50]).

Finally, questions about public health policy might also benefit from the use of nested models. An important aim of models of disease dynamics is to study the evolutionary responses of pathogens to treatments [30,51,52]. If the prevalence of a disease in the host population affects, for example, either the probability of an individual receiving treatment or the efficacy of this treatment, a nested model might be useful for capturing the feedback that would result between the within- and between-host dynamics.

Conclusion

Linking different scales of biological organization with nested models is an approach that has long been used in ecology. The use of nested models for linking levels of disease dynamics has recently become more common, focusing on nesting a model of within-host dynamics into a model of between-host epidemiological dynamics. Almost all of the studies that have used nested models of disease dynamics to date have not actually required nesting to accomplish their goals. Despite this, these models have provided important insights about pathogen evolution. Further, when there is reciprocal feedback between these two levels of biological organization, the nesting of models is the only way to capture this effect. The nesting of models will therefore continue to prove useful for tackling open questions in the evolutionary epidemiology of infectious diseases.

Acknowledgements

We thank S. Gandon and A. Hurford for comments on an earlier draft and the Queen's University Mathematical Biology Group for helpful discussion of the contents of this manuscript. We also thank David Earn for suggesting the distinction between nested models with and without reciprocal feedback, and Mike Gilchrist for championing the term and the use of 'nested models.' This research was funded by a Natural Sciences and Engineering Research Council of Canada Discovery Grant, support from the Canada Research Chairs Program and funding from the Mathematics of Information Technology and Complex Systems.

References

- Maynard-Smith, J. and Szathmari, E. (1997) *The Major Transitions in Evolution*, Oxford University Press
- Grenfell, B. and Harwood, J. (1997) (Meta)population dynamics of infectious diseases. *Trends Ecol. Evol.* 12, 395–399
- Anderson, R.M. and May, R.M. (1991) *Infectious Diseases of Humans: Dynamics and Control*, Oxford University Press
- Hethcote, H.W. (2000) The mathematics of infectious diseases. *SIAM Rev.* 42, 599–653
- Sasaki, A. (1994) Evolution of antigen drift/switching: continuously evading pathogens. *J. Theor. Biol.* 168, 291–308
- Nowak, M.A. *et al.* (1990) The evolutionary dynamics of HIV-1 quasispecies and the development of immunodeficiency disease. *AIDS* 4, 1095–1103
- De Boer, R.J. and Boerlijst, M.C. (1994) Diversity and virulence thresholds in AIDS. *Proc. Natl. Acad. Sci. U. S. A.* 91, 544–548
- Iwasa, Y. *et al.* (2005) Virus evolution within patients increases pathogenicity. *J. Theor. Biol.* 232, 17–26
- Nowak, M.A. and May, R.M. (1994) Superinfection and the evolution of parasite virulence. *Proc. Biol. Sci.* 255, 81–89
- May, R.M. and Nowak, M.A. (1995) Coinfection and the evolution of parasite virulence. *Proc. Biol. Sci.* 261, 209–215
- van Baalen, M. and Sabelis, M.W. (1995) The dynamics of multiple infection and the evolution of virulence. *Am. Nat.* 146, 881–910
- Frank, S.A. (1996) Models of parasite virulence. *Q. Rev. Biol.* 71, 37–78
- Anderson, R.M. (1994) Mathematical studies of parasitic infection and immunity. *Science* 264, 1884–1886
- Ewald, P.W. (1983) Host-parasite relations, vectors, and the evolution of disease severity. *Annu. Rev. Ecol. Syst.* 14, 465–485
- Sasaki, A. and Iwasa, Y. (1991) Optimal growth schedule of pathogens within a host: switching between lytic and latent cycles. *Theor. Popul. Biol.* 39, 201–239
- Ganusov, V.V. *et al.* (2002) Within-host population dynamics and the evolution of microparasites in a heterogeneous host population. *Evolution Int. J. Org. Evolution* 56, 213–223
- Gilchrist, M.A. and Sasaki, A. (2002) Modeling host-parasite coevolution: a nested approach based on mechanistic models. *J. Theor. Biol.* 218, 289–308
- André, J.-B. *et al.* (2003) Within-host parasite dynamics, emerging trade-off, and evolution of virulence with immune system. *Evolution Int. J. Org. Evolution* 57, 1489–1497
- Alizon, S. and van Baalen, M. (2005) Emergence of a convex trade-off between transmission and virulence. *Am. Nat.* 165, E155–E167
- Gilchrist, M.A. and Coombs, D. (2006) Evolution of virulence: interdependence, constraints and selection using nested models. *Theor. Popul. Biol.* 69, 145–153
- Antia, R. *et al.* (1994) Within-host population dynamics and the evolution and maintenance of microparasite virulence. *Am. Nat.* 144, 457–472
- Ganusov, V.V. and Antia, R. (2003) Trade-offs and the evolution of virulence of microparasites: do details matter? *Theor. Popul. Biol.* 64, 211–220
- Frank, S.A. and Schmid-Hempel, P. (2008) Mechanisms of pathogenesis and the evolution of parasite virulence. *J. Evol. Biol.* 21, 396–404
- Alizon, S. and van Baalen, M. (2008) Transmission-virulence trade-offs in vector-borne diseases. *Theor. Popul. Biol.* DOI: 10.1016/j.tpb.2008.04.003 (<http://www.sciencedirect.com/science/journal/00405809>)
- Anderson, R.M. and May, R.M. (1982) Coevolution of hosts and parasites. *Parasitology* 85, 411–426
- van Baalen, M. (1998) Coevolution of recovery ability and virulence. *Proc. Biol. Sci.* 265, 317–325
- Gandon, S. and Michalakis, Y. (2000) Evolution of parasite virulence against qualitative or quantitative host resistance. *Proc. Biol. Sci.* 267, 985–990
- Gandon, S. *et al.* (2001) Host life history and the evolution of parasite virulence. *Evolution Int. J. Org. Evolution* 55, 1056–1062
- Day, T. and Burns, J.G. (2003) A consideration of patterns of virulence arising from host-parasite coevolution. *Evolution Int. J. Org. Evolution* 57, 671–676
- Gandon, S. *et al.* (2001) Imperfect vaccines and the evolution of pathogen virulence. *Nature* 414, 751–756
- Ganusov, V.V. and Antia, R. (2006) Imperfect vaccines and the evolution of pathogens causing acute infections in vertebrates. *Evolution Int. J. Org. Evolution* 60, 957–969
- André, J.-B. and Gandon, S. (2006) Vaccination, within-host dynamics, and virulence evolution. *Evolution Int. J. Org. Evolution* 60, 13–23
- Levin, B.R. and Bull, J.J. (1994) Short-sighted evolution and the virulence of pathogenic microorganisms. *Trends Microbiol.* 2, 76–81
- Ebert, D. (1998) Experimental evolution of parasites. *Science* 282, 1432–1435

- 35 Mackinnon, M.J. and Read, A.F. (1999) Selection for high and low virulence in the malaria parasite *Plasmodium chabaudi*. *Proc. Biol. Sci.* 266, 741–748
- 36 de Roode, J.C. *et al.* (2005) Virulence and competitive ability in genetically diverse malaria infections. *Proc. Natl. Acad. Sci. U. S. A.* 102, 7624–7628
- 37 Bell, A.S. *et al.* (2006) Within-host competition in genetically diverse malaria infections: parasite virulence and competitive success. *Evolution Int. J. Org. Evolution* 60, 1358–1371
- 38 Coombs, D. *et al.* (2007) Evaluating the importance of within- and between-host selection pressures on the evolution of chronic pathogens. *Theor. Popul. Biol.* 72, 576–591
- 39 Mideo, N. and Day, T. (2008) On the evolution of reproductive restraint in malaria. *Proc. Biol. Sci.* 275, 1217–1224
- 40 Boldin, B. and Diekmann, O. (2008) Superinfections can induce evolutionarily stable coexistence of pathogens. *J. Math. Biol.* 56, 635–672
- 41 Alizon, S. and van Baalen, M. Multiple infections, immune dynamics and virulence evolution. *Am. Nat.* (in press)
- 42 Bonhoeffer, S. and Nowak, M.A. (1994) Mutation and the evolution of virulence. *Proc. Biol. Sci.* 258, 133–140
- 43 Ball, C.L. *et al.* (2007) Modeling within-host evolution of HIV: mutation, competition and strain replacement. *Bull. Math. Biol.* 69, 2361–2385
- 44 Day, T. (2002) On the evolution of virulence and the relationship between various measures of mortality. *Proc. Biol. Sci.* 269, 1317–1323
- 45 Graham, A.L. *et al.* (2005) Evolutionary causes and consequences of immunopathology. *Annu. Rev. Ecol. Syst.* 36, 373–397
- 46 Day, T. *et al.* (2007) Evolution of parasite virulence when host responses cause disease. *Proc. Biol. Sci.* 274, 2685–2692
- 47 Brown, S.P. *et al.* (2008) Evolution of virulence: triggering host inflammation allows invading pathogens to exclude competitors. *Ecol. Lett.* 11, 44–51
- 48 Schmid-Hempel, P. and Frank, S.A. (2007) Pathogenesis, virulence, and infective dose. *PLoS Pathog.* 3, 1372–1373
- 49 Sagar, M. *et al.* (2003) Infection with multiple human immunodeficiency virus type 1 variants is associated with faster disease progression. *J. Virol.* 77, 12921–12926
- 50 Arien, K.K. *et al.* (2007) Is HIV-1 evolving to a less virulent form in humans? *Nat. Rev. Microbiol.* 5, 141–151
- 51 Medley, G.F. (2002) The epidemiological consequences of optimisation of the individual host immune response. *Parasitology* 125, S61–S70
- 52 van Baalen, M. (2002) Dilemmas in virulence management. In *Adaptive Dynamics of Infectious Diseases: In Pursuit of Virulence Management* (Dieckmann, U. *et al.*, eds), pp. 60–70, Cambridge University Press
- 53 Day, T. (2003) Virulence evolution and the timing of disease life-history events. *Trends Ecol. Evol.* 18, 113–118
- 54 Diekmann, O. and Heesterbeek, J.A.P. (2000) *Mathematical Epidemiology of Infectious Disease*, Wiley
- 55 Day, T. (2001) Parasite transmission modes and the evolution of virulence. *Evolution Int. J. Org. Evolution* 55, 2389–2400