

Review

The Evolutionary Consequences of Stepwise Infection Processes

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Molecular and cellular studies reveal that the resistance of hosts to parasites and pathogens is a cascade-like process with multiple steps required to be passed for successful infection. By contrast, much of evolutionary reasoning is based on strongly simplified, one- or two-step infection processes with simple genetics or on resistance being a quantitative trait. Here we attempt a conceptual unification of these two perspectives with the aim of cross-fostering research and filling some of the gaps in our concepts of the ecology and evolution of disease. This conceptual unification has a profound impact on the way we understand the genetics and evolution of host resistance, ecological immunity, evolution of virulence, defence portfolios, and host–pathogen coevolution.

Complex Traits and the Progression of Stepwise Events

Many biological characteristics are determined by the progression of stepwise events. Mate choice [1], the progression of cancer [2], development [3], and metabolism [4] are all examples where the phenotype of an individual depends on a series of biological or molecular events that occur in chronological succession. An ordered series of stages appears to be an almost universal feature of complexity in living systems [5]. Recognising the pervasive nature of this organisation has led researchers to consider how an entire system, rather than just one or two components, is important for complex trait evolution. The resulting body of theory has shown that the stepwise nature of many biological systems is strongly linked to evolutionarily relevant phenomena such as protecting phenotypes from catastrophic change [5,6], altered strengths of selection [7], and the maintenance of genetic variation [8].

Stepwise processes are implicit in the progression of infectious disease (Box 1), but to date most evolutionary studies emphasise the broad outcomes of infection, such as infection success, mortality, or morbidity (i.e., health trajectories [9]). Insight into the pathways underlying infection have instead come from the reductionist approaches of molecular and cell biology [10], leaving a disconnect between the process of infection and the capacity of a stepwise process to influence adaptation. This distinction between a functional and an evolutionary interpretation is now changing: studies are beginning to explicitly address the role of individual infection steps in driving host and pathogen evolution [11–13]; multitrait theory has uncovered how the steps of infection can combine to enhance rates of evolutionary change [14,15]; and discord between classical quantitative genetic and genomic interpretations of host resistance has prompted discussion about the genetic architecture of disease susceptibility [16,17].

A conceptual unification of functional and evolutionary perspectives offers new opportunities to understand why differences in infection exist among individuals, genotypes, and populations. We draw parallels with analogous studies of complex system organisation, such as mate

Trends

Many biological traits are determined by the progression of stepwise events. Dissecting host–parasite interactions into steps offers great potential for understanding infectious disease biology and evolution.

The steps of infection are typically governed by unequal contributions of genetic (G), environmental (E), and G × E effects, allowing unique evolutionary trajectories at each step.

Variation at each step has different consequences for hosts and pathogens. A pathogen must pass through all steps until transmission starts or else its fitness is zero. For the host, the profitability of resistance at a given step declines with increasing virulence experienced by the host.

Red Queen coevolution driven by negative frequency-dependent selection can occur only at steps with host genotype–pathogen genotype interactions. By contrast, selective sweeps may occur at any step.

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Box 1. A Sequence of Attack and Defence Opportunities

Underlying the infection process is a sequence of attack and defence events that are separated in both time (i.e., duration post-exposure) and space (e.g., different tissues or body parts). The defence process begins with the behavioural, social, or life-history shifts that enable a host to avoid contact with infective agents altogether. Through grooming and hygiene, the avoidance of infected individuals, and adjustments in daily activity schedules, hosts can reduce the likelihood of infection by minimising exposure ([45], see examples in Table 4.1). Next are the barriers that prevent a pathogen from entering the host. These barriers can be physical (plant cuticles or animal epidermis), microbial (antimicrobial products or mucosal coatings), or chemical (enzymes or low-pH conditions) and act to either repel pathogens on contact or inhibit pathogen colonisation (e.g. [62,63]). Together these processes are often referred to as the first lines of defence as they minimise the opportunity for a pathogen to even encounter the host or infect the target tissue (*sensu* [64]).

Once the pathogen has entered the host it will be exposed to the immune response of the host (but see also [34]). During this step diverse defence reactions of the host can be triggered, such as phagocytosis, apoptosis of infected cells, encapsulation of the invader (phenoloxidase activity), B and T cell expansion, and many other forms of innate or induced defences (reviewed in [10]). In combination with pathways that control the availability of essential nutrients such as iron or cholesterol [65,66], these processes can act to clear an infection or limit the growth of a successful invading pathogen, albeit often over different timescales (i.e., hours versus days [67]; cf. [68]). The final step of the infection process is the production of transmission stages and their release, which at its most extreme will require the death of the infected host [27,61,69]. Here host barriers might arise that prevent a pathogen from migrating to the correct tissue to disperse, as in the case of the movement of various arboviruses to the saliva of an infected mosquito [70].

choice and development, and focus on three key evolutionary questions. (i) What are the consequences of multiple steps for the genetic variability of host and pathogen strategies? (ii) How does partitioning host and pathogen fitness in a sequential manner change our understanding of their coevolution? (iii) Can understanding the stepwise infection process help control the spread of infectious disease? Clearly the importance of understanding the steps of the infection process extends beyond a characterisation of the mechanisms that lead to individuals becoming diseased.

Biological Properties of a Stepwise Infection Process

The infection process begins whenever the opportunity for contact between a host and a pathogen occurs and ends with the transmission of the pathogen to the environment or a new host (Box 1). In principle one can subdivide such a process into a large number of small steps, even down to single molecules or the smallest time intervals. Here we define a step as all events that are necessary to fulfil a certain function, such as the entry of a pathogen into the host or the production of transmission stages. Some steps will be clearly distinct (e.g., entering the host) while others might be less easy to delimit (e.g., early and late phases of pathogen growth).

Genetic and Environmental Variation Is Unique to Different Steps

Multiple physiological, mechanical, and immune-based mechanisms contribute to the infection process and all vary in their resource cost, environmental sensitivity, and genetic architecture [16,18–20]. Immune function, for example, is thought to vary with the amount of resources a host can acquire and is therefore highly regulated by the environment. In turn, defence steps that are under tight developmental regulation, or that are under strong stabilising selection, will be genetically invariable. The resulting unequal distribution of genetic and environmental variation across the infection process allows the evolutionary potential of each underlying step to be unique. The greater the genetic variation available, and the less environmental variation that is present, the more likely a step will respond to selection (*sensu* [21]). As a whole, however, the overall signature of a multistep process will result from the interactions of the environment with all genes, steps, or pathways contributing to the entire infection process.

Much like any other complex trait related to lifetime fitness (e.g., mate choice [22,23]), the outcome of infection depends on a sequence of events separated in time, and even space (i.e., tissues) [24]. A single step is not necessarily representative of the total capacity of a host to

mitigate infection, due to the dependence of later-occurring episodes on those that occur earlier [7,25]. Traits that characterise the end points of infection, such as host mortality, represent the combined result of all previous steps. Thus, many genes contribute to end-point trait expression, in combination with sensitivity to environmental factors and, inevitably, gene-by-environment interactions. By contrast, for earlier steps in the infection process there is less potential for additional sources of genetic and environmental variation to influence the phenotype. Among the few illustrative examples of how the infection process can be subdivided into steps, revealing the step-specific pattern of genetic architecture and environmental sensitivity, are the study of interactions between the water flea *Daphnia magna* and its bacterial pathogen *Pasteuria ramosa* (Box 2 and Figure 1) and the defence portfolios directed at the European cuckoo [26].

Fitness Consequences for Hosts and Pathogens Are Asymmetric

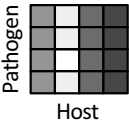
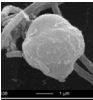
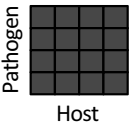
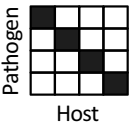
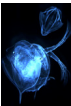
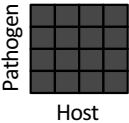
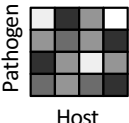
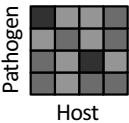
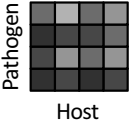
Infectious-disease life cycles are often presented as an orderly sequence of steps whereby all antagonistic interaction between a host and a pathogen is equally important in describing the process of infection (Figure 2, individual level). However, a more detailed analysis of the stepwise process reveals that the fitness consequences of any given step can be very different for the host and the pathogen. For the pathogen fitness is mostly determined by the likelihood of passing through all steps successfully and transmitting to a new host. In the case of viral infection of a cell, for example, loss of fitness can occur when the pathogen is prevented from entering the host, producing mature virus particles, or transmitting to a new host (Figure 2 and [27]). Failure to progress at any step would result in zero fitness for the virus (*sensu* [14]). Thus, the force of selection is high across all steps, as each step that occurs before the pathogen starts transmitting from the infected host is equally important.

This is not always valid for the host. If two defence steps occur independently, and close enough in time that the host does not suffer a reduction in fitness during the intervening period, selection is likely to act equally on each step. However, if host fitness declines as infection progresses, the benefit of resistance also decreases at later steps. In this context a transmission-blocking mutant that would prevent the further spread of disease would be favoured if the host has already suffered disease symptoms expressed earlier in the infection (but see [28]). Early steps can also act as filters that the pathogens have to pass through, with later steps experiencing less pathogen pressure and therefore reduced intensity of selection. For example,

Box 2. A Stepwise Infection Case Study Involving *Daphnia*

Underlying infection in the *Daphnia*–*Pasteuria* system are seven clearly distinguishable steps, from the initial encounter between the host and the pathogen to the killing of the host by the pathogen (Figure 1). Partitioning of the observed variation into the contributions of host and pathogen genotypes, the environment, and their interaction, has revealed a surprising diversity of effects across these seven steps. Steps such as the activation of pathogen spores have been shown to be invariable to diverse sources of genetic and environmental variability [43]. By contrast, the early within-host immune response is influenced by all possible sources of variability, from the genetic effects of hosts and pathogens to the environment and all of their interactions [71,72]. This differential sensitivity of individual steps to genetic and environmental influences is likely to be a universal feature of infection outcomes, suggesting that there are diverse ways for evolution to shape an infection process.

Only one step in the entire infection process facilitates rapid host–pathogen coevolution and can explain the outcome of previous time-shift infection experiments: the attachment of the pathogen to the host gut wall. Infection is possible only when compatible host and pathogen genotypes come together and attachment occurs (strong $G_H \times G_P$ interactions [43,73]). Variation in this step is not influenced by any so-far-tested environmental condition (temperature, host density, feeding conditions, microbiota composition) and the genetics for this step follow a matching-allele model [74,75]. By contrast, the subsequent sequences of events, involving the ability of the pathogen to establish and proliferate after entering the haemolymph, display much more quantitative variation. While the early within-host steps also show $G_H \times G_P$ interactions, the late steps are very sensitive to environmental factors and to $G \times E$ interactions [71,76]. Without knowledge of the stepwise structure of the infection process in this system, it would have been much harder to obtain solid evidence for a matching-allele-like form of coevolution in this system.

Key trait	Step	Host–pathogen interaction matrix	Environmental sensitivity
Likelihood of encounter with pathogen	Exposure step 		Yes
Likelihood to activate spore	Spore activation step 		No
Likelihood to attach to host	Attachment step 		No
Likelihood to enter host	Penetration step 		Yes
Likelihood to clear pathogen/spore development	Early within-host step 		Yes
Spore load	Late within-host step 		Yes
Time to host death	Host death 		Yes

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Figure 1. The Steps of Infection by *Pasteuria ramosa* in *Daphnia magna*. In this host–pathogen system, the relative contributions of environmental and genetic variation change profoundly over the course of infection, enabling different evolutionary trajectories or rates of change to occur at each step. The matrices shown in column three are a schematic representation of the variation as seen among host and parasite genotypes for a typical trait measured at this step. Darker fields indicate greater success for the parasite. In the exposure step, for example, all genetic variation is among hosts (the pathogen has a passive role in this step), while in the attachment step variation is binary and results from host–pathogen interactions. The right column indicates whether trait expression is also influenced by environmental factors such as food level, temperature, or host density. Modified from [61].

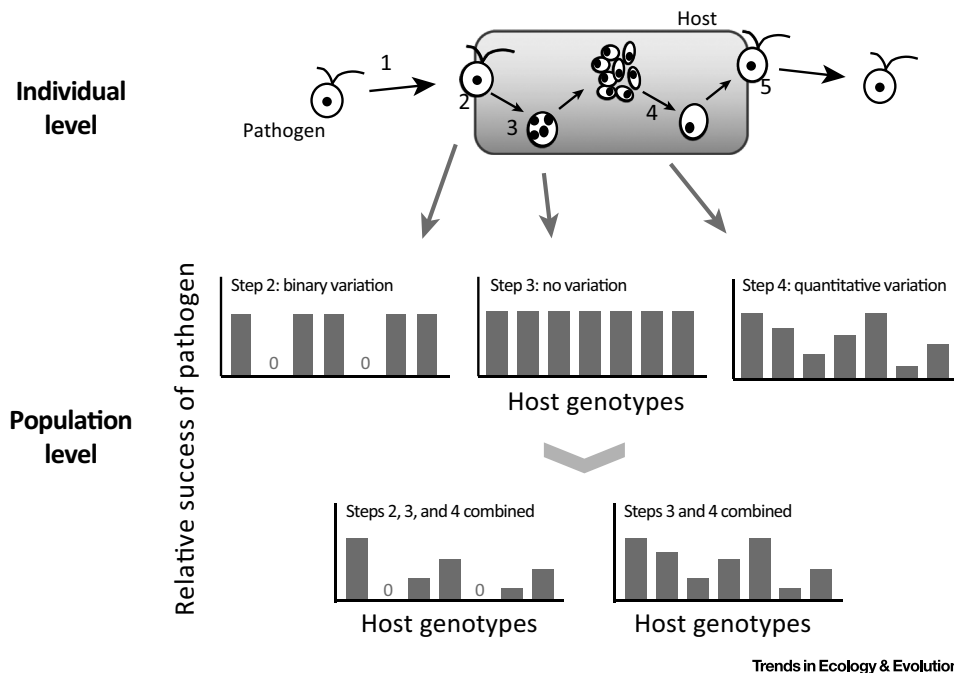


Figure 2. The Stepwise Infection Model at the Individual Level of the Host and Its Consequences for Patterns of Variation at the Population Level. At the individual level (top row), the infection process can be broken down into a chain-like process: (1) pathogen exposure; (2) entering the host (penetration step); (3) evading the initial immune response; (4) within-host growth and/or multiplication; and (5) transmission of new infective propagules. The middle row shows examples of the relative success of the parasite with seven arbitrary host genotypes for three isolated steps: entering the host (step 2) shows a binary pattern of variation with hosts being either permissible or not; immune evasion (step 3) shows no variation among host genotypes; and the growth phase (step 4) shows quantitative variation. Other steps are ignored (or experimentally excluded). The bottom row shows the combined assessment of parasite success for different combinations of steps (left: steps 2–4 combined; right: only steps 3 and 4 combined, with variation at step 2 excluded). This example highlights that the overall distribution of disease variability among genotypes depends on the steps considered. Redrawn after [31,45].

the within-host immune response occurs only when the pathogen successfully overcomes the first line of defence, reducing both the number of overall propagules and the number of genotypes that encounter this defence step.

The logic of these scenarios is most easily applied to systems where the pathogen has to kill the host for transmission (i.e., obligate killers), such as bacteriophages, parasitoids, and many insect viruses, but a reduction in the strength of selection can also occur when infections are terminated via host clearance. For less chronic infections, such as malaria or measles, prolonged infection reduces residual host fitness and should favour earlier clearance. This is analogous to arguments made in explaining the evolution of ageing via the concept of a ‘selection shadow’ [29]. Natural selection is predicted to become progressively weaker with increasing age, as older individuals have already attained most, if not all, of their reproductive potential. If residual fitness decreases during the infection process, so too should the strength of selection for resistance, potentially altering the epidemiology of disease at the population level in complex ways [30].

Evolutionary Consequences of a Stepwise Infection Process

Individual steps can have a profound influence on the evolution of infectious disease. Although the combined effect of all steps determines the overall resistance of a host [31], at any step hosts and pathogens will be experiencing different fitness costs or benefits and varying

Box 3. Constraints on a Stepwise Infection Process

Insight into the evolutionary limits of the infection process can be drawn from the study of analogous processes such as embryonic development or the progression of cancer. Theory suggests that having a multistep (or modular) architecture increases a system's ability to cope with environmental and genetic perturbations [5,6]. As failures arise they can be contained within one isolated step or module, allowing catastrophic consequences to be avoided due to the compensatory effect of other processes. However, a robust system that protects against persistent challenges also suffers from a number of inherent performance tradeoffs. Additional steps place increased resource demands on the individual, in terms of both maintaining the underlying mechanism and the costs associated with its use [5]. With each extra component, the rate at which natural selection removes deleterious mutations also slows, leading to a decrease in the performance of the individual components on average, relative to what would be achieved without the robust mechanisms [8,77].

Applying these concepts to the infection process, therefore, divulges the main benefits and costs associated with a cascade of defence steps. With each new infection step, the performance of the entire defence cascade increases as a pathogen faces one more challenge before being able to transmit to a new host (i.e., robustness, in the parlance of [5]). Multiple defence options also increase the evolutionary potential of the host relative to the pathogen, as the pathogen needs to overcome all steps while the host needs only to block the parasite once [14]. Infection steps, however, cannot be added indefinitely. Many components of the defence cascade will be costly to maintain and to use [78,79] (but see [20]) and a host only has a finite pool of available resources to spend (i.e., condition [80]). Not only does this limit the number of steps that can be added, it also gives rise to potential tradeoffs between components, such as those involved in resistance or tolerance [81]. Likewise, as the total number of steps is believed to increase with ongoing coevolutionary history [82], selection for each step will weaken and becomes less effective at maintaining the optimal performance of each component [83,84]. Thus, an evolutionary limit to the infection process will arise from tension between robustness and resource limitation and the threat of mutational degeneracy associated with increasing the number of control points underlying infection.

sensitivity to environmental or genetic factors. Specific steps in the infection process can have a particularly strong impact on overall disease expression. A meta-analysis of over 40 species of human pathogens, for example, showed that pathogens entering through the skin were associated with higher patient virulence than those that are inhaled or ingested [32]. Similarly, in *Drosophila melanogaster* inclusion of the first step of infection (oral infection) or its removal (systemic infections) led to vastly different patterns of evolutionary change [12]. We discuss the unequal contributions of individual steps to overall disease variability, the relationship between individual steps and host–parasite coevolution, and the evolutionary constraints that limit the number of steps in a defence cascade (Box 3).

Interactions among Multiple Infection Steps Define Disease Variability

A common observation in many disease systems is that interactions between hosts and pathogens are often highly specific, with hosts differing in their susceptibility to different pathogen types and pathogens differing in their ability to infect or grow within different hosts [31,33]. From the perspective of the host, however, not all underlying steps of infection necessarily contribute to trait variation in such a manner. Some defence components, such as avoidance behaviour or self-inoculation [34], are unlikely to be specific to individual genotypes of a pathogen species and instead reduce the average success of a pathogen. Other defence steps, such as antibody production, will be induced in a tightly regulated response to a specific pathogen strain [35]. Within any given infection process there will be a multitude of different defence components that act to shape the phenotypic distribution of pathogen reduction, ranging from binary, all-or-nothing responses to those that act in a nonspecific manner regarding the pathogen genotype or even species designation [31].

Considering that the overall infection phenotype results from a combination of steps, with variable specificity to a pathogen, several conclusions can be drawn (Figure 2). First, if one step shows specific variation for host resistance, every subsequent step will appear to be influenced by this pattern. Thus, any genetic variation among host genotypes that arises at one step will typically also be seen among hosts when the entire defence system is considered. Second, if a single step completely blocks infection, the host is resistant regardless of how permissive the

other steps are. Third, if one step describes a binary genetic outcome (resistance yes or no), it will influence the shape of genetic variation such that either hosts are totally resistant or, in the case that they are susceptible at the binary step, the genetic variation expressed at the other steps might produce quantitative variation for susceptibility (i.e., conditionally quantitative). Thus, the reporting of phenotypic and genetic variation in disease traits depends on the steps included in the study, with corresponding consequences for predictions about the evolution of the system [31].

Only a Few Steps Facilitate Rapid Host–Pathogen Coevolution

Host–pathogen coevolution is often described by two alternative models that have drastic differences for our understanding of the stepwise infection process. In selective sweep coevolution, beneficial mutants arising go to fixation through directional selection [36,37]. In organisms with genetic recombination, sweeps can occur in genes responsible for different steps at the same time, and hosts and pathogens can experience sweeps in different steps. This establishes a complex scenario whereby the ability of a mutation to sweep to fixation might be influenced by concurrent or previous sweeps. A sweep at one step, for example, could make genetic variation at another step irrelevant, but if the pathogen were to overcome this first step, other steps would become important again. Host–pathogen coevolution could thus be influenced by a complex interplay between sweeps in hosts and pathogens at a variety of infection steps.

The selective sweep model is in sharp contrast to Red Queen coevolution, which is based on negative frequency-dependent selection of host and parasite alleles [38,39]. Here, specific pathogen genotypes track the common host genotypes, providing a selective advantage to rare host genotypes. Unlike selective sweeps, the Red Queen hinges on the presence of host genotype-by-pathogen genotype ($G_H \times G_P$) interactions for patterns of resistance and infectivity [33,40]. However, it is not the entire genomes of host and pathogen that are coevolving, but genes involved in interactions at an individual step. Not all points of contact between the antagonists will match this requirement. $G_H \times G_P$ interactions are required such that no host genotype is able to outcompete all other host genotypes in the presence of pathogens and no pathogen is able to infect all hosts (no supergenotypes [33,40]). The strongest signature of Red Queen coevolution is expected for candidate steps that control infection in all-or-nothing capacity and are mostly genetically (G) determined, as environmental (E) factors and $G \times G \times E$ interactions will impede the evolutionary dynamics [18].

A candidate for a step where $G_H \times G_P$ interactions are found is the recognition of a pathogen antigen, which occurs early in the infection process and is likely to be less dependent on environmental conditions [20]. By contrast, immune defences expressed late in an infection process are poorer candidates for Red Queen coevolution, as here selection on the host is weaker and the expression of this form of resistance is more likely to depend on host condition [20]. The *Daphnia*–*Pasteuria* system (Box 2 and Figure 1) offers insight into how individual steps help shape the process of host–pathogen coevolution. A time-shift experiment (i.e. [41]) revealed how temporal variation in pathogen infectivity fluctuated over time, as predicted by Red Queen dynamics, while traits related to the severity of disease increased steadily [42]. Subsequent work on the estimation of variance components across all steps resulted in a single step – the attachment of the pathogen to the host – being identified as the principle candidate for the fluctuating changes in infectivity observed in the sediment core study [43,44].

Implications of the Stepwise Infection Process

Blending together functional and evolutionary perspectives has important implications for the advancement of our understanding of infectious disease evolution and the guiding of research and discussion in other, associated biological phenomena (see Outstanding Questions). Work

Box 4. Genome Engineering and the Future of Disease Control

An appreciation of the infection process has been used for many years to manage the invasion of pathogens in agricultural crop systems. Resistance loci have been discovered that either control the early recognition of a pathogen and confer complete resistance (qualitative or R-gene loci [56]) or subsequently limit the characteristics of disease at the adult stage (partial or quantitative loci [85]). Most focus was traditionally placed on R genes, not only because they were easy to assay in greenhouse studies due to their large effect size but also because they masked the phenotypic contribution of any subsequent quantitative resistance locus (*sensu* Figure 2). Although R genes enabled high levels of resistance to plant pathogens, they were not necessarily durable (short-term persistence), as the intense directional selection and large effective population sizes they generate allow pathogens to rapidly adapt and overcome this defence [86,87]. Modern approaches to durable resistance have turned to promoting variation in the R-gene-controlled steps and combining different qualitative and quantitative resistance loci (i.e., resistance pyramiding), thus reducing the strength of selection on a pathogen [85,87,88].

With the emergence of genome editing tools such as the clustered regularly interspaced short palindromic repeats (CRISPR) system, the ability to engineer more-resistant hosts or vectors is close to fruition for many animal systems, including humans (e.g. [89]). For dengue fever, for example, the risk of dengue outbreaks is the extrinsic incubation period (EIP), or the time between the acquisition of the virus by the mosquito and the point where the virus reaches the saliva and is able to be transmitted to a new host [90]. To transmit, however, the virus must successfully pass through six barriers, including passing, establishing, and replicating in the midgut, escaping the midgut and transmitting to new tissues, and, finally, infecting and escaping the salivary gland barrier [70]. Targeting the pathways involved in transmission at each of these barriers (see also [70,91,92]) offers the opportunity to tailor particular interventions for maximum results – spreading genes into a target vector population that most effectively reduced the force of infection – while bearing in the mind the lessons learned regarding the role of quantitative and qualitative steps in the evolution of durable resistance.

on coevolutionary dynamics between hosts and pathogens has benefited greatly from the limited work on this topic already and will continue to do so with new, theoretical explorations of the role of multistep infection processes in the evolution of infectious disease. However, evolutionary insight on the stepwise infection process also has a role to play in evaluating how disease interventions made today will potentially impact the evolution of a host or pathogen in the future – particularly in the context of recent advances in genome editing and disease control (Box 4).

Extending the Theory of Host–Pathogen Coevolution

The theory of host–parasite interactions has made considerable inroads regarding the role of coevolution in shaping local adaptation, the evolution of sex, and the maintenance of genetic polymorphisms [45]. In each of these cases, a single step of the infection process, typically described as a matching-allele interaction, has been applied to fundamental ecological and evolutionary phenomena. Only a limited body of theory, however, has considered the evolutionary consequences of the multistep infection process. Motivated by the recognition that a host must first detect the invasion of a parasite and then control its proliferation, theory has explored how different host strategies can combine and trade off to limit the growth of pathogens [46] or affect the evolution of pathogen virulence [47]. Other studies have explicitly modelled infection as a two-step process, combining the influence of specific and nonspecific defence components [48], matching-allele- and gene-for-gene-like mechanisms [49], or inverse-gene-for-gene and gene-for-gene models of infection genetics [15] within a single coevolutionary model.

Emerging from these models are several concepts regarding the evolutionary dynamics of a multistep infection process. Despite a complex arrangement of multiple steps, coevolutionary dynamics can reduce to a simple one-step process if the costs of resistance or virulence are high enough. In the most extreme case, evolution of resistance at one step can lead to complete relaxation of selection for resistance at other steps, a phenomenon called ‘strategy blocking’ [50]. This single step can ultimately dominate the oscillating dynamics, regardless of whether the infection process contains a mixture of qualitative or quantitative steps (gene-for-gene- or matching-allele-like mechanisms) [15,48,49]. However, once different costs of host

and pathogen traits are considered, coevolution under a multistep scenario can lead to correlated changes in hosts alleles occurring across seemingly independent steps [15,47]. In certain circumstances, multiple defence mechanisms (i.e., multidimensional trait space) might help to increase the evolutionary potential of the host and allow it to more readily escape an attacking pathogen [14].

Open questions now relate to the optimal number of steps that facilitate escape from pathogens and whether the order that qualitative or quantitative resistance loci are arranged in the infection cascade matters for pathogen evolution [26]. However, including additional steps beyond a one- or two-step understanding of infection is not the only avenue for progress. In multistep models variation in the sensitivity of different steps to the environment has not been considered. Spatial and seasonal variability are ubiquitous and likely to facilitate the long-term maintenance of polymorphisms in resistance and virulence [51,52] by diversifying host–pathogen coevolution at different spatial scales or times [18,53,54]. However, as discussed, not all steps of the infection process are equally sensitive to environmental variability and some will be completely robust to any change (Figure 1). Insight into this topic would provide a valuable link between the pattern of infection uncovered in the laboratory and the variability of disease observed in nature.

Resolving the Genetic Architecture of Resistance

To better understand how and why organisms often succumb to infection, an ever-increasing number of studies have explored the polymorphisms that underlie the host defence system [16,55–57]. Arising from this work is the finding that susceptibility to infection is under the control of a limited number of genes of often large effect [11,44,58,59]. A meta-analysis of QTL mapping studies across plant and animal studies, for example, revealed that host resistance can typically be explained by two or three loci, explaining 30–50% of the variation in susceptibility to pathogens [57]. This seems to conflict with our understanding of complex trait genetics, where quantitative characteristics such as human height are predicted to be under the control of many alleles, each contributing only a small amount to the total observed variability [60]. This has led to the suggestion that the genetic architecture of resistance might be fundamentally different to that of other complex traits [16] (but see [17]); however, we argue that an understanding of the entire infection process can help resolve this issue.

Typical metrics of disease – be they mortality or pathogen load – are inevitably influenced by a chain-like series of events where each step is likely to be controlled by a different genetic architecture. Due to the dependence of later steps on what occurs earlier (see above), if one step is influenced by all-or-nothing alleles (qualitative loci, R genes), all subsequent estimates of infection will reflect the variation imposed by this major-effect gene (Figure 2). This organisation masks the potential contribution of minor or quantitative alleles to disease variation. A major-effect QTL for infection success, for example, can dominate the majority of the variation describing the severity of disease, even if the candidate alleles are not directly involved in the pathways controlling the target phenotype. Thus, while some aspects of the infection process will deviate from a quantitative genetic understanding of complex traits (i.e., host–pathogen compatibility), this is not necessarily true for all resistance components.

Isolating individual steps, and removing unwanted variation, will help improve the annotation of candidate loci by firmly annotating their function within a biologically defined stage of the infection process. Circumventing pivotal steps experimentally (e.g., a barrier defence can be avoided by injecting the parasite into the host) can expose minor genetic variation at other steps and make them accessible for quantitative work (Figure 2). Isolating single steps – and by this removing variation from other steps – also allows greater precision in our estimates of disease and a greater chance of identifying candidate loci. This is already an established approach in

research on infectious disease; for example, where pathogens are injected into particular target tissues thereby removing variation caused by earlier steps (e.g. [12]). However, careful partitioning of infection phenotypes by forward and backward exclusions (excluding early or late steps, respectively) can be applied to most systems (Figure 1).

Concluding Remarks

The unification of functional and evolutionary perspectives holds great promise for our understanding of the evolution of infectious disease. While the relative contributions of genetic and environmental variation have always been central to the study of host–pathogen interactions, distinguishing between different models of antagonistic coevolution, and predictions of how pathogens react to our control measures, are possible only when the coevolving steps and their genetic basis are understood (see Outstanding Questions). Our review has focussed firmly on classical host–pathogen systems, but the general approach of partitioning variation along a sequence of events holds much promise for our understanding of the evolutionary ecology of any antagonistic interaction, from the conflict that arises from brood parasitic birds [26] to the perturbations that occur as cell proliferation runs unchecked during the progression of cancer [8].

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Supplemental Information

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References

- Andersson, M.B. (1994) *Sexual Selection*, Princeton University Press
- Frank, S.A. (2004) Genetic predisposition to cancer — insights from population genetics. *Nat. Rev. Genet.* 5, 764–772
- Scott, M.P. and Carroll, S.B. (1987) The segmentation and homeotic gene network in early *Drosophila* development. *Cell* 51, 689–698
- Desvergne, B. *et al.* (2006) Transcriptional regulation of metabolism. *Physiol. Rev.* 86, 465–514
- Kitano, H. (2004) Biological robustness. *Nat. Rev. Genet.* 5, 826–837
- Visser, J.A.G.M. *et al.* (2003) Perspective: evolution and detection of genetic robustness. *Evolution* 57, 1959–1972
- Hunt, J. *et al.* (2009) Male–male competition, female mate choice and their interaction: determining total sexual selection. *J. Evol. Biol.* 22, 13–26
- Frank, S.A. (2004) Genetic variation in cancer predisposition: mutational decay of a robust genetic control network. *Proc. Natl. Acad. Sci. U. S. A.* 101, 8061–8065
- Lough, G. *et al.* (2015) Health trajectories reveal the dynamic contributions of host genetic resistance and tolerance to infection outcome. *Proc. Biol. Sci.* 282, 20152151
- Flajnik, M.F. and Du Pasquier, L. (2012) Evolution of the immune system. In *Fundamental Immunology* (7th edn) (Paul, W.E., ed.), pp. 67–129, Lippincott Williams & Wilkins
- Martins, N.E. *et al.* (2014) Host adaptation to viruses relies on few genes with different cross-resistance properties. *Proc. Natl. Acad. Sci. U. S. A.* 111, 5938–5943
- Martins, N.E. *et al.* (2013) Host adaptation is contingent upon the infection route taken by pathogens. *PLoS Pathog.* 9, e1003601
- Dubovskiy, I.M. *et al.* (2013) More than a colour change: insect melanism, disease resistance and fecundity. *Proc. Biol. Sci.* 280, 20130584
- Gilman, R.T. *et al.* (2012) Coevolution in multidimensional trait space favours escape from parasites and pathogens. *Nature* 483, 328–330
- Fenton, A. *et al.* (2012) Two-step infection processes can lead to coevolution between functionally independent infection and resistance pathways. *Evolution* 66, 2030–2041
- Hill, A.V.S. (2012) Evolution, revolution and heresy in the genetics of infectious disease susceptibility. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 367, 840–849
- Hall, M.D. and Ebert, D. (2013) The genetics of infectious disease susceptibility: has the evidence for epistasis been overestimated? *BMC Biol.* 11, 79
- Mostow, R. and Engelstädter, J. (2011) The impact of environmental change on host–parasite coevolutionary dynamics. *Proc. Biol. Sci.* 278, 2283–2292
- Rauw, W.M. (2012) Immune response from a resource allocation perspective. *Front. Genet.* 3, 267
- Rigby, M.C. *et al.* (2002) Why should parasite resistance be costly? *Trends Parasitol.* 18, 116–120
- Lynch, M. and Walsh, B. (1998) *Genetics and Analysis of Quantitative Traits*, Sinauer Associates
- Hall, M.D. *et al.* (2008) Experimental evidence that sexual conflict influences the opportunity, form and intensity of sexual selection. *Evolution* 62, 2305–2315
- Hall, M.D. *et al.* (2010) Competitive PCR reveals the complexity of postcopulatory sexual selection in *Teleogryllus commodus*. *Mol. Ecol.* 19, 610–619
- Shaw, R.G. *et al.* (2008) Unifying life-history analyses for inference of fitness and population growth. *Am. Nat.* 172, E35–E47
- Eck, D.J. *et al.* (2015) An integrated analysis of phenotypic selection on insect body size and development time. *Evolution* 69, 2525–2532

Outstanding Questions

Are there general trends over the long-term coevolution of hosts and pathogens? For example, the number of steps may increase or decrease, resistance polymorphisms may be more often seen during early steps, and host–pathogen interactions (necessary for Red Queen dynamics) may be limited to a single step.

What is the genetic architecture of resistance across the steps of infection? With the help of population genomic approaches, the role of qualitative and quantitative resistance in shaping infection outcomes will become apparent, even for non-model systems.

Do analogous steps of infection across host–parasite systems correspond to specific genes or gene families? The underlying genetics of analogous steps in different systems may be conserved, providing options for genetic interventions based on insights from model species.

Can an understanding of the stepwise process help us predict host ranges? Often, multiple host species are susceptible to a certain parasite but other, related host species are completely resistant. Determining which steps are responsible for excluding a host from a parasite's host range would help us understand the evolution of resistance and host ranges.

26. Feeney, W.E. *et al.* (2014) Advances in the study of coevolution between avian brood parasites and their hosts. *Annu. Rev. Ecol. Syst.* 45, 227–246
27. Catalão, M.J. *et al.* (2013) Diversity in bacterial lysis systems: bacteriophages show the way. *FEMS Microbiol. Rev.* 37, 554–571
28. Iranzo, J. *et al.* (2015) Immunity, suicide or both? Ecological determinants for the combined evolution of anti-pathogen defense systems. *BMC Evol. Biol.* 15, 43
29. Kirkwood, T.B.L. and Austad, S.N. (2000) Why do we age? *Nature* 408, 233–238
30. Day, T. *et al.* (2011) Bridging scales in the evolution of infectious disease life histories: theory. *Evolution* 65, 3448–3461
31. Schmid-Hempel, P. and Ebert, D. (2003) On the evolutionary ecology of specific immune defence. *Trends Ecol. Evol.* 18, 27–32
32. Leggett, H.C. *et al.* (2012) Mechanisms of pathogenesis, infective dose and virulence in human parasites. *PLoS Pathog.* 8, e1002512
33. Lambrechts, L. *et al.* (2006) Coevolutionary interactions between host and parasite genotypes. *Trends Parasitol.* 22, 12–16
34. Parker, B.J. *et al.* (2011) Non-immunological defense in an evolutionary framework. *Trends Ecol. Evol.* 26, 242–248
35. Frank, S.A. (1994) Recognition and polymorphism in host–parasite genetics. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 346, 283–293
36. Levin, B.R. (1981) Periodic selection, infectious gene exchange and the genetic structure of *E. coli* populations. *Genetics* 99, 1–23
37. Buckling, A. and Rainey, P.B. (2002) Antagonistic coevolution between a bacterium and a bacteriophage. *Proc. Biol. Sci.* 269, 931–936
38. Hamilton, W.D. (1980) Sex versus non-sex versus parasite. *Oikos* 35, 282–290
39. Dybdahl, M.F. and Lively, C.M. (1998) Host–parasite coevolution: evidence for rare advantage and time-lagged selection in a natural population. *Evolution* 52, 1057–1066
40. Salathé, M. *et al.* (2008) The state of affairs in the kingdom of the Red Queen. *Trends Ecol. Evol.* 23, 439–445
41. Gaba, S. and Ebert, D. (2009) Time-shift experiments as a tool to study antagonistic coevolution. *Trends Ecol. Evol.* 24, 226–232
42. Decaestecker, E. *et al.* (2007) Host–parasite “Red Queen” dynamics archived in pond sediment. *Nature* 450, 870–873
43. Duneau, D. *et al.* (2011) Resolving the infection process reveals striking differences in the contribution of environment, genetics and phylogeny to host–parasite interactions. *BMC Biol.* 9, 11
44. Routtu, J. and Ebert, D. (2015) Genetic architecture of resistance in *Daphnia* hosts against two species of host-specific parasites. *Heredity* 114, 241–248
45. Schmid-Hempel, P. (2011) *Evolutionary Parasitology*, Oxford University Press
46. Shudo, E. and Iwasa, Y. (2001) Inducible defense against pathogens and parasites: optimal choice among multiple options. *J. Theor. Biol.* 209, 233–247
47. Carval, D. and Ferriere, R. (2010) A unified model for the coevolution of resistance, tolerance, and virulence. *Evolution* 64, 2988–3009
48. Frank, S.A. (2000) Specific and non-specific defense against parasitic attack. *J. Theor. Biol.* 202, 283–304
49. Agrawal, A.F. and Lively, C.M. (2003) Modelling infection as a two-step process combining gene-for-gene and matching-allele genetics. *Proc. Biol. Sci.* 270, 323–334
50. Britton, N.F. *et al.* (2007) Evolution of defence portfolios in exploiter–victim systems. *Bull. Math. Biol.* 69, 957–988
51. Burdon, J.J. and Thrall, P.H. (1999) Spatial and temporal patterns in coevolving plant and pathogen associations. *Am. Nat.* 153, S15–S33
52. Thrall, P.H. and Burdon, J.J. (2003) Evolution of virulence in a plant host–pathogen metapopulation. *Science* 299, 1735–1737
53. Thompson, J.N. (2005) *The Geographic Mosaic of Coevolution*, University of Chicago Press
54. Nuismer, S.L. (2006) Parasite local adaptation in a geographic mosaic. *Evolution* 60, 24–30
55. Chapman, S.J. and Hill, A.V.S. (2012) Human genetic susceptibility to infectious disease. *Nat. Rev. Genet.* 13, 175–188
56. Thompson, J.N. and Burdon, J.J. (1992) Gene-for-gene coevolution between plants and parasites. *Nature* 360, 121–125
57. Wilfert, L. and Schmid-Hempel, P. (2008) The genetic architecture of susceptibility to parasites. *BMC Evol. Biol.* 8, 187
58. Magwire, M.M. *et al.* (2012) Genome-wide association studies reveal a simple genetic basis of resistance to naturally coevolving viruses in *Drosophila melanogaster*. *PLoS Genet.* 8, e1003057
59. Jalvingh, K.M. *et al.* (2014) Genomic changes under rapid evolution: selection for parasitoid resistance. *Proc. Biol. Sci.* 281, 20132303
60. Hill, W.G. *et al.* (2008) Data and theory point to mainly additive genetic variance for complex traits. *PLoS Genet.* 4, e1000008
61. Ebert, D. *et al.* (2016) A population biology perspective on the stepwise infection process of the bacterial pathogen *Pasteuria ramosa* in *Daphnia*. *Adv. Parasitol.* 91, 265–310
62. McGuckin, M.A. *et al.* (2011) Mucin dynamics and enteric pathogens. *Nat. Rev. Microbiol.* 9, 265–278
63. Serrano, M. *et al.* (2014) The cuticle and plant defense to pathogens. *Front. Plant Sci.* 5, 274
64. van Baalen, M. (1998) Coevolution of recovery ability and virulence. *Proc. Biol. Sci.* 265, 317–325
65. Drakesmith, H. and Prentice, A.M. (2012) Hepcidin and the iron–infection axis. *Science* 338, 768–772
66. Bansal, D. *et al.* (2005) Role of cholesterol in parasitic infections. *Lipids Health Dis.* 4, 10
67. Haine, E.R. *et al.* (2008) Antimicrobial defense and persistent infection in insects. *Science* 322, 1257–1259
68. Bell, A.S. *et al.* (2006) Within-host competition in genetically diverse malaria infections: parasite virulence and competitive success. *Evolution* 60, 1358–1371
69. Cory, J.S. and Myers, J.H. (2003) The ecology and evolution of insect baculoviruses. *Annu. Rev. Ecol. Syst.* 34, 239–272
70. Franz, A. *et al.* (2015) Tissue barriers to arbovirus infection in mosquitoes. *Viruses* 7, 3741–3767
71. Hall, M.D. and Ebert, D. (2012) Disentangling the influence of parasite genotype, host genotype and maternal environment on different stages of bacterial infection in *Daphnia magna*. *Proc. Biol. Sci.* 279, 3176–3183
72. Vale, P.F. *et al.* (2008) Temperature-dependent costs of parasitism and maintenance of polymorphism under genotype-by-environment interactions. *J. Evol. Biol.* 21, 1418–1427
73. Luijckx, P. *et al.* (2010) Cloning of the unculturable parasite *Pasteuria ramosa* and its *Daphnia* host reveals extreme genotype–genotype interactions. *Ecol. Lett.* 14, 125–131
74. Luijckx, P. *et al.* (2013) A matching-allele model explains host resistance to parasites. *Curr. Biol.* 23, 1085–1088
75. Metzger, C.M.J.A. *et al.* (2016) The Red Queen lives: epistasis between linked resistance loci. *Evolution* 70, 480–487
76. Vale, P.F. and Little, T.J. (2009) Measuring parasite fitness under genetic and thermal variation. *Heredity* 103, 102–109
77. Frank, S.A. (2008) Evolutionary dynamics of redundant regulatory control. *J. Theor. Biol.* 255, 64–68
78. Sheldon, B.C. and Verhulst, S. (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* 11, 317–321
79. Zuk, M. and Stoehr, A.M. (2002) Immune defense and host life history. *Am. Nat.* 160, S9–S22
80. Rowe, L. and Houle, D. (1996) The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. Biol. Sci.* 263, 1415–1421
81. Råberg, L. *et al.* (2007) Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. *Science* 318, 812–814
82. Rothstein, S.I. (2001) Relic behaviours, coevolution and the retention versus loss of host defences after episodes of avian brood parasitism. *Anim. Behav.* 61, 95–107

83. Edelman, G.M. and Gally, J.A. (2001) Degeneracy and complexity in biological systems. *Proc. Natl. Acad. Sci. U. S. A.* 98, 13763–13768
84. Frank, S.A. (2003) Genetic variation of polygenic characters and the evolution of genetic degeneracy. *J. Evol. Biol.* 16, 138–142
85. Poland, J.A. *et al.* (2009) Shades of gray: the world of quantitative disease resistance. *Trends Plant Sci.* 14, 21–29
86. McDonald, B.A. and Linde, C. (2002) Pathogen population genetics, evolutionary potential, and durable resistance. *Annu. Rev. Phytopathol.* 40, 349–379
87. Zhan, J. *et al.* (2014) Achieving sustainable plant disease management through evolutionary principles. *Trends Plant Sci.* 19, 570–575
88. Burdon, J.J. *et al.* (2014) Guiding deployment of resistance in cereals using evolutionary principles. *Evol. Appl.* 7, 609–624
89. Gantz, V.M. *et al.* (2015) Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*. *Proc. Natl. Acad. Sci. U. S. A.* 112, E6736–E6743
90. Tjaden, N.B. *et al.* (2013) Extrinsic incubation period of dengue: knowledge, backlog, and applications of temperature dependence. *PLoS Negl. Trop. Dis.* 7, e2207
91. Dong, S. *et al.* (2015) Heritable CRISPR/Cas9-mediated genome editing in the yellow fever mosquito, *Aedes aegypti*. *PLoS One* 10, e0122353
92. Sim, S. *et al.* (2012) Dengue virus infection of the *Aedes aegypti* salivary gland and chemosensory apparatus induces genes that modulate infection and blood-feeding behavior. *PLoS Pathog.* 8, e1002631