Review

Darwinian interventions: taming pathogens through evolutionary ecology

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From the seemingly inevitable emergence of antibiotic resistance following drug use to the necessity of annual influenza vaccine updates, the ability of pathogens to evolve appears to preclude the development of effective, long-term interventions for many diseases. Despite this gloomy forecast, recent research explores how various principles and concepts from evolutionary ecology might inform practical attempts to bring these disease-causing agents under greater human control. By utilizing evolutionary and ecological information at various scales, these works investigate some promising avenues of disease intervention, from trapping pathogens in evolutionary dead ends, through slowing or inhibiting the process of pathogen adaptation, to more accurate forecasting of pathogen evolutionary trajectories. Thus, an evolutionary ecology perspective might ultimately provide powerful new tools in the pursuit of enduring solutions to the problem of treatment-driven pathogen evolution.

The war on pathogens

Following the epidemiological successes of antibiotic drug development and mass vaccination programs in the post World War II era, the prevailing attitude among experts was that the war between humans and their pathogens was essentially over [1]. This initial optimism has recently given way to a more sobering reality: human interests continue to be plagued by both novel and familiar pathogens, as evidenced by the growing lists of emerging (including HIV/AIDS, Hepatitis C, SARS, foot-and-mouth disease and avian influenza) and re-emerging (including malaria, cholera and tuberculosis) diseases [2].

Numerous ecological, environmental and social phenomena have been implicated as causal factors in the epidemiological shifts that favor the spread of these diseases [3]. Anthropogenic disturbances, such as the expansion of agricultural lands into previously undisturbed regions, increase the probability of contact and disease transmission between wild species and humans or domesticated livestock. Increased susceptibility to infection, driven either by intrinsic demographic changes such as the ageing of populations, or by extrinsic factors, including the presence of concomitant immunosuppressive infections such as HIV, affect disease emergence and spread in both developed and developing nations. Urbanization, by increasing local population densities, restructuring social contact networks, and creating the conditions for the development of unhygienic slum areas, has also been recognized as a major factor promoting the emergence and re-emergence of infectious diseases [3,4].

It is becoming increasingly apparent that these types of changes can have dramatic evolutionary consequences [5]. Of paramount interest is the alarming revelation that the very vaccines and chemical therapies used by the medical and agricultural industries are probably the main culprits driving the evolution of viral and microbial pathogens. The rise of resistant strains of many clinically important pathogens (such as *Staphylococcus aureus* and *Streptococcus pneumoniae*) is thought to be attributable largely to the injudicious use of antibiotics [5,6].

With their typically large population sizes, rapid rates of reproduction and mutation, combined with the strong selection placed on them by vaccines or antibiotics, pathogens provide ideal case studies of evolution in action. Indeed, in no other realm of human endeavor does the Darwinian paradigm of 'variation + selection = evolution' play out with more relevance or profound importance to humans. Correspondingly, nowhere is an evolutionary perspective more indispensable than in the development of effective and sustainable treatments of microparasitic diseases.

The recent proliferation of books [7] and reviews [8,9] emphasizing the importance of ecological and evolutionary perspectives in the study of pathogens is testament to the growing appreciation of such thinking in infectious disease epidemiology. Here, I review some recent works that demonstrate the value of these perspectives in the study of pathogen evolution in response to vaccines and antibiotics.

For illustrative purposes, discussion is framed around two contemporary evolutionary ecology ideas that have far-reaching utility, both in making sense of observed epidemiological and evolutionary patterns, and in illuminating the underlying processes that shape these patterns: (i) source–sink dynamics; and (ii) competitive interactions. The review focuses on studies that, by appealing to such concepts, identify aspects of the broader ecological and evolutionary context of the host–pathogen interaction that can be manipulated, or otherwise utilized, to more effectively control epidemiological and evolutionary outcomes. Ultimately, such research might help to demonstrate how general ecological principles and concepts can be informatively applied to very specific disease

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Box 1. Virulence as a life-history tradeoff

In a seemingly unremarkable coincidence, 1859 saw the release not only of Darwin's *On the Origin of Species*, but also of 24 European rabbits onto Thomas Austin's property in Victoria, Australia for the purpose of sport hunting. In less than a century, Australia was home to approximately 600 million rabbits. In 1950, after numerous failed attempts at control, the myxoma virus, causative agent of myxomatosis, was introduced into the now wild rabbit populations. Two years later, after massive mortality, approximately 100 million remained [51].

Although initially highly pathogenic, the myxoma virus appeared to evolve to a stable level of attenuated virulence over the course of the following decade. Subsequent work showed that increased virulence is associated with both a decreased recovery rate from infection in rabbit hosts and an increased rate of transmission of the virus [51,52]. In fact, detailed numerical studies show that myxoma virus fitness is maximized at intermediate levels of virulence [52], with the risk of host death through virulence being balanced by the benefits of longer, or more efficient, transmission. As such, the rabbit–myxoma system is a classic example of what has been termed the 'tradeoff' model of pathogen virulence [52].

The tradeoff model can be viewed as a specific manifestation of life-history theory that aims to understand the evolution of strategies that optimize reproductive success [7]. From this perspective, pathogen fitness-determining traits, such as transmission, infection duration and virulence are all linked through their mutual dependence on the rate at which pathogens exploit hosts. Selective regimes that change the tradeoff structure, such as vaccination, will thus induce evolutionary change in this exploitation strategy, which in turn alters pathogen traits [19].

Although explicit tradeoffs have been numerically constructed for a few diseases (including the myxoma system) [14,52], the chief drawback of the model lies in the paucity of empirical studies confirming the widespread existence of such tradeoffs. This has led some authors to question the generality of the tradeoff as applied to host–parasite systems, as well as the validity of the virulence management agenda with respect to tradeoff manipulation [19,23].

The issue is far from resolved, but recent work on malaria [14] and HIV [53] offers examples of diseases of great import to public-health issues where the life-history perspective could provide insights into how virulence is shaped. In turn, such insights might help to determine the evolutionary consequences of the vaccines currently being sought to combat these important contributors to the global infectious disease burden.

Box 2. Vaccine-driven virulence evolution

Beginning with the work of Gandon *et al.* [26,54], recent theory has examined how vaccines that offer only a degree of protection to hosts and curative therapies are expected to influence the evolution of parasite life histories and, in particular, virulence traits [17].

The earliest studies of this type found that treatments that reduce the rate at which infection is either acquired (infection-blocking) or transmitted (transmission-blocking) are nonselective, whereas treatments that diminish mortality owing to infection (antitoxin) or reduce pathogen exploitation (growth-suppressing) select for increased exploitation and, hence, greater virulence [26,54]. Other models have shown that increasing the rate at which hosts become infection-free owing to an immune response (clearance-augmenting treatment) also selects for increased virulence (see, for example, Ref. [55]).

More mechanistically explicit models of the interplay between infection and immunity produce predictions largely in accord with those of Gandon *et al.* [26]. However, Ganusov and Antia [56] make the case that, in acute infections, transmission-blocking treatments can select for increased pathogen replication rates, leading to higher virulence. This follows from the realistic assumption that host immune responses probably exhibit some time dependence as they develop.

The findings of these works are all based on imposing treatments on an initially homogeneous host population, where all individuals are equally likely to be infected and respond to infection in identical fashion. More recently, the evolutionary consequences of interventions that target particular groups of hosts when the population can be partitioned into classes based on susceptibility to infection and vulnerability to mortality once infected have been explored [57]. The results show that, as the most susceptible and/or vulnerable hosts typically select for less-intense exploitation, any intervention that increases the weight of these host types in the determination of total pathogen transmission will select for decreased virulence. For example, targeting the most susceptible or vulnerable host type with an antitoxin vaccine can, in contrast to earlier predictions [54], select for reduced exploitation by this mechanism. Indeed, it is possible to reverse the predicted effect of all previously considered vaccine types by targeted treatment of either the least or most susceptible/vulnerable hosts (Table 2).

The type of host heterogeneities required to allow for these possibilities are probably common in many clinically important diseases, as indicated in a Nigerian study of human malaria [14]. Here, it was shown that children under 8 years of age contribute most to parasite transmission, but also experience the highest rates of virulence. Thus, targeted treatment plans could provide a new tool in the design of virulence management strategies.

situations, while also aiding in the development of novel public health practices and policies within a rational Darwinian framework.

Throughout, special attention is paid to illustrating how various ideas and concepts are being brought to bear on questions of virulence (i.e. the degree to which pathogens harm their host) evolution (Boxes 1–4), as it is the mortality effects of pathogens that are often of paramount interest to researchers and policy makers. From a human health perspective, this is understandable, as 16 of the 20 leading causes of global human mortality are attributable to infectious diseases [3]. Finally, I conclude by speculating on the future directions of research that could fruitfully employ an evolutionary perspective in the pursuit of enduring solutions to the problem of treatment-induced pathogen evolution.

Treatment-induced pathogen evolution

From the emergence of multi-drug resistant 'superbugs' [10] to the seasonal adaptations of influenza [11], a rising tide of evidence supports the claim that vaccines and antibiotic treatments can impose strong selection on numerous pathogen species that ultimately undermines treatment efficacies [12] (Table 1). For example, the introduction of penicillin in 1943 was quickly followed by the steady rise in frequency of penicillin-resistant bacteria, a pattern that has, more or less, been repeated following the widespread dissemination of every new antibiotic [13]. In a similar, although less dramatic, fashion, vaccination also appears to have selected for new pathogen strains, raising the spectre of the evolution of less treatable pathogens in response to vaccination [12,14,15].

Historically, studies of treatment-driven evolution have focused on three eventualities: (i) the emergence of immune escape variants [16,17], which occurs when heritable mutations induce structural alterations in the pathogen proteins used by the host immune system to identify, target and eliminate pathogenic invaders. This promotes the emergence of strains that evade immune recognition, a process that has been observed in numerous clinically important pathogens, including influenza, HIV,

Box 3. Sources, sinks, and virulence

Within hosts, source–sink theory has been used to address a particular model of the evolution of pathogen virulence [58]. In this incarnation, virulent effects are the consequence of repeated, albeit transient, evolution of adaptations that allow for the utilization of novel host tissues or structures with resultant pathogenic effects [19]. For example, through recurrent mutations in genomic regions affecting the FimH adhesin, the typically commensal, large-intestine dwelling bacterium *Escherichia coli* can acquire the ability to colonize host urogenital tracts, resulting in acute, although rapidly cleared, infections [59]. Moreover, such mutants are at a selective disadvantage in the original intestinal environment, and are quickly outcompeted by wild-type bacteria in the event of back-infection (see Figure 1a). Similar source–sink mechanisms might also underlie the virulent effects of polio and bacterial meningitis [19].

The potential for virulent mutants to arise through within-host selection has led to a number of proposals to circumvent such short-sighted evolution [60]. The first of these suggests that effective vaccination (or other treatment) schemes should focus on those strains of microorganisms actively involved in the transmission cycle, rather than all strains or even just the sink varieties directly responsible for virulent effects. Furthermore, by targeting these source genotypes, interventions accrue the additional benefit of reducing the probability of a virulence-causing variant arising through mutation by diminishing the within-host population of microorganisms. A final issue concerns the development of attenuated pathogen strains for live vaccines. Here, it is not only important to knock out active virulence determinants, but also to modify quiescent virulence genes that could be activated through mutation in the course of short-sighted evolution. Such regulatory losses of virulence suppression mechanisms appear to underlie the high levels of toxin production in *Bordetella pertussis* relative to other *Bordetella* species [61].

A novel application of the source–sink concept concerns the design of therapeutic treatments that target viral agents through the use of ecological traps, sink environments that, through sensory limitations, are easily mistaken to be sources. As noted by Repik and colleagues [62], viruses will bind to any host cells that possess compatible receptors, regardless of whether they can support viral replication. This inability to assess cell quality provides the means to trap viruses, diminishing their reproduction and pathogenic effects. This effect has recently been demonstrated in an experimental study of Coxsackievirus-infected mice where, by introducing mouse erythrocytes engineered to bind the virus, researchers were able to reduce the within-cell density of active viral particles and thereby extend survival times [63].

Box 4. Within-host competition and virulence

How strain competition might modulate selection on pathogen phenotypes has received a good deal of theoretical attention in the context of virulence evolution. The general consensus of these studies is that within-host competition promotes greater virulence (reviewed in Ref. [64], but see Ref. [35]). The intuition underlying this expectation is clear: by more rapidly utilizing host resources to generate transmissible stages, exploitative pathogens experience elevated transmission benefits relative to their more prudent relatives, while sharing the same risk of host mortality.

Empirical investigations of this question have also proliferated in recent years, mostly with respect to malaria. These experiments demonstrate that, in mixed-clone infections of avirulent and virulent strains of the rodent malaria agent *Plasmodium chabaudi*, both strains suffer reduced growth and transmission, indicating competitive suppression, with the effect being most pronounced for avirulent types [64–66].

Initial work on the influence of host resistance on competitive suppression compared the within-host performance of avirulent and virulent clones of *P. chabaudi* in genetically resistant and non-resistant hosts [66]. Researchers found that host resistance exacerbates the competitive disadvantage of avirulent strains, although a lack of resistance allowed avirulent types to persist and even perform better than if established without a competing strain. These results suggest that immune-augmenting vaccines might magnify the competitive suppression of avirulent strains, potentially leading to the evolution of higher virulence [66].

In support of this proposition, Raberg *et al.* [67] demonstrated pronounced suppression of avirulent strains of *P. chabaudi* when assayed in mice previously immunodeficient but re-invested with immunity by the addition of T cells, relative to an immmunodeficient control. These findings could prove troublesome, as many of the malaria vaccines under development work by augmenting T-cell immunity [68].

However, more recent studies challenge the view that such vaccines could play much of a role in driving evolution [35,69]. Barclay *et al.* [69] report that reductions in the number of host $CD4^+$ T cells do not result in a relaxation of between-strain competition. Perhaps even more emphatically, Grech *et al.* [35] show that, in mice hosts immunized either by prior exposure to infection or by treatment with a recombinant antigen, competitive suppression remains largely unaffected.

Nevertheless, enthusiasm for such results needs to be tempered. While immune-augmenting treatments might not drive virulence evolution through their effects on strain–strain competitive interactions, they can select for its increase by reducing the costs of exploiting the host. This prediction arises from theory if the tradeoff model of virulence applies [55] (Box 1) and is consistent with the results found in some experimental malaria systems [14,70].

dengue and malaria [18]; (ii) changes in the rate at which pathogens exploit their hosts [17], where the focus is on developing a framework for accurately forecasting how different interventions influence the evolution of pathogen virulence [19] (Boxes 1 and 2); and (iii) the evolution of drug resistance [20,21] in response to antibiotics (see Table 1). Studies of this type investigate the genetic and environmental factors that promote the acquisition of genetic elements, either through mutation or horizontal transfer, allowing for continued growth and reproduction of pathogens despite antibiotic treatment.

Although this partitioning has proven useful in focusing and organizing theoretical and empirical results, it is, nevertheless, an oversimplification. In particular, these categories are not mutually exclusive, with actual evolutionary outcomes often combining elements of each [8]. For example, *Bordetella pertussis* exhibits both antigenic and virulence evolution in response to vaccination. Indeed, the epitopes of many pathogens also function as virulence-determining factors [12,22] (Table 2).

In the context of the tradeoff model (Box 1), which assumes that functional relationships between different traits constrain their mutual evolution, Koella and Turner [23] recently discussed the possibility that the relationship between pathogen-induced damage of host tissues (i.e. virulence) and within-host growth, reproduction, and subsequent transmission to new hosts, can change during the course of drug resistance evolution, and hence drive the evolution of virulence. In fact, concomitant increases in both drug resistance and virulence have been observed in a number of different pathogens species [10], perhaps indicating common selection for the acquisition of both phenotypes [13].

Finally, even if evolution proceeds in only one of these directions, it need not be repeatable across populations. For example, in the USA, the virus causing infectious bursal disease in poultry appears to have undergone epitope evolution in response to vaccination. By contrast, the

Table 1. Examples of vaccine- and drug-induced pathogen evolution[a]

Disease	Type of evolution	Treatment	Ref.
Acinetobacter baumannii	Resistance	Antibiotic	[10]
Bordetella pertussis	Virulence and Antigenic	Vaccination	[15,22]
Candida albicans	Resistance	Antibiotic	[8]
Clostridium difficile	Virulence	Antibiotic	[8]
Corynebacterium diphtheria	Antigenic	Vaccination	[15]
Escherichia coli	Virulence and Resistance	Antibiotic	[6]
Marek's disease	Virulence and Antigenic	Vaccination	[15]
Haemophilus influenza	Antigenic	Vaccination	[25]
Human influenza A virus	Resistance	Antibiotic	[31]
Hepatitis B	Antigenic	Vaccination	[8,15]
Human immunodeficiency virus 1	Resistance	Antibiotic	[8]
Infectious Bursal Disease	Virulence	Vaccination	[12,14]
Klebsiella pneumonia	Resistance	Antibiotic	[10]
Methicillin-resistant *Staphylococcus aureus (MSRA)*	Virulence and Resistance	Antibiotic	[8]
Mycobacterium spp.	Resistance	Antibiotic	[10]
Neisseria meningitides	Antigenic	Vaccination	[8,25]
Plasmodium chabaudi	Virulence	Vaccination	[70]
Plasmodium falciparum	Virulence and Resistance	Antibiotic and Vaccination	[8,70]
Pseudomonas aeruginosa	Resistance	Antibiotic	[10]
Streptococcus pneumoniae	Resistance and Antigenic	Antibiotic and Vaccination	[6,25]
Vancomycin-resistant *Enterococci (VRE)*	Virulence and Resistance	Antibiotic	[10]

[a]The left-most column indicates the pathogen or disease that has undergone treatment-driven evolution, and the second and third columns indicate the trait that has evolved (pathogen virulence, antibiotic resistance, and/or antigenic profile) and the selective agent responsible (antibiotic and/or vaccine), respectively. The fourth column provides references for each instance of treatment-driven evolution.

Table 2. Contrasting vaccine-driven virulence evolution in homogeneous and heterogeneous hosts [a]

Vaccine type	Evolutionary effect on virulence		
	Homogeneous hosts	Heterogeneous hosts[d]	
		Less vulnerable/susceptible	More vulnerable/susceptible
Infection-blocking	No effect [b]	−	+
Transmission-blocking	No effect [b]	−	+
Anti-toxin	+ [b]	+	+ or −
Growth-suppressing	+ [b]	+ or −	+ or −
Clearance-augmenting	+ [c]	+ or −	+

[a]The left-most column identifies the manipulated vaccine type, whereas the remaining columns give the predicted evolutionary effect of an increase in the efficacy of the vaccine type in question on pathogen virulence. A + (respectively, −) symbol indicates increased (respectively, decreased) virulence is the predicted outcome. In cases where + or − is indicated, evolutionary outcomes depend on initial epidemiological parameters. The second column shows vaccine effects when all hosts are initially identical, and the third (respectively, fourth) column gives the results assuming that vaccination targets the least (respectively, most) vulnerable/susceptible hosts in an initially heterogeneous population.
[b]From Ref. [54].
[c]From Ref. [55].
[d]From Ref. [57].

viruses circulating in European populations have evolved higher virulence [12]. Such examples not only illustrate the adaptive flexibility of pathogens, but also serve as an important reminder of the need for caution when applying general conceptual models to specific disease situations.

Evolutionary modeling

The categorical distinctions discussed above are also reflected in the modeling approaches utilized to study the different evolutionary questions. Resistance evolution studies often employ the formalism of population genetics [20], whereas studies of vaccine-driven evolution have largely employed one of two methods: (i) multi-strain differential equation models, or their stochastic analogues, to investigate the process of immune evasion [18]; or (ii) game theory models to study tradeoffs and life-history evolution [17,24] (Box 1).

Immune evasion models typically follow the post-vaccination, short-term temporal evolution of the frequencies of various strains of a pathogen that differ in their antigenic profiles [16,25]. By contrast, game-theoretic models typically assume that, at epidemiological equilibrium, host populations are dominated by a single wild-type pathogen, which occasionally competes with a rare 'mutant' strain exhibiting a phenotype only slightly different from that of the wild type. This process is repeated until a strain that cannot be invaded by any other mutant-type is achieved. This framework can be further subdivided based on whether one takes a between- or within-host perspective (Box 2).

Each of these approaches has spawned a vast theoretical literature, allowed for the discovery of important epidemiological and evolutionary insights, and provided the impetus for numerous empirical studies. However, understanding the relationships between the various aspects of treatment-driven evolution is hindered by the lack of a unified mathematical basis for modeling these phenomena. As a partial remedy to this problem, a number of recent studies have addressed the artificial dichotomy between escape and life-history mutant approaches to vaccine-induced evolution [16,22]. Other authors have proposed that a framework akin to population genetics can effectively subsume all of these modeling approaches while avoiding some of their shortcomings [17,24]. Although

much work remains, such efforts offer the promise of a more synthetic theoretical framework for the study of treatment-driven evolution.

Empirical evolutionary ecology

Empirical works are also exploiting a number of evolutionary ecology concepts in the study of pathogen evolutionary responses to treatment. In particular, both source–sink dynamics and ecological competition theory are being profitably employed in the study of pathogen populations at multiple levels of organization, from within single hosts to among hosts within a population and between different host populations.

Source–sink dynamics

An organism's environment often comprises multiple connected habitats, some of which provide the conditions required for population persistence ('sources'), and some which do not ('sinks'). While the source–sink concept is most commonly utilized in population-ecology studies, it also has pervasive utility in the study of treatment-induced infectious disease evolution (Box 3). Treatment strategies that aim to prevent pathogen spread must, by necessity, result in the treated hosts infecting, on average, fewer than one other host. Thus, in the language of ecological theory, such hosts are sink environments, in which pathogens are operating at a demographic deficit [26].

Theory has identified both high migration rate and limited environmental harshness as crucial elements in the fostering of adaptation to sink environments [27]. The importance of these factors in governing the emergence of antibacterial resistance has recently been borne out in an *in vivo* investigation of the hospital pathogen *Pseudomonas aeruginosa*, where it was shown that low antibiotic concentrations, coupled with a steady influx of pathogens into treated environments, resulted in rapid resistance evolution [28]. Such insights could have important implications for the management of antibiotic resistance in hospital settings by suggesting that resistance evolution might be forestalled by controlling the input of susceptible bacteria from community source environments into clinical sink settings [28].

Recent work investigating global spatiotemporal patterns of influenza A epidemiology and strain diversity indicates that broad-scale source–sink dynamics shape the ecological and evolutionary context of seasonal epidemics (see Figure 1b). Utilizing antigenic cartography, Russell *et al*. [29] determined the origin and fate of strains of the human influenza A virus, H3N2 subtype, by studying the evolutionary dynamics of the hemagglutinin surface glycoprotein. Analyses indicated that global seasonal epidemics are probably initiated by strains emerging from East or Southeast Asian source populations, thereafter circulating to other parts of the world before finally dying out in temperate sink regions. Similar patterns consistent with this source–sink interpretation were reported by Rambaut *et al*. [30] for both A/H3N2 and A/H1N1 subtypes. These findings could have important implications for the design of seasonal influenza vaccines: by focusing surveillance efforts on detecting newly emerged Asian influenza A strains early in the season, it might be possible to forecast more accurately which strains to include in vaccine formulation [29].

This broad-scale perspective on influenza evolutionary epidemiology also offers some insight into a puzzling global pattern in the frequency of A/H1N1 resistance to the antiviral agent oseltamivir. During the 2007–2008 influenza season, Norway, despite sparing use of oseltamivir, reported that 67.3% of H1N1 strains were resistant, whereas Japan, with the highest usage rates of oseltamivir in the world, found resistant viruses in only 3% of cases. One possible interpretation of these findings is that they reflect the effects of antiviral selective regimes in Asian source populations, followed by viral migration to regions of higher latitude, with the signature of selection in these temperate sink populations then being lost as a result of local strain extinction without back migration to the Asiatic source [31]. Recent work on the emergence and subsequent worldwide spread of resistance to the adamantane class of antiviral drugs by A/H3N2 influenza supports a similar model of source–driven selection for antiviral resistance [32]. Here phylogenetic analysis reveals that, despite at least 11 independent origins of resistance, most circulating viruses trace their lineage to a single emergence arising in Hong Kong in 2003.

Such examples illustrate how source–sink dynamics, operating at regional and global scales (and even within single hosts; see Box 3 and Figure 1), can play a critical role in shaping disease ecology and evolution. In particular, by de-emphasizing the importance of local selection pressures shaping locally adapted phenotypes, a source–sink perspective allows for the seemingly intractable complications arising from local evolution, genomic reassortment, continual antigenic change and circulation of influenza viruses to be synthesized into a coherent model of influenza global epidemiology. If this model can indeed be used to help to identify regional hotspots of viral activity and to suggest where to target limited resources aimed at disease treatment and prevention [4], it would provide a valuable blueprint to follow in future studies of disease evolutionary ecology.

Competitive interactions and pathogen evolution

Treatment-driven strain replacement [25], represented by an increase in the frequency of either immune escape variants in response to vaccination or of resistant clones in response to antibiotic therapy, requires that treatment induces a switch in different strains' abilities to compete for hosts at either the between- or within-host levels. This can arise if vaccines are strain specific: vaccinated hosts are then less vulnerable to infection by the epidemiologically dominant targeted strain types, but remain available to untargeted variants. If enough hosts are vaccinated, strain dominance can change owing to a population-level competitive advantage of untargeted types resulting from differential access to vaccinated hosts [25].

More recent theory has challenged the necessity of differential vaccine efficacy in driving strain replacement by considering within-host competition; even if all strains are equally susceptible to a vaccine, vaccination can still promote replacement if strains vary in their superinfectivity (ability to take over a host infected with a different

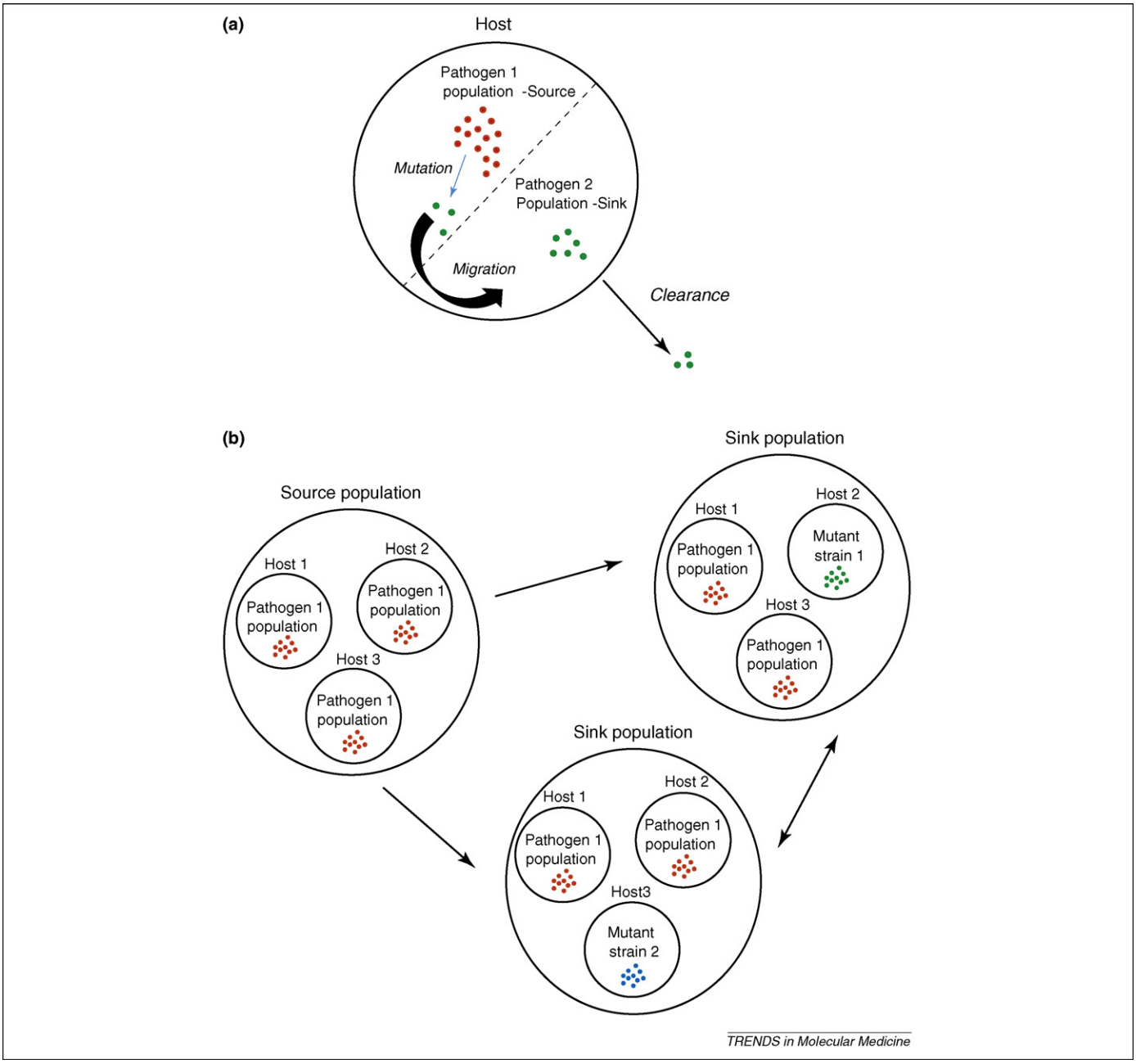


Figure 1. The evolutionary ecology context of source–sink dynamics. **(a)** Within-host context. Dashed line represents within-host compartmental separation. Left compartment allows for persistence of red pathogen. Mutation (green pathogen) allows for ephemeral colonization of right compartment before eventual clearance by host immune response. Back-migration by mutant to left compartment is negligible because outcompeted there by red pathogen. **(b)** Metapopulation context. Left-most source population allows for persistence of pathogen and migration to sink populations. Mutant strains appearing in sink populations (blue and green strains) are not exported out of sinks, while the signature of selection in source population can be detected in sinks owing to migration (red strain).

strain) [33]. In this case, the direct action of the vaccine is nonselective, but favors one type indirectly through its influence on the effective density of hosts available to be colonized by each strain, which is a function of superinfection abilities. Similar results are also possible if strains vary instead in their co-infectivity (ability to establish a coexisting infection in an already infected host) [25]. This theory demonstrates how the details of among-strain competitive interactions can be important determinants of escape mutant dynamics in response to vaccination.

Indeed, for the many infectious diseases that are characterized by a high degree of within-host genetic diversity [2], among-strain competition represents an important aspect of the within-host ecological context. Depending on their modes of action, therapeutic and prophylactic treatments can disrupt competitive hierarchies and thereby influence the selective regimes shaping pathogen evolution [21] (Box 4).

For example, strain-specific vaccines (or other treatments) could decrease the frequencies of targeted types by reducing their fitnesses while leaving untargeted types unaffected [34]. Alternatively, in mixed infections, untargeted strains might experience not only a relative fitness benefit owing to the decreased presence of targeted types, but an absolute increase in fitness as well, owing to the relaxation of within-host competition with these targeted strains. Such a process is known in the ecological literature as 'competitive release' [21].

Although the phenomenon of vaccine-induced competitive release is possible in principle [34], and several authors have suggested particular situations where its effects might be important, as in the case for vaccines designed to target cerebral [12] or pregnancy-associated malaria [35], the promotion of strain replacement through competitive release mechanisms has been discussed more fully with respect to the evolution of drug resistance. In the absence of treatment, resistance phenotypes are typically associated with decreased fitness relative to the sensitive wild-type strain [21]. The greater this competitive disadvantage, the greater the boost in fitness precipitated by the removal of sensitive competitors. This can greatly accelerate the spread of resistant strains, as well as the rate at which antibiotics are rendered ineffective [20].

Much of the work on this process has focused on malaria [20,34,36], although the relevance of competitive release for the evolution of viral and bacterial resistance has also been acknowledged [21]. Data from correlational human studies are broadly supportive of a role for competitive release in the emergence of chloroquine-resistant strains of *Plasmodium falciparum* [35]. Moreover, recent experimental studies using the mouse–*P. chabaudi* system have demonstrated competitive release following treatment with the antimalarial pyrimethamine [34,36].

In another experiment, using subcurative doses of pyrimethamine, susceptible clones were not totally removed from the infection, continued to competitively suppress the resistant strain and slowed its post-treatment growth [34]. An intriguing possibility discussed by the authors in light of this finding is the design of treatment plans that exploit within-host ecological structure in order to restrain the evolution of drug resistance [34].

Although the benefits of retaining competitive suppression through subcurative treatment would need to be weighed against the risk of increased persistence time, allowing for the evolution of resistance requiring multiple mutations [34], the concept is certainly an interesting prospective use of evolutionary ecology thinking. Moreover, if this were able to extend the time that a drug remained viable, it would offer an important counterexample to the reigning paradigm of 'use it and lose it' in regards to the inevitable loss of antibiotic efficacy following treatment [37].

Mixed *Plasmodium* species infections are common in endemic regions [38], so that malaria provides a natural focus for discussing competitive effects in disease determination. But the ubiquity of competitive interactions in ecological communities implies that interspecies interactions, as well as intraspecies interactions, might have general relevance in the evolutionary ecology of diseases.

A co-evolutionary arms race

To an infecting pathogen, treatment is akin to abrupt evolutionary change in the host, imposing strong selection on the pathogen and precipitating reciprocal evolutionary change. In turn, combating these newly evolved pathogens requires the development of novel chemical treatments and vaccines, again selecting for further pathogen adaptations, and so on. This process can be likened to an endless co-evolutionary arms race between host and pathogen, each partner continually evolving just to keep pace with the other, a process colorfully termed 'Red Queen' dynamics [2].

Staying ahead in this race requires gaining the upper hand in one or more facets of the co-evolutionary game. The discussions above show how two evolutionary ecology concepts, by shifting focus away from the strictly mechanistic aspects of the host–pathogen interaction and placing it within a web of broader ecological interactions, have shaped theory and practice with this end in mind. However, molecular techniques have now progressed to the point where we can begin to consider extending the reach of evolutionary ecology thinking in this endeavor to target directly the mechanisms that drive the generation of variation and the quality of selection that are fundamental to the micro-evolutionary process. Some of the more promising recent developments in this research program are discussed below.

Experimental evolution

In the realm of drug-resistance evolution, a first-line of effort consists of experimental evolution studies to determine both the possibility and probability of various evolutionary routes that a pathogen might take. One approach, termed the Barlow–Hall method [37], begins by subjecting target genes to site-directed mutagenesis and artificial recombination, then assaying the resultant mutant gene's function against the antibiotic of interest. The process is then repeated until evolutionary change in resistance ceases [37].

In vitro studies employing this technique have successfully reproduced the known *in vivo* evolutionary trajectory of the TEM-1 β–lactamase gene in response to β–lactam antibiotics, as well as to predict the same gene's eventual acquisition of greater resistance to the antibiotic cefepime in response to continued clinical use. The Barlow–Hall procedure can also be used to investigate when genes (such as IMP-1 β-lactamase or aac-6′-Iaa of *Salmonella enterica* serovar Typhimurim, for example) will not evolve further resistance, to impeninem or a number of aminoglycoside antibiotics, respectively [37]. This is possible because the Barlow–Hall mutant-generating protocol produces not only all the novel genotypes that are likely to arise *in vivo*, but also mutation combinations that are highly improbable in natural settings. Failure to produce resistant phenotypes under such permissive conditions thus allows researchers to conclude that the genes in question probably lack the evolutionary potential to acquire further resistance. By establishing the set of pathogen phenotypes that could result from a given treatment, the Barlow–Hall method functions as an early warning system, providing time for developing contingencies either to disrupt evolutionary outcomes or to treat them effectively. In addition, it can help to dispel the commonly held belief that resistance genes have a near limitless flexibility, while experimentally establishing the limits to this evolutionary plasticity [37]. Of course, such findings are primarily proof-of-concept, and the methodology is still in its infancy. To provide truly useful predictions, many genes within a given pathogen's genome that could potentially contribute to evolved resistance would need to be subjected to testing.

Although the costs of such an endeavor would probably be prohibitive for most organisms, phylogenetic analysis of the relationships between potential resistance genes might make it possible to test only representative candidates from within discernable gene families, rendering the problem more manageable [37].

Although the Barton–Hall method can be used to investigate resistance evolution in the pathogens of any organism of interest, including humans, extending the protocol to study vaccine-driven evolution is problematic. Because vaccines function by stimulating natural host immunity, studies of human pathogens would thus require human experiments, which is generally not feasible. However, the technique could, presumably, be used to study diseases of veterinary interest in an experimental context that included the relevant host species. This would still provide some valuable insight into the processes underlying vaccine-driven evolution and the likelihood of various evolutionary eventualities, and might also enable researchers to infer the evolutionary fate of related human pathogens [12].

Inhibiting the generation of variation

Pathogen mutation rates have presumably been shaped by selection to strike a balance between the need for replication fidelity and the ability to generate sufficient phenotypic variation to deal with environmental heterogeneities. Researchers have thus begun to explore the perturbation of pathogen mutation rates away from their presumed optima as a means to limit resistance evolution. Following drug treatment, bacteria can rapidly acquire mutations conferring resistance by activating proteins that increase mutation rates. This has spurred researchers to investigate the inhibition of these proteins as a means to slow resistance evolution [39]. For example, it has recently been demonstrated that suppression of the SOS response in *E. coli*, achieved by inhibiting the protease LexA, prevents the evolution of resistance to a number of widely used antibiotics [40]. Similar considerations have motivated studies on suppressing the horizontal transfer of resistance and virulence factors, as well as on inhibiting the induction of quiescent, transiently resistant phenotypes that would otherwise subsequently re-enter the growth phase and regenerate an infection once antibiotic treatment ceases [39].

The upregulation of mutation rates has also been investigated as an anti-resistance strategy. RNA viruses, such as HIV-1 or hepatitis C virus, achieve remarkable degrees of adaptability by replicating with very high error rates to produce 'clouds' of related genotypes known as viral quasispecies [41–43]. Such readily available variation allows viruses to respond rapidly to environmental changes, including the introduction of antiviral drugs. However, this adaptive flexibility implies that virus mutation rates are operating near their error thresholds, a theoretical limit beyond which copying fidelity is so low that the information content in viral RNA collapses and the quasi-species population goes extinct. This has led to the proposal of antiviral strategies predicated on lethal mutagenesis; that is, applying compounds that increase viral mutation rates beyond the error threshold [43,44].

Experimental investigation of this idea has been aided by the discovery of a number of potentially useful mutagenic agents [45]. Particularly well-studied is the nucleoside analogue ribavirin, a commonly administered antiviral with documented *in vitro* mutagenic effects on polio, foot-and-mouth disease, hepatitis C [42], West Nile and Haantan viruses [45]. However, whether ribavirin's *in vivo* mode of antiviral activity is due to its role as a mutagen or viral replication inhibitior has been the subject of great debate [45]. Despite this controversy, work with another mutagen, the neucleobase analogue 5-fluorouracil, has unequivocally demonstrated *in vivo* lethal mutagenesis in a mouse-lymphocytic choriomeningitis virus model system [46].

Given the mutability of RNA viruses, an obvious clinical concern is the risk of treatment-induced selection eroding treatment efficacies. In the present context, one way this could be realized is via selection for mutant virus strains with increased replication fidelity. However, such mutants would generate a less-diverse quasi-species and, hence, be less able to respond to changing selection pressures [45]. Indeed, in a recent study [47], a mutant poliovirus with a high-fidelity polymerase was found to replicate poorly within brain and spinal-cord tissues of infected mice, relative to wild-type poliovirus, with a concomitant reduction in viral pathogenesis. These traits might allow the host immune system to control the infection more effectively, or render traditional antiviral agents more effective [45]. It has also been shown that such attenuated strains, by offering protective immunity against wild-type viruses, could be utilized as live vaccines [45].

Another possible outcome of selection is the evolution of reduced viral sensitivity to the mutagen. However, as demonstrated in a recent study of foot-and-mouth disease virus resistance to ribavirin predisposed viruses to subsequent extinction following the sequential application of a second mutagen (5-fluorouracil) along with a viral inhibitor (guanidine hydrochloride) [42].

Of course, numerous questions must be addressed before lethal mutagenesis could become a viable clinical antiviral strategy, including the likelihood and degree of host-cell toxicity [45]. Nevertheless, successful implementation of lethal mutagenesis as an antiviral strategy would be a potent demonstration that the same evolutionary potential that appears to keep management goals for so many of our pathogens tantalizingly out of reach could be a decisive factor in bringing some of these diseases under control.

Constraining pathogen evolution

Another novel approach aims to take advantage of extant constraints on the pathogen's ability to evolve by tailoring treatments towards critical regulatory elements or highly conserved structural regions with limited evolutionary flexibility. In fungal pathogens, heat-shock protein 90 (Hsp90) is an important component in cellular protein-folding and cell signaling as well as in the phenotypic expression of resistance genes [48]. Combined drug–Hsp inhibitor therapy has proven effective and might also extend the useful life span of antifungals. Hsp90 inhibition

might also augment antimalarial treatments, extending the utility of this idea beyond that of fungal pathogens [48].

Influenza research has also benefited from the idea of exploiting constraints. Very recent work has identified antibodies that bind to a pocket region in the viral stem of the hemagglutinin surface protein, which effectively blocks the virus' ability to fuse with target-cell membranes. Preliminary work also suggests that escape mutants are unlikely to evolve, because conformational requirements for membrane-binding place powerful constraints on the evolution of sequences governing the structure of the pocket. This might explain why the stem region is highly conserved across influenza strains, and raises hope for the development of a broad-spectrum vaccine that does not require annual updating [49]. Investigations to identify non-evolutionarily labile vaccine targets for other diseases, including Aids and dengue, are also proceeding through the identification of conserved genomic regions [50].

However, given the evolutionary flexibility of pathogens, constraining adaptation in one trait might merely shift selection pressures to another, so that blocking evolutionary routes associated with antigenic change might carry some risks. An interesting question in the context of viral evolution asks whether limiting epitope evolution might promote evolutionary change in life history characteristics, such as virulence [12]. Nevertheless, there are no data to support this idea, and treatments aimed at constraining evolutionary options appear to be a very promising avenue for future exploration.

Future directions

Sixty years of experience with antibiotic resistance evolution, along with more recent examples of vaccine-induced evolution, are stark reminders that an unguided co-evolutionary arms race with our pathogens is a battle we are unlikely to win. Fortunately, the possibility of intervention strategies that slow this race, or avoid escalating it altogether, raises the hope of being able to affect more favorable outcomes. As much of the work discussed herein illustrates, there is a rising tide of sentiment that the principles of evolutionary ecology might provide the guide needed to fashion such strategies. Indeed, while it is too early to judge the practical value of many of the approaches considered here to applied problems of drug- and vaccine-driven evolution, the questions they highlight serve to demonstrate that any potential solution to such problems must be founded on a rational Darwinian framework.

Future work will need to utilize this perspective, and doing so will require weaving threads from microbiology, parasitology, virology and molecular biology into the broader web of evolutionary and ecological interactions that shape the environment of the pathogen of interest. By this route, the concepts of evolutionary ecology can continue to provide a deeper understanding of the process of treatment-driven evolution while hopefully also aiding in the on-going search for practical solutions to treatment-driven evolutionary problems.

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