Harnessing evolutionary biology to combat infectious disease

Tom J Little, Judith E Allen, Simon A Babayan, Keith R Matthews & Nick Colegrave

Pathogens have remarkable abilities to flout therapeutic intervention. This characteristic is driven by evolution, either as a direct response to intervention (for example, the evolution of antibiotic resistance) or through long-term co-evolution that generates host or parasite traits that interact with therapy in undesirable or unpredicted ways. To make progress towards successful control of infectious diseases, the concepts and techniques of evolutionary biology must be deeply integrated with traditional approaches to immunology and pathogen biology. An interdisciplinary approach can inform our strategies to control pathogens or even the treatment of infected patients, positioning us to meet the current and future challenges of controlling infectious diseases.

Over the last century, considerable progress has been made in controlling infectious diseases, but this success has been far from universal. Pathogens remain a major health burden, with over 9.8 million people per year globally dying from infections (comprising over 16.5% of all annual deaths), half of which are children[1]. Thus, despite intense investment of effort and money in the development of vaccines and therapeutics in the twentieth century, old (existing and resurgent) and new (emerging) pathogens remain a major threat. Some treatments or pathogen control efforts begin effectively and then lose ground. Other diseases are associated with control problems that seem to be intractable, and pathogen control programs fail at an early stage. What causes such failures?

The authors are at the Centre for Immunity, Infection and Evolution, School of Biological Sciences, University of Edinburgh, Edinburgh, UK.
e-mail: tom.little@ed.ac.uk

What underlies the variability in the short- and longer-term successes of control measures? A major factor is evolution. And evolution, aside from being pervasive, often works in complex ways and results in outcomes that are difficult to predict.

This is the age of biology, but to fully exploit the moment, we need to recognize the scale and complexity of the problems posed by evolution and find a way to productively integrate evolutionary biology with traditional biomedical research. The fact that these fields typically approach such problems in markedly disparate ways is a challenge that must be overcome if we are to understand, control and even manipulate evolutionary processes to our benefit.

Evolution: the scale of the problem

The threat posed by evolution is vast and falls into two broad categories. First, intervention drives evolution. The evolution of bacterial resistance to antibiotics and vector resistance to pesticides are the classic (and ongoing) examples of intervention-driven evolution[2–4], though many other interventions may provoke undesirable evolution[5–8]. It is crucial to note that, in the majority of instances, resistance did not evolve as expected, and our intuition stemming from an overly simplistic view of evolutionary processes has been a poor guide in identifying pathogenic threats and exploiting new opportunities for the control of infectious diseases (**Box 1**).

Second, past evolution confounds current intervention. Complex traits that have been fine-tuned by eons of natural selection and co-evolution can incidentally thwart current efforts at pathogenic control. Such pathogen traits include the classic examples of hiding with a non-antigenic cloak[9], antigen switching[10] and directly manipulating the immune system[11–15], each of which has hindered our efforts to identify targets for vaccination. Evolved traits can also become intervention-driven threats. For example, helminth parasites seem to have the capacity to monitor host immune status and shift their reproduction into high gear accordingly, thereby enhancing their transmission[7]. Such plasticity in reproductive schedules, which the parasite has the capacity for because of a long evolutionary interaction with immune systems, can also be direct responses to immune effectors that are boosted by vaccination[7]. Host adaptations can also confound treatment. For example, it was thought that anemia was a pathological consequence of bacterial infection, however, patients with anemia that are administered iron die because the anemia is actually an adaptation by the host to remove the iron on which the infecting bacteria rely. The role of iron remains an unresolved issue in the treatment of a variety of parasites and pathogens[16,17]. To make sure that the appropriate treatment for infection is administered, we must understand why an adaptation such as anemia evolved. A similar reasoning applies to the treatment of fever. Should we always treat mild fever during an infection? The answer to that question depends on whether the fever, or any pathology, is an evolutionary adaptation, and if so, an adaption for whom—the host or the parasite (**Box 1**)?

Integrating evolutionary biology

Creating new, long-lasting therapies against historically elusive pathogens (for example, *Plasmodium* or helminths) or sustaining control initiatives against resurgent and new pathogens (for example, tuberculosis, influenza or opportunistic bacteria) will require the integration of pathogen biology and immunology with evolutionary biology. From the outset, this integration will involve overcoming language barriers—evolutionary biologists and traditional biomedical researchers often speak very different scientific languages, and we should

begin preparing young scientists to become fluent in diverse terminologies.

These disciplines also differ substantially in their respective approaches to research. Infectious disease biologists have traditionally interrogated systems at the molecular and cellular levels, whereas evolutionary biologists more often consider the fitness of the whole organism, polymorphism, and changes in populations through time (**Fig. 1**). For instance, immunologists strive to reduce variation (for example, environmental or genetic variation) to elucidate mechanistic pathways, but for ecologists and evolutionary biologists, variation is the subject, as they analyze changes in fitness in relation to genetic and environmental heterogeneity. There is room for the biomedical sciences to shift away from a focus on inbred model organisms in the laboratory to an emphasis on the responses of natural hosts in the wild[18,19]. Equally, there is room for both evolutionary biology and ecology to strengthen their appreciation of the mechanistic underpinnings of traits and to transform this knowledge into predictive models. We may require the development of more study systems, as humans and mice may not be ideal subjects for evolutionary studies, but the role of traditional model systems could also be expanded to test whether the patterns observed in the laboratory can be generalized to other environments (for example, see refs. 19–21).

We do not expect this integration to be easy. Disentangling the complex molecular biology of host-pathogen interactions is not straightforward, and although the elegant simplicity of Darwin's theory of natural selection can tempt us into thinking evolutionary outcomes will be easy to predict, in reality, they are not (**Box 1**). Resistance to even a single drug may involve multiple mechanisms and mutational targets, and predicting the spread of resistance in natural populations will require complex, parameter-rich models in which estimating each parameter will be a major challenge. This complexity means we cannot use a cursory understanding of evolutionary biology to guess at evolutionary outcomes. Technological transformations, particularly in genomics, bioinformatics and computing power, are already aiding integration by creating new opportunities to map genotypes and molecular mechanisms onto phenotypes[22] in both models and the real world. We will require scientists with mastery of these new technologies, but perhaps the most important skill required to move integration forward will be the ability to contextualize the ever-expanding quantities of data.

With the right data and outlook, we can begin to ask biomedically relevant questions at multiple levels, creating a synergy that will allow biological insights to be translated into pathogen control strategies. For example, a key unresolved issue is why natural selection hasn't completely purged from natural populations genotypes that are susceptible to infection. Genetic work has revealed possible insight into this issue by showing that there are evolutionary trade-offs in which parasite-resistant individuals suffer from chronic diseases. A recent example, obtained by combining data

BOX 1 Threats and opportunities

Evolutionary outcomes can be difficult to predict. This fact has generated many unforeseen threats to human health, but an appreciation of the evolutionary strategies used by pathogens can also foster the development of successful therapies and strategies to control their transmission.

- **Resistance at a bargain.** The ability of microbes to resist antibiotics should negatively impact their fitness in the absence of treatment, as resistance traits are likely to be detrimental to fitness. However, mutations that confer resistance are often quickly followed by additional mutations elsewhere in the genome that compensate for the costs of resistance. Compensatory mutations can even increase the fitness of the resistant genotype above that of the original sensitive genotype, creating 'superbugs' that outcompete other bacteria both in the presence and absence of antibiotics.
- **Old enemies.** A standard view has been that long-term host-pathogen associations inevitably co-evolve to become less harmful. In fact, parasites face an evolutionary dilemma: reproducing too quickly may indeed kill the host before transmission takes place, but sometimes there are advantages to reproducing rapidly (and thus harming the host), such as after co-infection with a competing pathogen. These counteracting demands lead to the evolution of an intermediate level of virulence that maximizes parasite transmission[2]. There is a clear potential for treatments that reduce virulence without directly harming pathogens to favor fast-growing parasites[5,6].
- **Family matters.** Many aspects of pathogen biology only make sense in light of the evolutionary theory of kin selection. For example, some *Salmonella* bacteria induce an immune response that effectively empties the host gut of competing pathogens. This immune response will also harm *Salmonella*, but this suicide strategy makes sense if it helps nearby kin who share genes with the original *Salmonella* strain[42].
- **Hit 'em late, hit 'em soft.** Evolutionary theory has suggested a blueprint for 'evolution-proof' insecticides: target old rather than young individuals[43,44]. This seems to counter the intuition that the best way to control a pest is to hit it quickly and hard, however, an insecticide that kills young mosquitoes maximizes natural selection for resistance. Evolutionary biology predicts that an insecticide that kills later, once most pathogen reproduction has occurred, will minimize natural selection for resistance, as the strength of natural selection decreases with age. In parasites where transmission generally occurs from older vectors, such as *Plasmodium*, there may be an age window that could be targeted when selection for insecticide resistance is weak and that is before transmission takes place.
- **Ribosomally synthesized antimicrobial peptides.** Ribosomally synthesized antimicrobial peptides (RAMPs) are part of the innate immune system of all multicellular organisms, and bacteria do not seem to have resistance to RAMPs, raising hope that these peptides might be used therapeutically. However, resistance to RAMPs easily evolves[45] when they are studied under the conditions that evolutionary theory predicts will provoke the evolution of resistance. Coincidentally, these conditions are similar to those the bacteria would experience if RAMPs were used therapeutically.
- **Deceptive epitopes.** Immunodominance is the preference of the immune system toward a limited set of epitopes. By strategically positioning immunodominant epitopes, the influenza virus can 'lock' the immune system into a relatively ineffective and strain-specific response. Thus, evolutionary theory predicts that attempting to mimic the natural immune response may play into the pathogen's hands. Instead, vaccine constructs that mask the immunodominant deceptive epitopes may allow the immune response to be refocused toward less antigenic but more conserved epitopes, generating more effective and wide-ranging immunity[46,47].

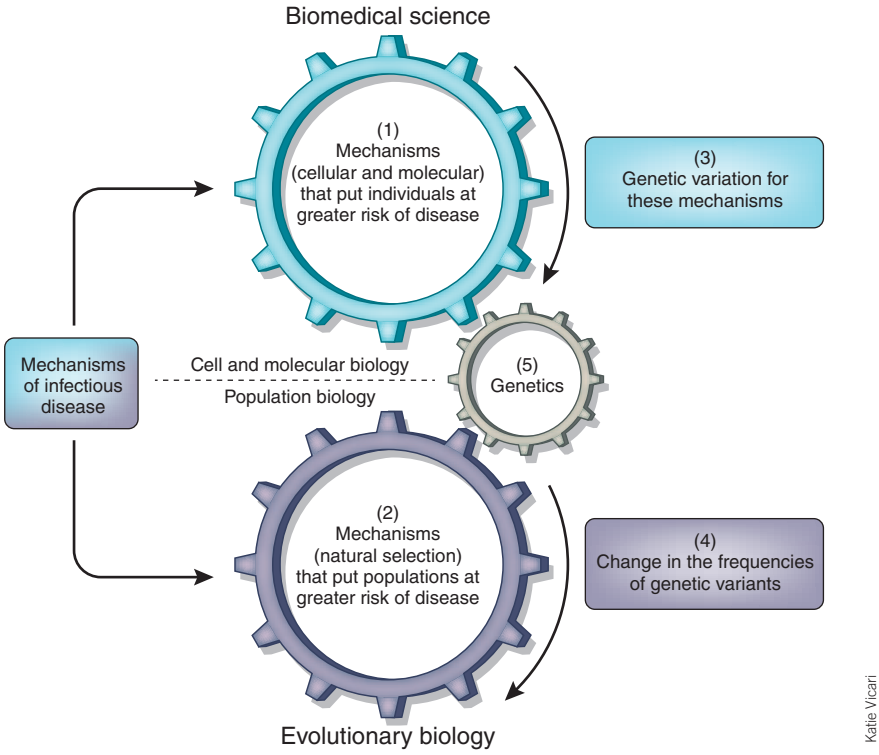

Figure 1 A central aim of all biomedical research is to elucidate the mechanisms associated with infectious disease. Much basic biomedical research, for example, in immunology or pathogen biology, has its roots in cellular and molecular biology and, as such, has sought to identify cellular or molecular mechanisms (for example, pathogen virulence factors or host immune deficiencies) that place an individual at risk of contracting or expressing a disease (1). The study of evolution, by contrast, is principally a form of population biology, and, in the context of infectious disease, evolutionary biology seeks to identify mechanisms (prominently, natural selection) that change whole populations toward either a greater or lower average risk of infection (2). There has been substantial progress made toward identifying genetic variation that modifies the cellular and molecular mechanisms that put individuals at greater risk of disease (3); evolutionary biology then studies how the frequencies of these genetic variants may change over time as a result of natural selection (4). Thus, genetics, either molecular or quantitative, provides a key link between traditional biomedical science and evolutionary biology (5).

from association studies and characterizing signatures of natural selection, is a polymorphism in the gene encoding the human apolipoprotein L-1 (APOL1) protein that confers trypanosome resistance but also risk of kidney disease[23]. Sickle cell anemia and malaria is the entrenched example of such a trade-off, but even this relationship is being reassessed, as recent data have provided a new understanding of why sickle cells are protective for malaria. Rather than reducing the malarial parasite load, it is now surmised that sickle cells release more heme than normal red blood cells, which induces the production of heme-neutralizing systems that improve both sickle cell pathology and cerebral malaria[24]. This form of protection, where pathology is reduced without reducing parasite numbers, puts fundamentally different selective pressures on parasite populations[25].

Ultimately, analyses of natural selection and the resulting polymorphisms would be able to generate predictive biomarkers that would assist in analyses of disease spread. Although genotyping and phenotyping in the field remain challenging in geographical areas that have a poor health infrastructure, as genome sequencing studies continue to analyze more dispersed human populations, it might be possible to use polymorphisms in genes that encode immune factors to predict disease susceptibility, and, with an understanding of the local pathogen diversity, therapies could even be tailored to specific geographical populations.

In addition to these human studies, multigenerational datasets on wild mammals are now allowing us to associate immunological data with comprehensive measures of Darwinian fitness in the context of optimal immunity. For example, the new application of existing immunological tools has shown that highly immune-responsive feral sheep suffer from autoimmunity, which reduces reproductive success in these sheep but enhances survival as a result of their lower parasite burdens[26]. The balance of the immune system is also influenced by co-infection: removing one set of pathogens in a co-infection or altering commensal populations can increase a host's susceptibility to other pathogens[27], leading to an imbalanced immune response[28–30] and the potential for autoimmunity, allergy or asthma. Hence, pathogen exposure and long-term co-evolutionary interactions may not always select striking and discrete polymorphisms such as those at *APOL1* and, instead, may select for complex and graduated genetic responses (which are best studied using the tools of quantitative genetics) that respect the demands of polyparasitism. New pathogen control strategies must address optimal immunity in the natural world outside of the laboratory, where multiple infections in one host are the norm, to avoid adverse immune consequences or the emergence of new or previously rare pathogens.

Thus, analyses of how pathogens and pathogen diversity evolve in response to control measures are crucial. New approaches are making it possible to rapidly identify medically relevant features of pathogens and the timescales on which they arise. For example, population genomic analyses of ongoing epidemics are elucidating the timing of the emergence of drug resistance and changes in host range and pathogenesis and are identifying source populations and species that are likely to seed the next epidemic[31–33]. The pace at which we can now sequence genomes is key, and, ultimately, such phylodynamic approaches may provide the early warning signals for disease emergence or drug resistance, allowing us to help predict and limit the spread of these diseases[34–39]. Similarly, laboratory selection of parasite resistance to new drugs, coupled with genome sequencing and other molecular techniques, is proving useful in identifying the mechanisms of drug action and of cross-resistance to other drugs, as well as the potential for infection-control failure in both *Mycobacterium tuberculosis*[40] and *Plasmodium*[41], among other deadly pathogens. Biomedical researchers can and should follow the identification of key traits in pathogens as well as their hosts that are crucial for pathogen survival and persistence with mathematical models that can generate predictions for the impacts of pathogen control strategies.

If we could travel back in time to the beginning of the antibiotic era and use our knowledge of evolutionary biology to apply these drugs in a manner less likely to provoke undesired evolution, we would. The lessons learned through integrative strategies can and must be applied to pathogen control in

the future because the cost of developing and testing new therapeutics is so high that any strategy to prolong their efficacy, however slightly, is both morally and economically compelling.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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