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Discussion

Evolutionary epidemiology 20 years on: Challenges and prospects

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ABSTRACT

The advent of molecular biology has deeply impacted the study of infectious diseases and their epidemiology in particular. However, evolutionary biology, which provides an essential conceptual framework to understand the observed patterns of genetic and phenotypic diversity, is still lacking the attention it deserves from the medical community. In 1988, Paul Ewald coined the term evolutionary epidemiology to describe a systematic approach to the evolution of pathogen virulence. This review seeks to cast a new light on evolutionary epidemiology beyond Ewald's seminal project. While ecologists and evolutionary biologists have developed powerful theoretical tools to help us understand pathogen evolution, more work is needed across traditional disciplines in order to analyse and tackle the unabated spread of hypervirulence, drug resistance and antigenic escape. I review a number of cases where evolutionary biology has played or could play a leading role in the design of novel medical approaches.
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1. Introduction

Specialisation and reductionism have dominated scientific research for several decades, but there is now a growing recognition that major challenges such as infectious disease control need integrative approaches. This involves various disciplines, from the basic to the applied, including mathematics, computational biology, evolutionary biology, ecology and population biology, microbiology, genetics, cell biology, immunology, epidemiology, veterinary science, clinical care and public health policy. Some of these have grown and developed together for a long time, but others virtually ignore one another (REX Consortium, 2007; Rosvall and Bergstrom, 2008).

Evolutionary biology has a central role to play as it provides the key to understanding observed patterns and predicting the potential effects of behaviours, treatments, policies or environmental changes. A number of recent publications (MacCallum, 2007; Stearns and Koella, 2007; Nesse and Stearns, 2008) have promoted evolutionary approaches to medicine in general and advocated better teaching of evolution in the medical curriculum. Infectious diseases, caused by microscopic living organisms with short generation time and huge demographic variations, are arguably a natural playfield for evolution. But despite this widespread recognition, I often encounter misconceptions on the mechanisms of evolution among the medical community, regarding for example the evolution of virulence or group selection (see detailed arguments on those issues by May and Anderson, 1983; Frank, 1996).

The term 'evolutionary epidemiology' first appeared in the mainstream medical literature twenty years ago, in a seminal paper by Ewald (1988) which considered the diversity of pathogens in their ability to cause severe disease from a new perspective, integrating public health, ecology and evolutionary biology. Twenty years on, has the concept of evolutionary epidemiology been embraced by a large scientific and medical community? Has it led to practical solutions for public health? What progress has been made? What are the current challenges and promising trends?

This review seeks to highlight the importance of evolutionary biology, beyond virulence management, to address modern challenges in infectious diseases research and control. Following a brief update on virulence management, I will focus on the three main areas where pathogen evolution is posing unabated threats to public health (hypervirulence, drug resistance and antigenic escape); then I will review recent progress and remaining issues in translating evolutionary theory into 'Darwinian medicine'.

2. Virulence management: gold standard or red herring?

Since the early ages of experimental microbiology and epidemiology, the plasticity of pathogen's virulence (generally defined as the amount of detrimental effects of infection on the

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host) has been the subject of investigation and concern (Pasteur, 1880; Topley, 1921). However, the frequent observation that serial passage of pathogens in laboratory animals resulted in increased levels of virulence (Dowdeswell, 1883; Terry, 1910) did not appear to hinder the widely-held view that adaptation of any pathogen to its host should lead to decreased virulence and even mutualism. Although Ball (1943) dismissed the latter paradigm as preposterous in an address to the Western Society of Naturalists, it proved a diehard in the medical community. Roy Anderson, Robert May and Paul Ewald (among others) successfully stormed the stronghold in the 1980s (Anderson and May, 1982; May and Anderson, 1983; Ewald, 1988). A decisive argument was provided by the case of myxomatosis in Australian rabbits (Fenner, 1983), which provided the first example of directly-observed virulence evolution in the field associated with epidemiology: it demonstrated that intermediate levels of virulence could be beneficial to the pathogen if the cost associated with host death was balanced by an increase in the instantaneous rate of transmission. Illustrating important notions of individual level selection and trade-offs (Lenski and May, 1994; Frank, 1996), this novel idea was a godsend to evolutionary biologists, whose influence in the field of infectious diseases has grown since then. One of them was particularly instrumental in bringing together evolutionary biology and medical epidemiology.

For twenty years now, Paul Ewald has actively promoted 'evolutionary epidemiology' (Ewald, 1988, 1994, 1996), with the aim to understand and control how ecology and human (especially medical) interventions shape the evolution of virulence in pathogens. His 'comparative method' consists of using evolutionary theory to interpret empirically observed diversity and variations among pathogens. While this approach has generated interesting predictions (see, for example Walther and Ewald, 2004) and given ecology and evolution a central role in epidemiology, it falls short of explaining the short-term evolution of specific pathogens and how it can be affected by human behaviour. As pointed out by van Baalen and Sabelis (1995), interpreting patterns of association (e.g. between mode of transmission and virulence) observed across a range of unrelated organisms as the result of adaptive evolution can be mistaken if one does not know the evolutionary history as well as the biological constraints of each species considered—it is yet another example of the old debate in evolutionary biology about the 'adaptationist programme' (Clutton-Brock and Harvey, 1979; Gould and Lewontin, 1979).

A more rigorous theoretical framework for virulence evolution has since been developed within the wider field of adaptive dynamics. Dieckmann et al's (2002) book provides a detailed overview of the recent progress in the application of adaptive dynamics to infectious diseases and in particular to the issue of virulence evolution. However, this progress has occurred mostly on the theoretical front. The main advances in testing the predictions of the mathematical models have come from experimental ecology and evolution rather than medicine. Since the early, simplistic models of parasite evolution proposed by Levin and Pimentel (1981), Anderson and May (1982) or Bremermann and Pickering (1983), the uninterrupted (though sometimes controversial) dialogue between theoreticians on the one hand and ecologists working in the field or in the lab on the other hand, has led to a much better understanding of some of the factors that affect the evolution of virulence, in particular the different modes of transmission, within-host competition, genetic associations between traits, phenotypic plasticity as well as variability among hosts and environments (Ebert, 1994, 1998; Frank, 1996; Ebert and Mangin, 1997; Hochberg, 1998; Boots et al., 2004; Mackinnon and Read, 2004b; De Roode et al., 2005b).

However, adaptive dynamics have gained limited input from epidemiology until very recently (see Section 3.1 below for an update) and its practical value for effective virulence management remains mostly untested. In particular, one key assumption in most theoretical models is the existence of trade-offs between virulence and other traits of pathogens (Frank, 1996) that determine the adaptive response of pathogens to external constraints. Unfortunately, these trade-offs are likely to be specific to pathogens or even host–pathogen associations, and limited information is available to date (Ebert and Bull, 2003; Elliot, 2003; Ganusov, 2003; Ganusov and Antia, 2003; De Roode et al., in press; Frank and Schmid-Hempel, 2008). Therefore adaptive dynamics, though a valuable theoretical framework, has had limited influence so far in public health.

Yet interesting prospects lie ahead. Currently there are at least two major human diseases that could potentially benefit from theoretical insight into the evolution of virulence, namely malaria and HIV-AIDS. Even though practical implications for disease control remain hypothetical, the theory of virulence evolution is stimulating innovative research on important pathogens. First, several experimental studies (Mackinnon and Read, 1999, 2004a; Mackinnon et al., 2002) on *Plasmodium chabaudi* in mice have established genetic correlations between parasite growth, virulence and transmission potential, complemented by field data on human malaria (Mackinnon and Read, 2004b). Recent experiments have established a link between virulence and competitive ability when mice are co-infected with different clones of *P. chabaudi* (de Roode et al., 2005a), even in mice previously immunised against malaria (Grech et al., 2008). Those results support the development of mathematical models for virulence evolution in an epidemiological context, such as the one proposed by Gandon et al. (2001) to investigate the potential selective pressure created by imperfect vaccines. Naturally the main caveat is that rodent malaria differs from human malaria caused by *P. falciparum* in many respects (Mackinnon and Read, 2004b; Langhorne et al., 2008). But at least some important mechanisms have been uncovered experimentally, enabling theoreticians to make more specific predictions which can hopefully be tested with relevant systems. In addition, these developments force epidemiologists to think more carefully about potential unexpected effects of vaccination (Gandon et al., 2001; Smith, 2002; De Roode et al., 2005b). The second example, also still hypothetical to date, concerns HIV. Despite limited and contradictory reports on the temporal trends of virulence (Ariën et al., 2005; Müller et al., 2006), two recent studies proposed theoretical models for virulence evolution in HIV: Fraser et al. (2007) reported a correlation between viral titre, virulence (time to death) and infectivity; they then built a trade-off model that predicts an optimal intermediate level of virulence, close to the observed value in field studies. Based on experimental competition assays of different HIV isolates, Ariën et al. (2007) argued that virulence may have decreased over time as a result of within-host selection combined with transmission between host with different HLA alleles. These examples represent a promising trend in reconciling adaptive dynamics and epidemiology with the ultimate objective to develop efficient strategies to counter pathogen evolution.

While novel mathematical models are being developed to incorporate realistic epidemiological features into evolutionary frameworks (see Section 3.1 below), the main challenge on the road to virulence management is the reconciliation between within- and between-host dynamics. Indeed, in order to understand the possible links between virulence, which is expressed inside hosts, and transmission between individuals, the gap between microbiology and epidemiology needs to be filled. Here too mathematical approaches are very helpful (see Section 3.1) but more experimental and other empirical effort is needed to quantify the different aspects of pathogen fitness over the whole

transmission cycle (Levin and Antia, 2001; Brown et al., 2006a,b; Grant et al., 2008).

3. Evolutionary challenges to the control of infections

3.1. Virulence evolution

While virulence management has yet to be put to practice as we have just seen, virulence remains a major concern for public health. In particular an increasing number of reports (Razavi et al., 2007) point to sudden and sometimes dramatic changes in the virulence of known pathogens, either in the lab or in natural populations. Modern molecular techniques often make it possible to identify the underlying genetic mechanisms, which appear to be quite diverse, as illustrated below. This demonstrates the versatility acquired by pathogens during their rapid evolution, and underlines the importance of combining evolutionary concepts with epidemiology and microbiology.

The fact that genetically controlled variation in virulence has been reported in many pathogens justifies the principle of virulence management. However, the quantitative approach inherited from adaptive dynamics (see previous section) which considers virulence as a continuous trait susceptible to infinitesimal variation often appears inconsistent with empirical observations. Indeed in most cases, pathogen variants cluster into discrete sets with sharp differences in virulence levels, sometimes caused by single mutations or genetic transfers. For example, Conenello et al. (2007) compared the PB1-F2 sequences of human influenza A virus strains isolated during major outbreaks, including Hong Kong 1997 and the 1918 pandemic and identified a single mutation responsible for a sharp increase in viral growth and virulence in mice. Similar cases include equid herpes virus (Goodman et al., 2007) and *Staphylococcus epidermidis* (Wang et al., 2007). Gene transfer was found to be responsible for shifts in virulence in methicillin-resistant *Staphylococcus aureus* (Diep et al., 2007) and invasive group A streptococcal infections (Vlaminckx et al., 2007). The phylogenies of large sets of pathogen isolates, such as *Escherichia coli* O157:H7 (Manning et al., 2008) or *Clostridium difficile* (Stabler et al., 2006) have revealed that high levels of virulence could be confined to specific clades. Interestingly, some of these 'hypervirulent' clades, such as *Shigella*, were initially classified as different species, which is now being questioned based on new genetic information (Lan and Reeves, 2001).

Beyond variability in virulence levels, single mutations may have an impact on the whole course of an infection: Szmaragd et al. (2006) identified mutations on a single gene (viral polymerase) of the hepatitis B virus which explained a large part of the variation in disease outcomes among patients, such as between acute mild infection, 'fulminant' and chronic hepatitis. It has also emerged recently that a number of pathogens possess so-called 'anti-virulence genes' which, if mutated, can result in sharp increases in the virulence, colonisation ability and other fitness components of these pathogens (Foreman-Wykert and Miller, 2003). One such gene, *Mycobacterium tuberculosis fadB4*, was identified and studied *in vivo* by Scandurra et al. (2008): its deletion enhanced bacterial replication, immunosuppression and virulence in lab mice. These features represent yet another challenge to our understanding of pathogen evolution.

The possibility that treatment, vaccination or other control measures might select for increased pathogen virulence, in a sort of arms race, has been considered theoretically (Gandon et al., 2001; Porco et al., 2005), and empirical evidence has started to appear. Mackinnon and Read (2004a) performed serial passage of malaria parasites in immunized mice and in naïve ones and found that strains evolved in immunized animals had higher virulence in naïve mice, even after mosquito-mediated transmission, in line with theoretical predictions. Using phylogenetic analysis of isolates of *Clostridium difficile*, Stabler et al. (2006) suggested that recent hypervirulent strains (identified by their toxin expression profile) may have been selected by the use of antibiotics. Besides, phenotypic responses to treatment have been documented: antibiotics favouring gut colonisation by virulent pathogens including *C. difficile* (Owens et al., 2008) and *Salmonella enterica* (Lawley et al., 2008) and low doses of antifungal drug triggering higher virulence in *Candida albicans* (Navarathna et al., 2005).

A further complication comes from the fact that virulence is not a simple uniform property of a given pathogen; its expression depends on many factors and varies during infection (Frank and Schmid-Hempel, 2008), between hosts and environments. Phase shifts have been described for several decades in bacterial pathogens on the basis of changes in the physical properties of cultures grown in different conditions. These are typically stress responses to environmental changes which, among other effects, can cause shifts in virulence levels during the course of infection. Examples include the repression of toxin production by *C. difficile* in response to varying glucose levels (Dupuy and Sonenshein, 1998), the regulation of several virulence genes in *Vibrio cholerae* triggered by host signals (Peterson, 2002) or variations in the overall level of gene transcription in *Bordetella pertussis* mediated by glutamate concentration (Nakamura et al., 2006). Gal-Mor et al. (2008) identified a novel secretion pathway in *S. enterica* which plays a critical role in regulating the level of virulence during infection; its expression, modulated by zinc concentration, is inhibited in systemic sites and is maximised during faecal shedding.

Thus the emerging picture of virulence is complicated, changing and polymorphic. It strongly indicates that the evolution of virulence cannot be understood, and therefore controlled, unless it is properly integrated in the whole life cycle of the pathogen, both within and between hosts.

3.2. Drug resistance

Drug resistance is arguably one of the first instances where the direct relevance of evolution in medicine has been recognised (Palumbi, 2001), yet this is still not properly acknowledged in the medical literature (Antonovics et al., 2007). Many reviews on the topic have been published, so the aim of this section is primarily to point out recent findings that illustrate issues with the epidemiology and evolution of drug resistance, rather than the molecular mechanisms. I will consider separately the cases of bacteria, viruses, fungi and malaria parasites. Table 1 presents a list of factors that affect the evolution of drug resistance, across taxa, and that are particularly relevant to the design of mathematical models on the topic.

3.2.1. Antibiotic resistance in bacteria

Despite decades of research, antibiotic resistance is still a major threat for the control of many bacterial infections. Recent reviews include Wright (2007) on molecular and evolutionary aspects, and Levy and Marshall (2004) with a focus on proximal causes and management. Two main issues are often cited as possible causes for the failure of controlling the spread of antibiotic resistance: poor management and paucity of the chemical arsenal.

Firstly, there appears to be a discrepancy between the progress of basic research on antibiotic resistance and the implementation of simple preventive measures in the field. The main culprits are hygiene problems and, most importantly, the misuse of antibiotics and other sterilising agents. Dagan et al. (2008) reported a positive correlation between the seasonal variations of antibiotic prescription and levels of resistance in Israeli children, suggesting that

Table 1
Important factors that affect drug resistance evolution, from genes to ecosystems.

Category	Mechanism	Impact on resistance	References
Molecular regulation	Regulation of mutation rates	Genes involved in resistance appear particularly prone to increases in mutation rates, ensuring rapid emergence of resistance	Martinez and Baquero (2000), Woodford and Ellington (2007)
	Phenotypic switch to bacterial persistence	Following antibiotic treatment of a bacterial clone, a few 'persister' cells, which had entered a dormant stage, can survive and start growing again afterwards	Balaban et al. (2004), Connolly et al. (2007)
	Small-colony variant phenotype	Slow-growing subpopulations of bacteria with unusual morphology and chemical properties have been isolated clinically; resistance appears to be caused by the reduced activity of the pathways targeted by antibiotics	Proctor et al. (2006)
Genetic exchange	Mobile elements	Methicilin resistance in *S. aureus* is caused by transfer and integration of large DNA sequences (or cassettes) into the bacterial chromosome, carrying genes for antibiotic resistance	Hiramatsu et al. (2001)
	Protozoa-mediated gene transfer	Resistance gene transfer across bacteria species was observed in the rumen of cattle, sheep and goats, mediated by predator protozoa	McCuddin et al. (2006)
	Rapid plasmid transfer across bacteria species	Rates of plasmid transfer between different bacterial species measured in the intestines of rats were much higher than *in vitro*, resulting in widespread antibiotic resistance	Feld et al. (in press)
Cost of resistance	Key assumption in evolutionary models	Mathematical models typically assume that resistance is costly and therefore cannot be maintained in the absence of treatment	Koella and Antia (2003), Perron et al. (2007)
	Mutation-specific cost	Specific mutations conferring resistance can be associated with diverse fitness costs or even benefits in the absence of drugs	Hastings and Donnelly (2005), Luo et al. (2005)
	Compensatory mutations	Secondary mutations that compensate the initial cost of drug resistance often appear	Zhang et al. (2008)
	Phenotypic switching	Single clones of *S. aureus* can alternate between resistant and sensitive states, thus avoiding fitness costs in the absence of antibiotics	Massey et al. (2001)
Pathogen ecology	Community-wide resistance	Antibiotic-inhibiting molecules excreted by certain bacteria can protect surrounding clones that lack intrinsic resistance	Dugatkin et al. (2005)
	Co-infection	For reasons still poorly understood, co-infection with HIV appears to favour the spread of drug-resistant malaria, tuberculosis, hepatitis B, etc.	Cotton et al. (in press), Levy and Grant (2006), Wells et al. (2007), White (2004)
	Soil reservoir	The natural occurrence of resistance in many soil bacteria across taxa could represent an underestimated reservoir for resistance genes	Dantas et al. (2008)
Environmental change	Antibiotic cycling	This classical clinical method to fight antibiotic resistance appears to have weak efficacy; it should be reviewed in the context of the diverse underlying genetic mechanisms of resistance	Farr et al. (2001), van Loon et al. (2005), Fridkin et al. (2003), John and Rice (2000)
	Hospital vs. community	Movements of individuals between these tow very different environments create a metapopulation structure that affects the spread, persistence and evolution of resistance	Cooper et al. (2004), Gorwitz (2008), Liu et al. (2008)

antibiotic-restriction policy could have a rapid beneficial effect on the incidence of drug resistance. Finch et al. (2004) proposed ways to improve behaviours in hospitals and the community, while McEwen and Fedorka-Cray (2002) reported evidence that antimicrobial use in farm animals can select for resistance in commensal and pathogenic bacteria in those animals, and Aiello and Larson (2003) alerted the medical community about the risks posed by the misuse of cleaning products in our hygiene-obsessed societies. Clearly, better education and information is needed at all levels, beginning with clinical practitioners who should be provided with clear clinical guidelines (Barlow and Nathwani, 2005). Given that even basic principles are still poorly applied, one may ask whether improving practice might not, in itself, contribute to substantial progress in reducing the prevalence of antimicrobial resistance, without the need for further research? Murphy (2008) argued that the spread of drug-resistant tuberculosis had been successfully controlled in New York City thanks to the efficient implementation of a range of control measures across the public health system, and that this example could inspire other countries with a robust pre-existing public health infrastructure. However, other large scale schemes have had mixed results: a ten year follow-up of the antibiotic use reduction programme in Sweden showed significant decrease in resistance in some bacterial species but an increase in others (Mölstad et al., 2008).

Secondly, it has been argued that the available chemical arsenal is too limited to hope defeat the extraordinary ability of pathogens to evolve resistance, and that the situation is not likely to improve (Barrett, 2005; Becker et al., 2006). Nevertheless, some authors are more optimistic about the development of new classes of antimicrobials in the near future. For example, promising new compounds have been identified against *Mycobacterium tuberculosis* (Sacchettini et al., 2008), methicillin-resistant *Staphylococcus aureus* (Van Bambeke et al., 2008), as well as nematodes (Kaminsky et al., 2008) and trematodes (Sayed et al., 2008). Cationic antimicrobials peptides (CAMPs) which are naturally produced by living organisms have been claimed by some to be less prone to elicit antibiotic resistance than classical antibiotics (Hancock, 2001; Zasloff, 2002). However, experience calls for prudence on this matter: arguments by Bell and Gouyon (2003) and experimental evidence by Perron et al. (2006) strongly indicate that resistance to CAMPs can evolve in bacterial populations. In the absence of a 'silver bullet', progress may come from improved clinical and public management of resistance, as well as the combination of antagonistic molecules (Chait et al., 2007)—although this latter strategy needs to be carefully investigated as some drug associations may actually favour the evolution of resistance (Hegreness et al., 2008).

3.2.2. Antiviral resistance

Antiviral resistance, although ubiquitous, has been less publicised than antibacterial resistance because of differences in the treatment, ecology and history of viral compared to bacterial

infections (Richman, 2006). Typically, drug resistance is more of a challenge in rapidly adapting RNA viruses rather than in less variable DNA viruses such as herpesviruses. Accordingly, I concentrate here on two major RNA virus infections, human influenza A and HIV.

In human influenza A, the spread of resistance to the main antiviral class (adamantane) in populations was first reported by Hayden et al. (1989) during a clinical trial. By 2006, 95–100% of H3N2 isolates worldwide were adamantane resistant, but only 15% of isolates of H1N1 (Deyde et al., 2007), both strains being the major agents of current seasonal epidemics. Now the emergence of resistance to another neuraminidase inhibitor, oseltamivir (Lackenby et al., 2008), is causing major concern, especially in Europe where the prevalence of resistance in H1N1 isolates exceeded 20% in eight countries as of August 2008 (European Centre for Disease Prevention and Control, 2008). While resistance had already been documented in clinical contexts, this rapid spread was unexpected given the limited use of oseltamivir. According to Rameix-Welti et al. (2008), it may have been caused by the amplification of pre-existing resistant strains of influenza A virus which did not suffer any competitive cost in the absence of oseltamivir. Since this drug, also known as Tamiflu, is at the centre of planned measures in the event of a pandemic, there have been recent calls to re-evaluate the potential effect of drug resistance on pandemic control. Indeed, although the recent resistance outbreak was observed in H1N1 strains, the occurrence of oseltamivir resistance has also been documented in a few H5N1 (avian influenza) human patients (De Jong et al., 2005). Mathematical models have shown that drug resistance could spread during an influenza pandemic with possibly severe consequences on control measures (Regoes and Bonhoeffer, 2006; Alexander et al., 2007; Lipsitch et al., 2007). However, there are still many uncertainties about the within-host dynamics of influenza infections which could affect the spread of resistance in populations (Handel et al., 2007).

In HIV-1, antiretroviral resistance is frequent and is now threatening the recent 'combination' therapies that are typically used to treat HIV infection. Costagliola et al. (2007) reported an overall frequency of resistance mutations of 80–90% among treated patients nationwide in France, with some completely resistant to three drugs. Another study (Napravnik et al., 2007) found that 8% of treated patients in the USA had triple-class drug resistance, a figure down to 3% among highly active antiretroviral therapy (HAART) recipients. While the emergence of resistance mutations is particularly important in chronic infections with a long exposure to drugs, their transmission must also be monitored, especially in a pandemic context. However, transmitted resistance appears to remain relatively low: recent studies reported around 10% of resistance among naïve patients in Switzerland (stable) (Yerly et al., 2007), in New York State (Parker et al., 2007) and in the UK (decreasing) (UK Collaborative Group on HIV Drug Resistance et al., 2007). These levels are higher than in developing countries (5–8%) (Shekelle et al., 2007) probably due to lower treatment coverage. Poor adherence to treatment is a major concern in those countries (Shekelle et al., 2007), although a review of recent introduction of HAART in 14 African countries found overall high levels of compliance (Akileswaran et al., 2005). The actual effect of adherence on resistance selection needs to be carefully monitored (Bangsberg, 2008). Worryingly, clinical resistance to a new molecule still in trial, enfuvirtide (a peptide that prevents viral fusion to host cells), has already been observed and different evolutionary routes have been identified (Ray et al., 2007). In response to those concerns, worldwide resistance monitoring is now at the centre of the World Health Organisation's (WHO) strategy against HIV (Bertagnolio et al., 2008).

3.2.3. Antifungal resistance

As fungal infections are on the rise, particularly among immunocompromised patients, there is growing concern about the spread of drug resistance (Rogers, 2006). Fungal pathogens have evolved a number of stress response strategies which enable them to develop rapid tolerance to drugs (Cannon et al., 2007). But progress in understanding the molecular mechanisms, and in particular the role of the heat-shock protein Hsp90 in promoting phenotypic plasticity, may provide new targets to fight drug resistance (Cowen, 2008). Another source of concern with fungal as well as some bacterial infections is the formation of highly resistant biofilms that cling to medical devices, facilitating nosocomial infections (Donlan, 2001). The frequent association between biofilm formation and drug resistance is not fully understood yet. It may relate to the physical properties of biofilms as well as changes in bacterial gene expression, mediated by bacterial chemical communication (known as quorum sensing) (Blankenship and Mitchell, 2006).

3.2.4. Antimalarial drug resistance

The emergence and spread of resistance to anti-malarials have followed closely the introduction of most molecules since the mid-20th century (Hyde, 2005), and resistance to the latest artemisin-based combination treatments has already been reported in Eastern Asia (Wongsrichanalai and Meshnick, 2008). Regional variations in transmission intensity, mortality and public health policies strongly affect the spatial spread of particular mutations conferring drug resistance (Björkman and Bhattarai, 2005; Lynch et al., 2008). The genetic bases of resistance are diverse and can now be studied systematically based on *Plasmodium falciparum* genome sequence (Ekland and Fidock, 2007). Switching and combination of drugs have been the main strategies for several years. Recent studies show that this has led to loss of resistance to old drugs (Nkhoma et al., 2007), offering new hopes for sustainable treatment strategies. In addition, resistance reversal to some specific agents can be triggered by treating patients with other molecules (Egan and Kaschula, 2007).

To conclude, recent studies on antimicrobial resistance suggest that a rational use of drug combination together with progress in understanding the molecular mechanisms involved are the two main avenues in the fight against drug resistance. However, it would be unrealistic to hope to prevent the evolution of resistance. So the main objective now should be to ensure that the spread of infections does not get out of control and we still have a range of available measures that can, at least for some time, maintain some efficacy. This requires a good knowledge of the mechanisms of pathogen evolution. It is even possible to devise new approaches to counter the adaptive strategies of pathogens with the help of evolutionary biology, as we shall see later (Section 3.3).

3.3. Antigenic escape

When neither our innate immune system nor antimicrobials can stop an infection, our adaptive immune system provides protection, notably in the event of a second encounter with the same pathogen. This very efficient second line of defence can exert strong selective pressures on pathogens causing acute infections that need to constantly infect new hosts to survive, but also on those causing chronic infections once antibodies have been produced. The huge diversity of patterns of antigenic variation among pathogens is not fully understood. Two recent review papers have proposed classifications of those patterns based on pathogen population dynamics and immune pressure (Grenfell et al., 2004; Lipsitch and O'Hagan, 2007). In particular, the case of

measles virus which appears to lack antigenic variation is still puzzling scientists. The main hypothesis is that the wide range of antibodies produced against measles virus prevents successful escape by single mutations (Birrer et al., 1981). Frank and Bush (2007) argued, with the help of a mathematical model, that the epidemiology of measles may promote selection for increased transmission in naïve individuals rather than antigenic escape.

At the other end of the spectrum, rapid antigenic shifts in certain pathogens can be responsible for recurrent outbreaks in populations. Influenza A presents a unique pattern of uninterrupted punctuated antigenic evolution, causing annual epidemics through drift (Boni et al., 2006) and irregular larger outbreaks through antigenic shift (Smith et al., 2004) (see Box 1 for more details on influenza). In dengue, recurrent epidemics associated with alternation of the main four serovars can be explained by a combination of antibody-dependent enhancement (increased viral replication in patients infected successively with two different serovars), short-lived cross-immunity and seasonal variation in vector demography (Wearing and Rohani, 2006). Irregular outbreaks of *Neisseria meningitidis* may be the result of both antigenic and pathogenicity diversity among circulating strains (Stollenwerk et al., 2004).

Antigenic variation enables some pathogens and parasites to persist inside hosts for long periods in the face of adaptive immunity. Within-host evolution has been well characterised in immunodeficiency viruses (human-HIV and simian-SIV). In particular, it has been shown that epitope-specific cytotoxic lymphocytes (CTL) select for virus escape mutants in patients with a certain HLA genotype ($B57^+$) (Geels et al., 2003), which can hinder vaccine protection (Loh et al., 2008). In addition, SIV escape mutants selected in one animal also appear to be protected against CTL response when transmitted to naïve hosts (Friedrich et al., 2004). However, recent research indicates that epitope-specific CTL may not be the main cause of infected cell death in HIV (Asquith et al., 2006), which raises questions about the effectiveness of selective pressure on the virus in humans. Interestingly, a follow-up study in macaques infected with SIV found higher rates of CTL-mediated killing than in HIV-infected humans (Asquith and McLean, 2007). This – and other similar reports – emphasises the need for caution when extrapolating results from animal models to human infections. Other pathogens, especially eukaryotic ones, have acquired a number of different copies of genes coding for antigens. This antigenic repertoire enables them to respond very rapidly (by switching gene expression) to the host's immune response. Frank and Barbour (2006) have reviewed a number of examples, including bacteria (*Borrelia* spp.) and protozoa (*Trypanosoma* and *Plasmodium* spp.), with a description of the mechanisms involved.

High levels of antigenic diversity in pathogens are a challenge for the development of vaccines. In the case of seasonal influenza in humans, virus evolution is essentially linear (Smith et al., 2004) and so vaccines can be evaluated, and if necessary updated, on an annual basis. This contrasts with swine influenza, where, despite near identical viruses to man, many strains co-circulate (Marozin et al., 2002); the divergent patterns between the two host species is likely due to population structure and demography. For other pathogens which present a number of co-circulating serotypes, conjugate vaccines can be produced to protect against several of the most prevalent strains: a heptavalent vaccine against *Streptococcus pneumoniae* was introduced in the United States in 2000 with good results (Centers for Disease Control and Prevention, 2008). Tetravalent vaccines against the four main serotypes of dengue virus are now being tested with great hopes thanks to recent massive investment (Edelman, 2007). However, the development of vaccines against HIV (Thomson et al., 2002; Fernandez et al., 2007; Loh et al., 2008; Moriya et al., 2008) and malaria (Takala et al., 2007) is still held back by the high level and plasticity of antigenic diversity in the pathogens.

For those vaccines that have been developed and used for many years or even decades, there is concern that vaccination itself may select for antigenic evasion. Martcheva et al. (2008) reviewed a number of initially successful vaccination campaigns that were eventually followed by increases in prevalence with confirmed or putative antigenic escape of the pathogens, including *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Bordetella pertussis*. There have also been reports involving viral pathogens, notably hepatitis B virus. Following vaccination campaigns, a number of mutations conferring antigenic variation have been identified in infected vaccine recipients in Italy (Carman et al., 1990), Taiwan (Hsu et al., 1999) and Western

Box 1. A success story: the phylodynamics of influenza A virus in humans.

Following the onset of vaccination campaigns against influenza in the 1940s, it was soon realized that rapid antigenic escape by the virus necessitated regular update of vaccine composition (Salk and Suriano, 1949). The red blood cell agglutination assay previously developed by Hirst (1941) enabled the quantification of this antigenic escape through time, revealing a pattern of punctuated evolution with periods of 'antigenic drift' followed by major 'shifts' responsible for pandemics (Webster and Laver, 1972). Molecular epidemiology identified mutations and recombinations as two major mechanisms of influenza virus evolution (Young et al., 1979; Young and Palese, 1979). Thanks to advances in molecular biology, it then became possible to construct phylogenies of influenza viruses based on gene sequences (Fitch et al., 1997). However, two major issues remained: (i) the mechanisms responsible for the observed punctuated evolution of the virus, and (ii) the integration of serological and molecular data.

Mathematical modelling has been the key to significant progress in the last six years. A first step was the development of frameworks to explore the dynamics of multiple strains of influenza viruses in a population with immune memory (Gog and Grenfell, 2002), demonstrating that antigenic escape can naturally result in a succession of strain clusters. By matching phylogenetic and epidemiological data with computer simulations, Ferguson et al. (2003) refined the model of cross-immunity to account for both antigenic drift and shifts. But both models relied on a simplistic mapping between genetic and antigenic changes and did not take serological data into account. The main difficulty was that, unlike gene sequences, antibody binding assays do not allow a direct comparison between viral strains, as the antigenic 'distance' between two strains is always relative to a specific serum.

This problem was solved by Smith et al. (2004) who developed an algorithm that could locate together all viral strains and sera on a 'map' (hence the term 'antigenic cartography'). It then became possible to estimate numerically the antigenic distance between any pair of strains, and highlight discrepancies with their genetic distance. The idea that different mutations in different contexts can have different antigenic effects was the basis for a further model for influenza evolution coupled with seasonal transmission (Koelle et al., 2006), which provided the most comprehensive explanation to date for the punctuated evolution of influenza A viruses. Lately, Russell et al. (2008) combined genetic analysis and antigenic cartography to reconstruct the global circulation of the virus on a yearly basis: their demonstration that seasonal influenza is seeded worldwide from strains circulating within South-East Asia will have important consequences for vaccine selection. Thus phylodynamics (as defined by Grenfell et al., 2004) is providing a way to start predicting the direction of pathogen evolution.

Africa (Karthigesu et al., 1999). Another worrying example is that of avian influenza H5N2: recent isolates from Mexican chickens (2002–2006), following a vaccination programme, exhibited significant molecular drift when compared with the H5N2 vaccine-strain or other field isolates (1994–2000) (Escorcia et al., in press). However, it should be stressed in the interest of public health that, despite the occurrence of antigenic escape, mass vaccination remains a very efficient way of controlling the spread of infectious diseases. Research into ways to maintain vaccine protection in the long term is therefore very important, along with economic solutions to ensure that the cost of novel vaccination strategies does not prevent their implementation in the poorest regions which suffer the most from infectious diseases.

The question of the existence of fitness costs associated with antigenic mutations (as those associated with drug resistance mutations) has surprisingly received limited attention, either from biologists or from theoreticians (but see Gog, 2008). It is however an important issue, to determine when and how quickly an escape mutant can invade, or whether those mutations may be lost when introduced into a naïve host or population. Typically, mutations conferring antigenic escape reduce antibody binding to a specific site of a surface or excreted protein of the pathogen. If this site also plays a critical role in the pathogen's infectivity, then a cost is likely to arise. Most of the available information concerns CTL epitope mutations in immunodeficiency viruses, but results are contrasted. *In vitro* experiments with SIV (Peyerl et al., 2004) and HIV (Martinez-Picado et al., 2006) measured the replicative fitness of a number of CTL escape mutants and showed that costs were common. However, compensatory mutations can subsequently restore part of this fitness loss (Crawford et al., 2007). Other reports, based on human transmission surveys, found no or low costs of escape mutations (Goulder et al., 2001; Asquith et al., 2006). While the various escape mutations identified may differ in their fitness costs, those costs and the associated benefits are also likely to vary in different conditions and according to the transmission routes.

3.4. Combined patterns of evolution

The three patterns of evolution (virulence, drug resistance and immune escape) described previously are not necessarily independent, although they have mostly been studied as such. However, there are a growing number of cases where two or more traits appear to evolve together, even though the underlying genetic relations are not fully understood. Emerging infectious diseases (EID) involve all aspects of evolutionary epidemiology. Jones et al. (2008) analysed 335 occurrences of EID recorded since 1940, which include novel diseases of animal origin, but also new strains of known pathogens which have caused significant outbreaks due to changes in virulence, antigenicity or drug resistance. In addition to the changes previously described, adaptation to different hosts, including vectors, represents a major evolutionary hurdle for the emergence of pathogens via expansion of host range (Heeney, 2006; Coffey et al., 2008). Influenza A viruses have received particular attention as they are known to cause devastating pandemics when new recombinants acquire the ability to spread among human hosts together with high levels of virulence. An often quoted (but still unproven) hypothesis is that avian and human viruses may co-infect pigs, which would facilitate recombination (Webby and Webster, 2001). Areas where high densities of humans, pigs and birds coexist, such as South East Asia, might therefore act as crucibles for the emergence of new pandemic strains.

Even with established hosts, pathogens can exhibit patterns of increased virulence combined with drug resistance, such as *C. difficile* (Razavi et al., 2007) or *Aspergillus fumigatus* (Romano et al., 2006), or with antigenic escape, such as SIV (Kimata et al., 1999). It was also shown that the severity of meningitis in rabbits varies between serotypes (*i.e.* strains with different antigenic properties) of *S. pneumoniae* (Ribes et al., 2008). Combined increase in drug resistance and antigenic escape has been reported too in HIV (Karlsson et al., 2003; Mueller et al., 2007), while conversely some drug resistance mutations appear to result in enhanced recognition by the immune system (Mason et al., 2004).

Those combined effects of simple genetic changes (mutations or recombinations) are interesting for a number of reasons. First, they may reveal common mechanisms involved in antigenic recognition, virulence and drug resistance. Second, they highlight the importance of considering all aspects of host–pathogen interactions in order to understand pathogen evolution: for example, the use of drugs or vaccines may alter selective pressures on virulence, not only through direct effects (e.g. Gandon et al., 2001) but also through pleiotropic effects of mutations.

4. The long march from evolutionary theory to Darwinian medicine

As we have seen, traditional medical approaches to infectious diseases, despite decades of public health improvement, appear to have allowed or even exacerbated the antagonistic evolution of many pathogens. Evolutionary medicine promises to substitute the short-sighted use of drugs and vaccines with sustainable solutions: if we cannot eradicate infections by frontal assault, we may be able to keep them at bay durably provided we can anticipate the next move of pathogens. However, there is a long way from evolutionary theory to practical treatment and policies. In this section I will firstly review some of the latest trends in theoretical evolutionary epidemiology; secondly cast light on the increasing use of molecular biology in combination with epidemiology and evolutionary biology; thirdly describe new treatments and control measures that seek to address some of the challenges raised in the previous section; lastly and briefly, broach the topic of host evolution and its potential impacts in medicine and agriculture.

4.1. New trends in mathematical modelling

Mathematical models have been used from the early ages of epidemiology in the 19th century (Fine, 1979) and have gained a wide recognition, now playing a major part in influencing public health policies (Grassly et al., 2001; McKenzie, 2004; Matthews and Woolhouse, 2005). Evolutionary models have been less successful in permeating the medical and veterinary communities, with the possible exception of models for antibiotic resistance (Temime et al., in press). One reason is that evolutionary models have traditionally relied on simplistic epidemiological frameworks (Galvani, 2003). This has prompted harsh criticism of recent attempts by evolutionary biologists to make specific predictions on pathogen evolution (e.g. Gandon et al., 2001; Smith, 2002). While evolutionary models have often been developed independently of applied research, novel approaches aim to produce results that can be directly tested and applied in microbiology and epidemiology. Promising trends have appeared that rely on integrating previously separate modelling approaches.

A number of authors have started to address the main caveat of adaptive dynamics, namely the separation of ecological and evolutionary time scales. Day and Proulx (2004) and Day and Gandon (2007) have developed an analytical framework inspired by quantitative genetics which can be used to monitor the simultaneous dynamics of epidemics and virulence evolution, as well as the selective pressure caused by vaccination (Gandon and

Day, 2007). Boni et al. (2006) used a similar approach to model the antigenic evolution of influenza A virus during a single seasonal outbreak. The most popular technique however relies on stochastic simulations, made easier by constant progress in computer power. Stochastic models are particularly relevant in the context of unsteady dynamics such as epidemic outbreaks, as they can account for random strain extinctions: because real populations are of finite size, even strains that would be expected to spread in a deterministic model (which assumes an infinite population size) may fail and disappear while they are at low prevalence. Stochastic epidemic simulations have been used to investigate the short-term evolution of pathogen virulence and life history traits (van Ballegooijen and Boerlijst, 2004; André and Hochberg, 2005; Read and Keeling, 2007), antigenic variation (Gog and Grenfell, 2002; Abu-Raddad and Ferguson, 2004; Kirupaharan and Allen, 2004; Stollenwerk et al., 2004; Restif and Grenfell, 2007) or combinations of both (Restif and Grenfell, 2006). They also cast new light on the role of evolution in emerging infectious diseases of zoonotic origin (Antia et al., 2003; André and Day, 2005; Day et al., 2006), focusing on the early stages when the pathogen's reproductive ratio (R_0) is lower than unity in the human population. Those studies emphasize the role of chance and transient dynamics in epidemiology and pathogen evolution, and encourage predictions for public health to be made in terms of probabilities associated with different possible outcomes.

Another challenge for modellers, and biologists alike, is to reconcile within- and between-host levels of selection. Traditionally, models have focused either on host population dynamics (epidemiology) or *in vivo* dynamics (microbiology). However, some modellers have proposed a 'bottom-up' approach, starting with models of within-host dynamics and assuming that the transmission potential at any time is a simple function of the current pathogen load. An interesting outcome of this approach is the emergence of trade-offs between pathogen life history and transmission (Alizon and Van Baalen, 2005; Alizon, 2008; Mideo and Day, 2008), which had previously been introduced as *ad hoc* assumptions in evolutionary models. It is then possible to design 'nested models', where the transmission term of an epidemiological model is derived from an underlying within-host model, and investigate the evolution of virulence driven by multi-level selection: André et al. (2003) extended a simple predation model for within-host bacterial dynamics (Antia and Lipsitch, 1997), while Gilchrist and Coombs (2006) considered viral dynamics for persistent infections like HIV. However, those frameworks are complex and are only tractable with simplistic assumptions. Heffernan and Keeling (2008) developed an elegant method to incorporate waning immunity in the population dynamics of acute viral infections and applied it to measles, but they have not yet used it in an evolutionary context. The main difficulty in designing those models is the lack of quantitative experimental data linking the two levels. A few studies on veterinary infections have combined clinical records and transmission measurements, notably in foot and mouth disease (Alexandersen et al., 2003) and equine influenza (Park et al., 2004). The latter provides a rare assessment of the quantitative effects of antigenic escape on the duration and variability of the latent and infectious periods of infection in the natural host, which are critical elements for the design of realistic mathematical models. In contrast, for most human infections, the only feasible experimental measurements of antigenic diversity rely either on *in vitro* antibody assays or on *in vivo* clearance assays where an animal model is available. They give no information on the actual variations in susceptibility or infectivity in human hosts, and mathematical models have to rely on unproven assumptions. In other words, there is still a discrepancy between the definitions of 'cross-immunity' in microbiology and epidemiology. The first step towards reconciling these two levels may be to improve our quantitative understanding of the *in vivo* population dynamics of pathogens and immune defences (Bonhoeffer et al., 1997; Levin and Antia, 2001). Recent progress has been made through the combination of sophisticated microbiology techniques and mathematical models (Brown et al., 2006a; Grant et al., 2008), but more work is needed to get reliable models that can account for evolution of pathogens over their entire life cycles.

4.2. From molecular epidemiology to phylodynamics

Tremendous progress in molecular biology over the last 30 years means that the genotype of virtually any pathogen isolated from a patient anywhere in the world can be analysed quickly and cheaply. The term 'molecular epidemiology' emerged in the 1970s when it was realized that gel electrophoresis techniques could be used to compare and classify virus isolates from different origins more reliably than existing serological assays (e.g. Pereira et al., 1976). The advent of polymerase chain reaction (PCR) in the late 1980s allowed comparisons at the genetic level, making it possible to identify the precise nature of variability among pathogen isolates. Whatever the technique used to produce molecular data, the principle of molecular epidemiology has remained the same: genetic differences between isolates reflect their temporal divergence from a common ancestor (which is a foundation of evolutionary theory). The key addition here is the fast rate of mutations in pathogens, which can accumulate over the same time scale as epidemic spread. Molecular epidemiology is in essence a forensic science which can, for example, help to relate multiple occurrences of antibiotic resistance or identify routes of transmission (McDade and Anderson, 1996).

Unfortunately, as technology becomes more and more readily available, molecular epidemiology is sometimes seen by practitioners as just another diagnostic tool (Traub et al., 2005)—indicative of a still widespread approach to epidemiology that focuses on risk factors in a static framework. As a result, the vast amount of data collected on pathogen molecular diversity is largely under-exploited. It has become apparent that a correct and valuable interpretation of those data requires the integration of knowledge from various disciplines (Levin et al., 1998; Tibayrenc, 1998), including molecular biology (nature of the marker), population genetics of the pathogen species considered (mutation, selection, recombination and genetic transfer) and its ecology (spatial range, seasonality, migration/dispersion and host range). Recent progress has been made towards this integration. de Meeus et al. (2007) described a number of existing tools from population genetics that can be and have been used to address specific questions in epidemiology. Chevillon et al. (2008) combined genetic and ecological knowledge to contrast the life histories of chikungunya and dengue viruses and assess the risk of chikungunya re-emergence. Table 2 lists a number of pathogens whose recent evolutionary history has been reconstructed thanks to molecular epidemiology. It highlights the importance of host ecology as well as recent control measures in shaping the evolution of pathogens.

Most of the aforementioned studies have considered neutral genetic variability to track pathogen spread in time and space. However, there have been recent attempts to reconcile molecular epidemiology with adaptive evolution of pathogens. For example, evolutionary and ecological input into molecular surveys can help understand global phenomena such as antibiotic resistance across taxa of bacteria (Aminov and Mackie, 2007). As we saw earlier, immune escape is another major driving force of pathogen evolution. Grenfell et al. (2004) proposed an integrated approach

Table 2
Using molecular epidemiology to reconstruct the evolutionary history of pathogens.

Pathogen	Patterns	References
Dengue virus	Unclear origin; the four serotypes have emerged from an early stage, possibly in monkey hosts, and remain endemic in human populations. Complex patterns of cross-immunity and antibody-dependent enhancement allow the ongoing cyclic circulation of the four serotypes	Holmes and Twiddy (2003)
G12 rotaviruses	Recent emergence and worldwide spread, associated with gene acquisition and rapid diversification	Rahman et al. (2007)
Human influenza A virus	Recent spatiotemporal patterns of antigenic diversity within the H3N2 strain suggests permanent circulation and evolution in southeast Asia and seasonal spread to Europe, Oceania and Americas	Russell et al. (2008)
Paramyxoviruses	Recent divergence between main viral species, followed by drastic population bottlenecks linked to the epidemiology of measles, mumps and canine distemper viruses	Pomeroy et al. (2008)
Listeria monocytogenes	Clonally clustered phylogeny with ancient divergence, possibly associated with different environments or routes of transmission. Extremely low rates of recombination	Ragon et al. (2008)
Methicillin-resistant *Staphylococcus aureus*	The main MRSA clones have emerged from the most successful clones of methicillin-susceptible *S. aureus*. The *mec* resistance cassette was then transmitted horizontally	Enright et al. (2002)
Mycobacterium bovis	Highly clonal structure, forming 6 main lineages worldwide with local spread; local adaptation to different host populations	Gagneux et al. (2006)
Mycobacterium leprae	Outbreaks caused by expansion of single clones; the disease originated in East Africa or Near East and spread worldwide with human migrations	Monot et al. (2005)
Salmonella enterica serovar Typhi	Long-term persistence of several genotypes, until recent use of antibiotics, which caused independent selection of various mutations and stimulated the clonal expansion of one genotype. Acute infections play a key role in the pathogen's evolution. Genetic drift and gene loss are dominant over selection at the genomic level, consistent with the hypothesis that human carriers are the main reservoir	Roumagnac et al. (2006), Holt et al. (2008)

to pathogen evolution, which they named phylodynamics, that combines immune selection with pathogen population dynamics, in order to interpret the various shapes of molecular phylogenies. Since then, this integrative theory has been useful in the consideration of the recent evolution of human influenza A viruses (see Box 1), thanks notably to the development of antigenic mapping (Smith et al., 2004), which enables a direct quantitative comparison of genetic and phenotypic evolution.

4.3. Delivering evolutionary medicine: new answers and new questions

Concerns about drug resistance, antigenic escape and virulence evolution have motivated the development of alternative control methods against infectious diseases, which hopefully have the potential to inhibit antagonistic evolution or even take advantage of evolution. However, the fact that a new treatment may remove the selective pressures created by previous ones does not mean that it will not select for adapted defence mechanisms in the pathogen via other routes. If anything, previous experience should teach us caution and encourage us to try and anticipate the opponent's next move. Here I propose a critical review of some of the latest ideas in evolutionary medicine.

Biological control is a fairly old idea to combat various pests of human health and agriculture, although it has seldom been practised with an evolutionary perspective. Indeed, the main focus in the context of biological control has been on population dynamics (Murdoch et al., 1985). Elliot et al. (2002) begin their review on virulence management in biological control by acknowledging that the only example to date where virulence evolution has been monitored in a real system is the spread of myxomatosis in the Australian rabbit population, studied by Fenner (1983). In a medical context, biological control of pathogens has been attempted for a long time though mostly on an empirical basis. Phage therapy of bacterial infections (relying on bacteriophages, which are viruses infecting bacteria) was first tried eighty years ago but its clinical use has been replaced by antibiotics since the 1940s (Levin and Bull, 2004), except in the former Soviet Union and Poland (Alisky et al., 1998). Now, in the anxious search for solutions to antibiotic resistance, phage therapy is in the spotlight again. A number of recent studies have started to investigate its feasibility and safety in farm animals (e.g. Sheng et al., 2006) and in mouse models of human infections (e.g. Capparelli et al., 2007). However, two important issues appear to have been largely overlooked so far, concerning the ecology and evolution of bacteria-phage systems. First, naturally occurring phages (including activated prophages) are conspicuous in infections potentially targeted by phage therapy, such as septicaemia, where they play an important role in mediating competition between the different bacterial species or strains present (Gaidelyte et al., 2007). Experimental models that include some bacterial diversity are therefore necessary to appreciate the complex dynamics of phage systems (Brown et al., 2006b; Joo et al., 2006). Second, *in vitro* studies have highlighted that cultures of bacteria and phages undergo rapid coevolution (Buckling and Rainey, 2002). Although phage evolution itself might ensure their continuous therapeutic success, more detailed studies, both theoretical and experimental, are needed to fully understand these coevolutionary dynamics (Levin and Bull, 2004).

Other strategies of biological control are being considered against bacterial infections, such as the use of probiotics, *i.e.* commensal bacteria with a potential competitive advantage over invasive pathogens. Probiotics have received considerable clinical interest in the treatment of infectious and non-infectious pathologies, notably for their anti-inflammatory properties, but the mechanisms involved are still poorly understood (Boirivant and Strober, 2007; Colbère-Garapin et al., 2007). Even less is known about the ecological and evolutionary interactions between probiotics and pathogens. Corr et al. (2007) showed that the probiotic strain *Lactobacillus salivarius* UCC118 expressed a bacteriocin active against the invasive foodborne pathogen *Listeria monocytogenes*. Recent experimental (Stecher et al., 2007; Lawley et al., 2008) and theoretical (Brown et al., 2008) studies have highlighted the complex interactions between pathogens, commensal bacteria and host inflammatory responses with important implications for the use of probiotics. Another example is the use of avirulent bacteria as live vaccines against related virulent strains. Beside historical attenuated vaccines against polio or measles, new candidates include bacterial strains of *B. pertussis* (Ho et al., 2008), *M. tuberculosis* (Martin et al., 2006) or *S. enterica* (Guzman et al., 2006). Once more, careful attention needs to be given to the ecology and evolution of the full system, as those studies have focused on the immune reactions. Indeed, Foster et al. (in press) observed that the *in vivo* growth rate of a virulent strain of *S. enterica* in mice can increase in the presence of an attenuated strain. And one cannot exclude either that co-infections following

live vaccination might select for increased virulence in the pathogenic strain. Finally, the possibility for some live immunizing agents (e.g. polio virus) to be transmitted between hosts has to be carefully monitored and controlled, particularly if reversion to virulence can occur.

From another perspective, some of the molecular mechanisms of pathogen adaptation that have been recently identified (see Section 2) are seen as new potential targets for chemotherapy. The goal is to counteract the adaptive tricks used by pathogens to resist drugs (see Table 1) or escape immune response, or even to curb their ability to evolve. For example, by preventing bacteria from switching to persistent stage (Smith and Romesberg, 2007) or by blocking quorum sensing (Hentzer et al., 2003; Bjarnsholt and Givskov, 2007), it should be possible to improve antibiotic efficacy against bacteria. It may also be possible to reduce the rate of mutation of some viruses (Vignuzzi et al., 2008) and bacteria (Cirz et al., 2005) to reduce their chances of becoming drug resistant. Promising as these ideas may be, it is too early to know what selective pressures they may impose on pathogens. In any case, they underline the importance of improving our understanding of the mechanisms involved in the evolution and the life history of pathogens. This requires to consider the infection cycle as a whole (Schmid-Hempel and Ebert, 2003; Merrel and Falkow, 2004) and assess the links between growth, virulence, immune evasion and transmission (Frank and Schmid-Hempel, 2008).

4.4. Host evolution: the next frontier?

Because most human pathogens have a very short generation time compared to ours, host evolution has been overlooked in the medical context. However, the development of human genetics and genomics has shed new light on our evolutionary history in relation with infectious diseases. In a veterinary context, biotechnologies even offer solutions to manipulate host susceptibility to infections.

There appears to be widespread polymorphism in human populations for susceptibility to a range of infectious diseases. This can help to understand the global epidemiology of those infections and offer new targets for treatment or vaccine development. The human leukocyte antigen (HLA) gene complex, which plays a crucial role in pathogen recognition, is a well-known source of diversity within and between populations. It has long been argued that pathogens must be the main driving force of this persistent polymorphism, although direct evidence is lacking (Jeffery and Bangham, 2000). Using genetic data from human populations worldwide, Prugnolle et al. (2005) found that geographical variations in HLA diversity were driven mainly by human colonization history and local pathogen richness. It has also been proposed that the evolution of selective mate choice in mammals (including humans) has ensured the maintenance of polymorphism of the major histocompatibility complex (MHC, the equivalent of HLA in other animals), as a way to improve immune defence among offspring (see review by Milinski, 2006). Variability at an increasing number of other genes has been found to correlate with protection against specific pathogens. In particular, a single deletion ($\Delta 32$) on the gene coding for the CCR5 chemokine receptor confers resistance to HIV and has a high prevalence in Europe. The evolutionary history of this mutation in human populations is still debated, in relation with the recent HIV pandemic as well as older pandemics of smallpox and bubonic plague (Galvani and Novembre, 2005; Sabeti et al., 2005; Zawicki and Witas, 2008). Other phenotypes, such as HIV 'spontaneous control' (untreated patients chronically infected but maintaining very low viral levels), are not well understood yet (Pereyra et al., 2008). Hennig et al. (2008) identified single nucleotide polymorphisms in three genes (other than HLA) associated with variation in antibody levels following hepatitis B vaccination in infants, which could affect long-term protection. Inherited resistance to malaria, linked to haemoglobin genotype, has also been documented: sickle haemoglobin has long been associated with resistance (Allison, 1954); and Cholera et al. (2008) recently identified the cellular mechanism that confers protection. Individuals homozygous for another genotype, haemoglobinopathy α^{+}-thalassaemia, are also protected against severe malaria (Lell et al., 1999). Fowkes et al. (2008) showed that thalassemia was associated with increased erythrocyte count and microcytosis (small erythrocyte size), which may be responsible for the observed protection.

Identifying and manipulating the genetic bases of host resistance have received particular attention in farm animal management. Initial approaches were mostly empirical, relying on breeding for resistance: Webster (1924) first proposed the idea in mice based on observed variability in resistance to typhoid infection. Selective breeding has been used empirically in agriculture, but with conflicting production interests (Donald, 1994). Although some genetic mechanisms have been identified, resistance phenotypes can be quite complex and may involve several genes: for example, Hutchings et al. (2007) compared the behaviours of a sheep breed resistant to parasitic infections with a susceptible breed, and found that the resistant animals avoided grazing tussocks which were more likely to harbour parasites. The identification of target genes is not a simple process. Mx proteins (a family of GTPases) are known to play a major role in susceptibility to orthomyxoviruses (including influenza viruses) in lab rodents (Haller et al., 2007; Tumpey et al., 2007). Various alleles of the Mx gene have been reported in chickens worldwide (Balkissoon et al., 2007), suggesting that breeding for resistance could be implemented if a protective role of Mx against avian influenza was established (but see Benfield et al., 2008). More unusual is the hypothesis proposed by Arnaud et al. (2007), based on sheep population genetics: the fixation of endogenous retroviruses (ERV) in animal genomes may reflect antagonistic coevolution between hosts and exogenous (pathogenic) retroviruses, as ERV have been found to protect hosts against infection by exogenous viruses (Best et al., 1997). Once genetic factors have been identified, biotechnologies offer new ways to introduce them in herds: transgenic farm animals have recently been produced, including cows resistant to *S. aureus* mastitis (Wall et al., 2005) and goats expressing human lysozyme which produce milk with enhanced health standards (Maga et al., 2006). However other attempts have not had the expected results: Hyvönen et al. (2006) found that transgenic cows producing lactoferrin in milk were not protected from experimental *E. coli* intramammary infection. An important stage of research in this field is to assess the impact of host resistance on the epidemiology of infectious diseases in an agricultural context. Mathematical models can be used to estimate what proportion of resistant animals need to be introduced in order to control the spread of infections (Geenen et al., 2004; Nath et al., 2004). Finally, it is essential to keep in mind that pathogens can evolve in response to any change in host populations, and this needs to be taken into account when assessing the value of potentially costly host enhancement programmes.

5. Perspective

Evolution plays a key role in some of the main challenges facing modern medicine in the field of infectious diseases: hypervirulence, drug resistance and antigenic escape. However, while the biomedical community has swiftly embraced genetics as a technology, it has been less prompt to recognize the practical

value of evolutionary biology. Most biologists can quote Dobzhansky's famous maxim about the enlightening value of evolution, but few actually see it at work. What I hope to have demonstrated in this review is that evolutionary biology is essential to properly understand infectious diseases, make sense of the enormous amount of information provided by molecular biology, and devise new strategies for sustainable control. The fundamental change it brings to our perception is a focus on the pathogen rather than on the disease: disease and patient, albeit at the centre of the medical agenda, are just one part of the life cycle of the microorganism at stake. Any control measure we take against infection will modify the selective pressures on the pathogen, but in a way that depends on the other constraints it is facing, both inside and outside the host targeted by the treatment. Without considering evolutionary biology, we may just keep on designing new drugs that will become obsolete within a few years, constructing phylogenic trees without understanding what processes shaped them, or end up selecting for harmful traits while focusing on specific elements of pathogenesis in isolation.

Mathematical models have a pivotal role to play in the integrative science needed to address these complex issues. Modelling frameworks have undergone huge progress over the past 20 years and their analytical and predictive values are increasingly acknowledged across the scientific, medical and political communities. Even before providing quantitative information on a system, models represent a rigorous basis to think about the underlying processes and identify key questions that need to be addressed experimentally.

So, twenty years on, evolutionary epidemiology may not have delivered its promises yet. I would argue that early simple mathematical models, and the elegant case study of myxomatosis in Australian rabbits, motivated an undue optimistic view that pathogen evolution could be easily understood and therefore controlled. Now, evolutionary epidemiology has hopefully reached the age of reason: its value does not reside in easy, universal answers, but in the provision of a new way to tackle infectious diseases at all levels, using integrated, multi-disciplinary approaches. Evolutionary epidemiology will become a reality if, whenever a new approach to infectious disease control is being considered, we seek to address the question: "How will the pathogen evolve, and what can we do about it?"

I conclude with a list of open questions which, although central to the development of dynamic models for infectious diseases, still require more empirical investigation.

- Transmission rates can be inferred from epidemiological data, however the temporal variations of infectivity at the individual host level are still poorly known for most diseases, and the relationship of transmission with pathogen load and host immune status has hardly been touched upon.
- The phylodynamic framework seeks to integrate antigenic diversity and epidemiological dynamics. This cannot be fully achieved until cross-immunity (typically measured *in vitro* by antibody binding assays or *in vivo* as within-host pathogen growth) can be measured in terms of host susceptibility or infectivity. Some information is available from animal experiments (Park et al., 2004), but the technical challenge must be overcome.
- While colossal amounts of genetic and genomic information are being produced on a wide range of pathogens, the genotype–phenotype map needs to be addressed. Here phenotype goes beyond protein expression, where good progress has been made: we need to unravel the full chain of translation into host–pathogen interactions (host colonisation, immune evasion, virulence, transmission, within- and between-host ecology). Antigenic cartography (Smith et al., 2004) provides one way forward. Of particular relevance for evolutionary issues are genetic relations between those phenotypic traits, which can affect selective pressures and create trade-offs.
- The main focus of this review has been on antagonistic evolution of pathogens, enabling them to counter host defences and cause disease. However, evolution also matters in the maintenance of good health which, for most plants and animals, relies on a wide range of symbiotic micro-organisms. Recent evidence points to a fine and moving line between 'mutualism', 'commensalism' and 'pathogenicity' in microbial communities (Dethlefsen et al., 2007; Mazmanian et al., 2008; McBride et al., 2008), which may require a change of paradigm in our approach to virulence.

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