Reconciling Pasteur and Darwin to control infectious diseases

Samuel Alizon

Laboratoire MIVEGEC (UMR CNRS 5290, IRD 224, UM1, UM2)

911 avenue Agropolis, B.P. 64501, 34394 Montpellier Cedex 5, France

email: samuel.alizon@cnrs.fr

Keywords: virulence, microbes, public health, vaccination, resistance, coevolution

2215 words in the main text, 60 references, 1 table and 3 figures.

Abstract

The continual emergence of new viruses and the increased spread of antibiotic resistance in bacterial populations keeps reminding us that microbes are living entities that coevolve with public health interventions. Following the historical thread of the works of Pasteur and Darwin shows how reconciling clinical microbiology and ecology & evolution can be instrumental to understand pathology, develop new therapies and prolong the efficiency of existing ones.

Surgeon General William H. Stewart told Congress in 1969 that it was time to "close the book on infectious diseases," to declare the war against pestilence won and to shift national resources to such chronic disease problems as cancer and heart disease.

Garrett L, *Newsday*, Discovery Section (May 30, 1989)

The burden of infectious diseases on global health has alleviated over the last century [1] and until the end of the 1980s, it was thought the pasteurian ideal of controlling infectious was beyond grasp, especially with the eradication of smallpox through vaccination. Today, the famous and probably apocryphal [2] line attributed to William H. Stewart reads as overly optimistic after the emergence of Human Immunodeficiency Virus (HIV) and the ongoing spread of antibiotic resistance. This essay shows how re-uniting Pasteur's experiments with Darwin's theories may be decisive for controlling infectious diseases (Figure 1).

A missed rendez-vous?

Although the French microbiologist and the English naturalist were at the top of their career at the same time, they barely interacted and have rarely been compared [3–5]. This distance can be blamed on all the differences listed in Table 1. In 1988, molecular biologist Joshua Lederberg referred to this as a '*lost opportunity*' [4] and microbes are indeed perfect organisms to test evolutionary theories [6]. But are Pasteur and Darwin really to blame for the current lack of evolutionary biology in public health?

Contrarily to a still common belief [e.g. 7], Darwin did write about microbes and even thought about how to integrate them in his theory [8]. On the microbiology side, the conclusion reached by a detailed history of science study [9] is that '*Work on virulence and vaccines made the 1880s bacteriological laboratory perhaps the earliest place of sustained experimental cellular-level in vitro research on phenomena understood as biological variations and evolutionary mechanisms.*' Harnessing ecology and evolution to control infectious diseases therefore somehow means going back to the origins.

Emergence of new threats

Pasteur's hypotheses rarely strayed away from facts obtained in well controlled settings. One of the few exceptions is when he formulates hypotheses regarding the origin of the virulence of some infectious diseases [9, 10]. This must have cost him because of his religious beliefs and also because Darwin's book was used by proponents of the spontaneous generation [3–5]. But his intuition was correct in that pathogen emergence offers a perfect illustration of the role evolution can play in public health.

The only illustration in *The Origin of the species* strongly resembles a phylogeny. With the progresses in DNA sequencing and computer sciences, phylogenies are now commonplace, especially in epidemiological studies. The analysis of genetic sequences from population of microbes infecting one or several individuals has led to a field named phylodynamics, which postulates that the way infectious diseases spread leaves footprint in their genomes [11, 12]. For instance, analysing avian influenza virus genomes reveals the importance of environmental transmission in the life cycle of the virus [13].

The 2014-2016 Ebola epidemics in West Africa marked a quantitative shift in sequencing with the publication of full virus genomes sampled from 78 infections within the first months of the outbreak [14]. Overall, more than a thousand full genomes have already been analysed, thus giving us a detailed vision of the spread of the epidemics between countries [14, 15]. Furthermore, phylodynamics approaches have been used to infer key epidemiological parameters such as the basic reproduction ratio (R_0) or infection duration [16, 17].

Emergence often involves adaptation to new hosts. Pasteur argued that attenuated forms of virulent parasites already exist in the population and that their '*virulence can be progressively reinforced*' if the environmental conditions are adequate [9, 10]. The outbreak of Chikungunya virus that occurred in La Réunion island in 2005-2006 illustrates how such 'reinforcement' may occur. At the end of 2005, there was a first limited outbreak that caused a few thousand cases. In 2006, it was followed by a huge outbreak with hundred thousands of cases. The main reason for the size difference was that in 2006 most viruses bore a key mutation in position 226 of the E1 protein, which allowed them to better exploit *Aedes albopictus* (tiger) mosquitoes as a vector [18]. This explains the second outbreak, especially because of *A. albopictus*' preference for human blood meals. Invoking the 'evolutionary rescue' framework offers an even more

dynamical picture [19]: the virus population that emerged at the end of 2015 was bound to go extinct rapidly but the evolutionary event (the substitution in position 226) allowed it to persist and generate a major outbreak. By combining epidemiology and evolution, it is possible to quantifies the probability for such a major outbreak to occur [20] (Figure 2C).

Virulence evolution

Microbial virulence is a key concept to grasp the revolution that occurred at the end of the 19$^{\text{th}}$ century in the bacteriological laboratories [9]. Theobald Smith was the first to formalise how virulence should evolve in the field [21]. His theory, although more complex than usually presented [22], is that virulent strains should be counter-selected because killing their host is detrimental to their epidemiological fitness [23].

In the 80s, the avirulence theory was challenged by showing that virulence, defined as the host's parasite-induced mortality rate, can be adaptive if trade-offs are involved [24, 25]. This hypothesis was criticised for its lack of empirical support but one of the clearest proofs eventually came from studying HIV-1 in humans. HIV virulence is classically measured in absence of treatment as the inverse of the time to AIDS, a trait controlled both by host and virus genetics [26]. Therefore, by definition, HIV virulence decreases infection duration. However, increased virulence also comes with increased probability of transmission per sexual contact. The trade-off originates from these two opposite forces: milder viruses cause longer but poorly contagious infections, whereas virulent viruses cause short but contagious infections. As a result, HIV seems to have evolved towards intermediate levels of virulence that maximise the number of secondary infections an infected host causes [27, 28].

The existence of a transmission-virulence trade-off has direct implications for public health interventions, especially vaccination, which was Pasteur's main instrument to fight infectious diseases. Indeed, if more virulent strains tend to transmit more per unit of time, modifying the life cycle of the parasite may affect the nature of the fittest strain [29] (Figure 2B). This was shown experimentally using the rodent malaria parasite *Plasmodium chabaudi* in mice: a replication-blocking vaccine leads to the evolution towards higher levels of virulence during serial passage experiments [30]. According to theory, interventions that only reduce infection virulence, therefore changing infected treated hosts into healthy carriers, are the most dan-

gerous on the long term. This is supported by the consequences of the implementation of virulence-blocking vaccines against Marek Disease Virus (MDV), an avian herpesvirus that is a major threat to the poultry industry. Until recently, the evidence was mainly correlational but experiments demonstrated that vaccinating chicks allows highly virulent strains otherwise incapable to generate any secondary case to spread [31].

Another theoretical implication of the trade-off is that virulent strains should be favoured early in an epidemics due to population dynamics feedbacks [32]. This short-term effect was shown *in vitro* by generating an outbreak in a bacterial population with a mixture of virulent and non-virulent phages. The initial 1:1 ratio first increases to 100:1 before decreasing to 10:1 in favour of the virulent phage [33]. Field data on the spread of a pathogenic bacteria in North American house finch also supports this theoretical prediction [34]. This makes emerging infectious diseases even more dangerous (not to mention the lack of host immunity).

Resistance to the evolution of resistance

We mentioned how vaccines can shape parasite populations [35] but the example of antibiotics is equally striking. In 2000, the frequency of *Steptococcus pneumoniae* isolates sampled in European hospitals that were resistant to penicilin in 2000 were shown to correlate with the amount of antibiotics prescribed by the hospital's country [36]. A bayesian analysis of prevalence data from Cuba, Venezuela and Estonia even suggested that the quality of a national tuberculosis prevention program may affect the fitness cost paid by *Mycobacterium tuberculosis* resistant strains [37]. Larger hospital sizes have also been shown to be associated with more antibiotic resistance in datasets from the US and Ireland, one interpretation being that a network of small hospitals maximises the risk of stochastic extinctions of newly emerged resistant variants [38].

Parasite virulence has been under scrutiny by clinical microbiologists since Pasteur [39] but it has has also been a central trait for evolutionary biologists [40, 41]. Although there are few exceptions [42, 43], this is not the case for antibiotic resistance. Evolutionary biology studies investigating how to best combine antibiotics [44] or alternate between them [45] are recent compared to the time since these practices have been attempted in the medical field. Even the idea, illustrated in Figure 3, that it is sometimes preferable to use lower doses to contain an infection instead of attempting to eliminate is gaining momentum in the field [46] although

similar 'watch and wait' strategies are already routinely implemented by clinicians, for instance to treat chronic lymphocytic leukemia [47].

Are medical doctors to blame?

Since 1990s, many insist that evolutionary biology should be taught to medical doctors [48, 49]. Indeed, the biomedical literature seems to avoids the 'e-word' [50]. But, from a historical standpoint, medical doctors were always very (if not too) enthusiastic for Darwin's ideas [51].

The contrast between the importance of virulence and antibiotic resistance in evolutionary biology suggests another hypothesis to the lack of evolution in public health. The concept of virulence was forged by the likes of Louis Pasteur and Theobald Smith, whose research relied on phenotypic variation and isolates from the field. Antibiotic resistance was discovered later, at a time where the mechanistic approaches were taking over in biology. Actually, along with phages, antibiotic resistance was instrumental in the development of molecular biology. Part of the problem is that part of the work on the evolution of infectious diseases was lost during the modern synthesis. In his book Theodosius Dobzhansky acknowledges that there was work on '*bacterial variation*' before the 1940s but dismissed it because of its '*Lamarkian flavour*' [52]. Lederberg's 1988 essay on the evolution of pandemics is even more enlightening [4]. He writes that the synthesis between Darwin is finally '*fully integrated*' because '*the study of the mechanisms of virulence is a top priority*'. If anything, this shows that mechanistic studies took over process-based studies. When he does mention virulence evolution, he explains that '*From the virus's perspective, its ideal would be a virtually symptomless infection*', thus missing all the work on the adaptive nature of virulence that took place in the years before [24, 25]. Finally, Lederberg raises the question '*Will AIDS get even worse?*' His question was extremely timely and relevant since meta-analyses suggest that HIV virulence did increase over the last decades [53] but his answer, although again visionary, has little to do with evolutionary biology since he states '*there is a fair possibility that some potential carriers are still uncounted*'.

In summary, although evolutionary biology can undeniably open medical doctors to ultimate causations (e.g. Is fever adaptive?), the instances where this can help them in their daily practice seem limited. Even for drug resistance, which is probably the most relevant evolutionary process they may face, there are national guidelines to follow. Overall, if the space devoted to ecology

and evolution needs to be expanded, it should perhaps first be in public health and molecular & cellular biology curriculae.

Controling coevolution

Microbial evolution in response to public health interventions is unavoidable. In return, the nature of the microbial population shapes the public health response since, for instance, mild strains are less reported and treated than virulent ones. In contrast with Sir Alexander Fleming, who warned against the risk of evolution of antibiotic resistance in his Nobel prize speech in 1945, Neither Pasteur nor Darwin did anticipate this feedback loop. We cannot avoid this coevolutionary process but we can act so that its outcome tends more towards a peaceful coexistence than an arms race [54].

Over the years, the fields that Darwin and Pasteur contributed to create have drifted apart. Evolutionary ecologist tend to focus on processes and phenotypic traits at the expenses of the underlying molecular and cellular mechanisms, whereas clinical microbiologist do the opposite. A synthesis is necessary to understand pathogenesis, but also to develop and preserve therapies. The evolutionary approach also reveals that the stakes are higher than loosing drugs. There is a risk that policies may trigger an arms race favouring more virulent strains, as witnessed for MDV in poultry. Similar concerns apply to the use of anti-microbial peptides in clinical or agricultural practice. Since these peptides are part of our own defence mechanisms, evolution of bacterial resistance could potentially weaken all our immune system [55]. Some studies already point to this risk of joint evolution of antibiotic resistance and virulence [56].

In conclusion, it is urgent to switch from an eradication to a control perspective. Instead of hoping to find 'magic bullets', we should aim at domesticating microbes. In this spirit, interventions such as microbiota transplantation, original administration of drugs (varying doses, alternating or combining molecules) or phage therapy represent the future of evolutionary ecology in public health [57, 58].

Acknowledgments

I thank Mircea Sofonea for helpful suggestions.

Funding

SA is supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 648963), by the CNRS and the IRD.

References

[1] Jones DS, Podolsky SH, Greene JA. The burden of disease and the changing task of medicine. N Engl J Med. 2012;366(25):2333–8.

[2] Spellberg B, Taylor-Blake B. On the exoneration of Dr. William H. Stewart: debunking an urban legend. Infect Dis Poverty. 2013;2(1):3.

[3] Farley J. The Social, Political, and Religious Background to the Work of Louis Pasteur. Ann Rev Microbiol. 1978;32(1):143–154.

[4] Lederberg J. Pandemic as a Natural Evolutionary Phenomenon. Social Research. 1988;55(3):343–359.

[5] Gayon J. Darwin and Darwinism in France before 1900. In: Ruse M, editor. The Cambridge Encyclopedia of Darwin and Evolutionary Thought. Cambridge University Press; 2013. p. 243–312.

[6] Buckling A, Maclean CR, Brockhurst MA, Colegrave N. The *Beagle* in a bottle. Nature. 2009;457(7231):824–9.

[7] Kolter R, Maloy S, editors. Microbes and evolution: the world that Darwin never saw. Washington DC: ASM Press; 2012.

[8] O'Malley MA. What *did* Darwin say about microbes, and how did microbiology respond? Trends Microbiol. 2009;17(8):341–7.

[9] Mendelsohn JA. 'Like all that lives': biology, medicine and bacteria in the age of Pasteur and Koch. Hist Philos Life Sci. 2002;24(1):3–36.

[10] Pasteur L, Chamberlain CE, Roux E. Compte rendu sommaire des expériences faites à Pouilly-le-Fort, près Melun, sur la vaccination charbonneuse. Comptes-rendus des séances de l'Académie des Sciences. 1881;92:1378–83.

[11] Grenfell BT, Pybus OG, Gog JR, Wood JL, Daly JM, Mumford JA, et al. Unifying the epidemiological and evolutionary dynamics of pathogens. Science. 2004;303(5656):327–32.

[12] Volz EM, Koelle K, Bedford T. Viral phylodynamics. PLoS Comput Biol. 2013;9(3):e1002947.

[13] Roche B, Drake JM, Brown J, Stallknecht DE, Bedford T, Rohani P. Adaptive Evolution and Environmental Durability Jointly Structure Phylodynamic Patterns in Avian Influenza Viruses. PLoS Biol. 2014;12(8):e1001931.

[14] Gire SK, Goba A, Andersen KG, Sealfon RSG, Park DJ, Kanneh L, et al. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science. 2014;345(6202):1369–72.

[15] Holmes EC, Dudas G, Rambaut A, Andersen KG. The evolution of Ebola virus: Insights from the 2013-2016 epidemic. Nature. 2016 10;538(7624):193–200.

[16] Stadler T, Kühnert D, Rasmussen DA, du Plessis L. Insights into the Early Epidemic Spread of Ebola in Sierra Leone Provided by Viral Sequence Data. PLoS Curr. 2014 Oct 6;(1).

[17] Saulnier E, Gascuel O, Alizon S. Inferring epidemiological parameters from phylogenies using regression-ABC: A comparative study. PLoS Comput Biol. 2017;13(3):e1005416.

[18] Schuffenecker I, Iteman I, Michault A, Murri S, Frangeul L, Vaney MC, et al. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. PLoS Med. 2006;3(7):e263.

[19] Gonzalez A, Ronce O, Ferriere R, Hochberg ME. Evolutionary rescue: an emerging focus at the intersection between ecology and evolution. Philos Trans R Soc Lond B. 2013;368(1610).

[20] Hartfield M, Alizon S. Epidemiological feedbacks affect evolutionary emergence of pathogens. Am Nat. 2014;183(4):E105–17.

[21] Smith T. Parasitic Bacteria and their Relation to Saprophytes. Am Nat. 1887;21(1):1–9.

[22] Alizon S. A Visionary Pioneer of Parasite Ecology and Evolution. Am Nat. 2016;187.

[23] Méthot PO. Why do Parasites Harm Their Host? On the Origin and Legacy of Theobald Smith's 'Law of Declining Virulence' — 1900-1980. Hist Phil Life Sci. 2012;34:561–601.

[24] Anderson RM, May RM. Coevolution of hosts and parasites. Parasitology. 1982;85:411–426.

[25] Ewald PW. Host-parasite relations, vectors, and the evolution of disease severity. Annu Rev Ecol Evol Syst. 1983;14:465–485.

[26] Fraser C, Lythgoe K, Leventhal GE, Shirreff G, Hollingsworth TD, Alizon S, et al. Virulence and pathogenesis of HIV-1 infection: an evolutionary perspective. Science. 2014;343(6177):1243727.

[27] Fraser C, Hollingsworth TD, Chapman R, de Wolf F, Hanage WP. Variation in HIV-1 set-point viral load: epidemiological analysis and an evolutionary hypothesis. Proc Natl Acad Sci USA. 2007;104(44):17441–17446.

[28] Blanquart F, Grabowski MK, Herbeck J, Nalugoda F, Serwadda D, Eller MA, et al. A transmission-virulence evolutionary trade-off explains attenuation of HIV-1 in Uganda. Elife. 2016;5:e20492.

[29] Gandon S, Mackinnon MJ, Nee S, Read AF. Imperfect vaccines and the evolution of pathogen virulence. Nature. 2001;414:751–756.

[30] Barclay VC, Sim D, Chan BHK, Nell LA, Rabaa MA, Bell AS, et al. The evolutionary consequences of blood-stage vaccination on the rodent malaria Plasmodium chabaudi. PLoS Biol. 2012;10(7):e1001368.

[31] Read AF, Baigent SJ, Powers C, Kgosana LB, Blackwell L, Smith LP, et al. Imperfect Vaccination Can Enhance the Transmission of Highly Virulent Pathogens. PLoS Biol. 2015;13(7):e1002198.

[32] Day T, Proulx SR. A general theory for the evolutionary dynamics of virulence. Am Nat. 2004;163(4):E40–E63.

[33] Berngruber TW, Froissart R, Choisy M, Gandon S. Evolution of virulence in emerging epidemics. PLoS Pathog. 2013;9(3):e1003209.

[34] Hawley DM, Osnas EE, Dobson AP, Hochachka WM, Ley DH, Dhondt AA. Parallel Patterns of Increased Virulence in a Recently Emerged Wildlife Pathogen. PLoS Biol. 2013;11(5):e1001570.

[35] Gandon S, Day T. Evidences of parasite evolution after vaccination. Vaccine. 2008;26S:C4–7.

[36] Goossens H, Ferech M, Stichele RV, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet. 2005;365(9459):579–587.

[37] Luciani F, Sisson SA, Jiang H, Francis AR, Tanaka MM. The epidemiological fitness cost of drug resistance in *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA. 2009;106(34):14711–14715.

[38] Kouyos RD, Abel Zur Wiesch P, Bonhoeffer S. On being the right size: the impact of population size and stochastic effects on the evolution of drug resistance in hospitals and the community. PLoS Pathog. 2011;7(4):e1001334.

[39] Casadevall A, Pirofski LA. Host-pathogen interactions: redefining the basic concepts of virulence and pathogenicity. Infect Immun. 1999;67(8):3703–13.

[40] Schmid-Hempel P. Evolutionary Parasitology: The Integrated Study of Infections, Immunology, Ecology, and Genetics. Oxford, UK: Oxford Univ Press; 2011.

[41] Alizon S, Michalakis Y. Adaptive virulence evolution: the good old fitness-based approach. Trends Ecol Evol. 2015;30(5):248–254.

[42] Baquero F, Blázquez J. Evolution of antibiotic resistance. Trends Ecol Evol. 1997;12:482–487.

[43] Bonhoeffer S, Lipsitch M, Levin BR. Evaluating treatment protocols to prevent antibiotic resistance. Proc Natl Acad Sci USA. 1997;94:12106–12111.

[44] Pena-Miller R, Laehnemann D, Jansen G, Fuentes-Hernandez A, Rosenstiel P, Schulenburg H, et al. When the Most Potent Combination of Antibiotics Selects for the Greatest Bacterial Load: The Smile-Frown Transition. PLoS Biol. 2013;11(4):e1001540.

[45] Fuentes-Hernandez A, Plucain J, Gori F, Pena-Miller R, Reding C, Jansen G, et al. Using a Sequential Regimen to Eliminate Bacteria at Sublethal Antibiotic Dosages. PLoS Biol. 2015;13(4):e1002104.

[46] Hansen E, Woods RJ, Read AF. How to Use a Chemotherapeutic Agent When Resistance to It Threatens the Patient. PLoS Biol. 2017;15(2):e2001110.

[47] CLL Trialists' CollaborativeGroup. Chemotherapeutic Options in Chronic Lymphocytic Leukemia: a Meta-analysis of the Randomized Trials. J Natl Cancer Inst. 1999;91(10):861–868.

[48] Williams GC, Nesse RM. The Dawn of Darwinian Medicine. Q Rev Biol. 1991;66(1):1–22.

[49] MacCallum CJ. Does Medicine without Evolution Make Sense? PLoS Biol. 2007;5(4):e112.

[50] Antonovics J, Abbate JL, Baker CH, Daley D, Hood ME, Jenkins CE, et al. Evolution by any other name: antibiotic resistance and avoidance of the E-word. PLoS Biol. 2007;5(2):e30.

[51] Méthot PO. Research traditions and evolutionary explanations in medicine. Theor Med Bioeth. 2011;32(1):75–90.

[52] Dobzhansky TG. Genetics and the origin of species. 3rd ed. No. XI in Columbia biological series. New-York: Columbia University Press; 1951.

[53] Herbeck JT, Müller V, Maust BS, Ledergerber B, Torti C, Di Giambenedetto S, et al. Is the virulence of HIV changing? A meta-analysis of trends in prognostic markers of HIV disease progression and transmission. AIDS. 2012;26(2):193.

[54] Van Baalen M. Coevolution of recovery ability and virulence. Proc R Soc Lond B. 1998;265:317–325.

[55] Bell G, Gouyon PH. Arming the enemy: the evolution of resistance to self-proteins. Microbiology. 2003;149:1367–1375.

[56] Beceiro A, Tomás M, Bou G. Antimicrobial Resistance and Virulence: a Successful or Deleterious Association in the Bacterial World? Clin Microbiol Rev. 2013;26(2):185–230.

[57] zur Wiesch PA, Kouyos R, Engelstädter J, Regoes RR, Bonhoeffer S. Population biological principles of drug-resistance evolution in infectious diseases. Lancet Infect Dis. 2011;11(3):236–47.

[58] Hauser AR, Mecsas J, Moir DT. Beyond Antibiotics: New Therapeutic Approaches for Bacterial Infections. Clin Infect Dis. 2016;63(1):89–95.

[59] Pichot A. Introduction générale. In: Écrits scientifiques et médicaux de Louis Pasteur. Paris, France: Flammarion; 2012. p. 7–18.

[60] Day T, Huijben S, Read AF. Is selection relevant in the evolutionary emergence of drug resistance? Trends Microbiol. 2015;23(3):126–133.

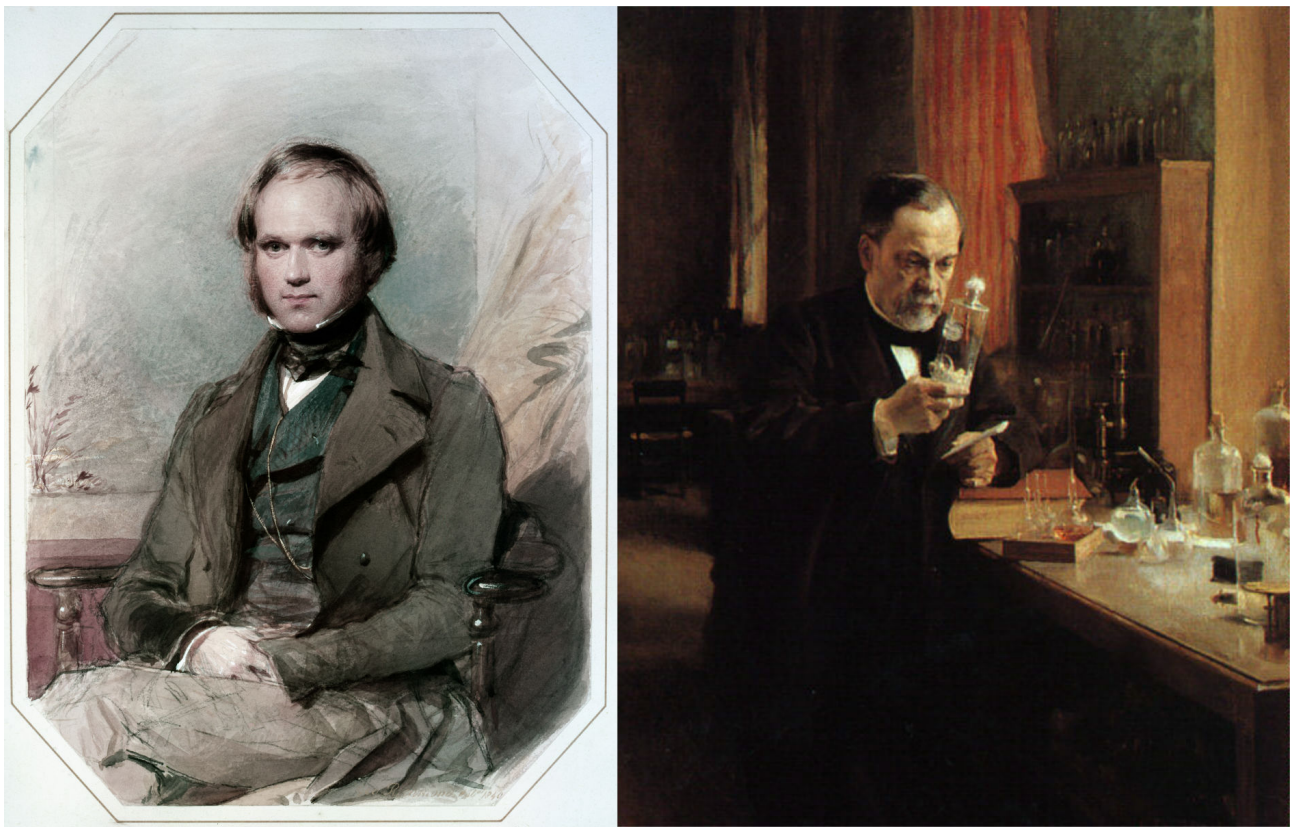

Figure 1: **Charles Darwin the naturalist and Louis Pasteur the microbiologist.** Facts in this table originate from the literature [3, 5, 59]. Charles Darwin's painting is from George Richmond in the late 1830s after his return from his voyage on HMS Beagle and the painting of Louis Pasteur in his laboratory is from Albert Edelfelt in 1885 (Musée d'Orsay, Paris).

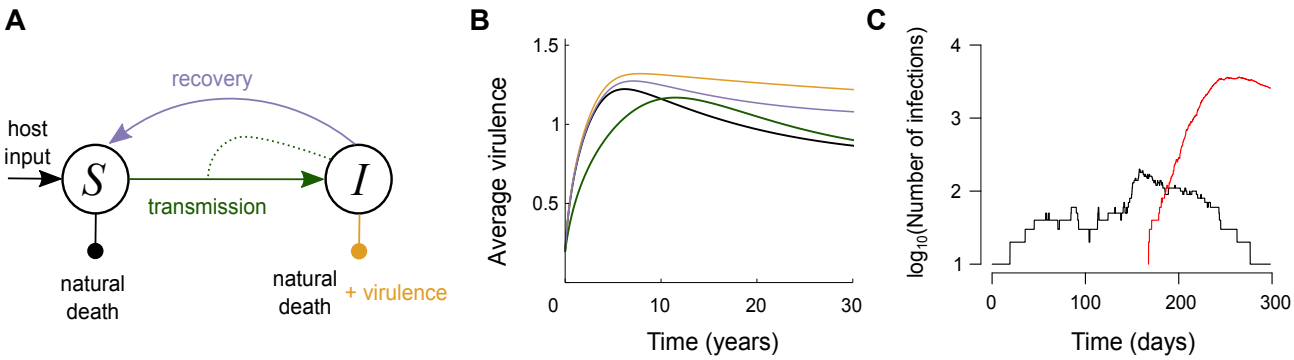

Figure 2: **Combining the epidemiology and evolution of infectious diseases.** A) Representation of the Susceptible-Infected-Recovered (SIR) epidemiological model, B) Virulence evolution in response to different types of interventions and C) Evolutionary rescue of a parasite via mutation. In B, the predictions are obtained using the Price equation formalism and the assumptions from Figure 2 in [32]. The colour of the curves corresponds to the arrows in panel (black is the untreated case). Even in absence of treatment, the virulence evolves on the short-term because its initial value is far from its optimal value. The virulence-blocking treatment (in yellow) leads to the highest increase virulence, whereas the treatment-blocking (in green) first favours less virulent strains. Increasing host recovery rate (in grey) also increases virulence. In C, the resident strain (in black) cannot generate a large outbreak (its $R_0 < 1$) but it can still persist long enough for a mutation event to occur that can lead to a well adapted mutant (in red) [20].

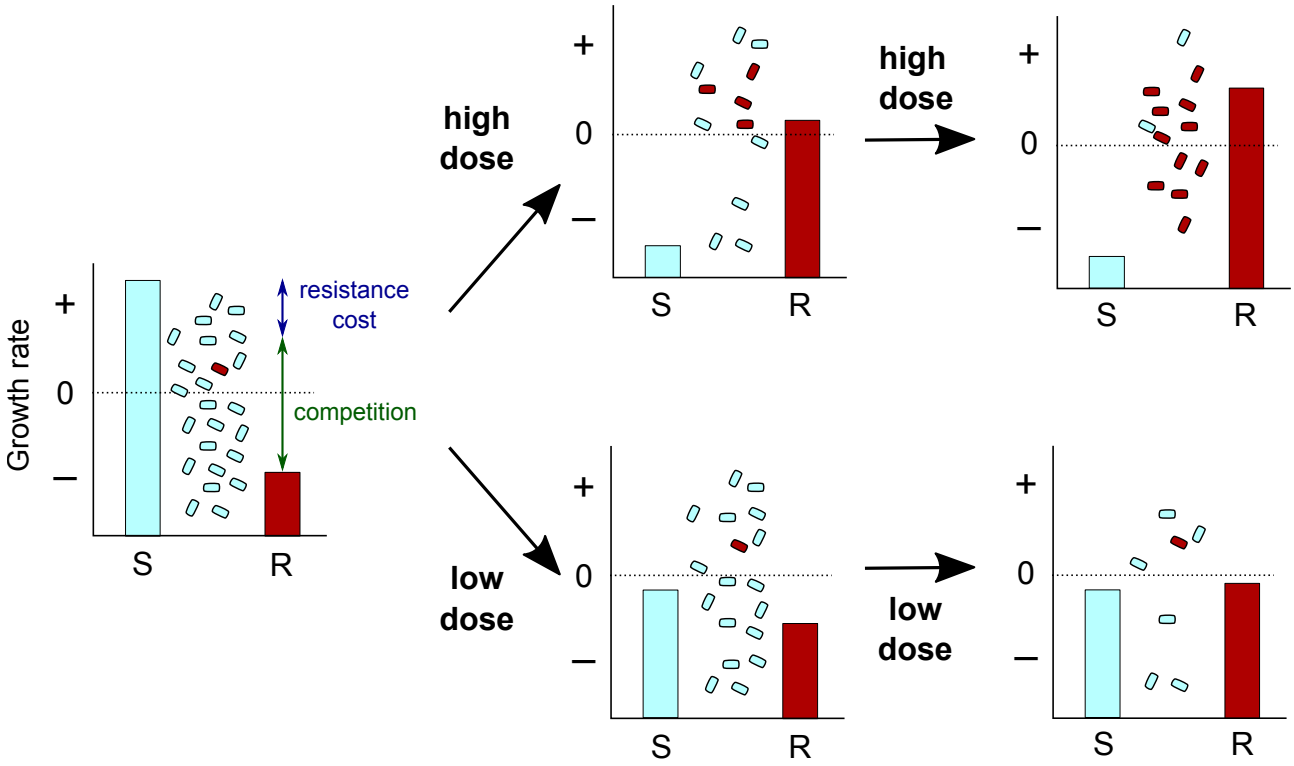

Figure 3: **How high drug doses can lead to selection of pre-existing drug-resistant mutants via 'competitive release'.** Charts show the within-host growth rate of drug susceptible (in cyan) and resistant (in red) bacteria populations at three time points using a high (top) or a low drug dose (bottom). Population sizes and the fraction of resistant bacteria are shown between the bars. Note that depending on the fitness landscape, there might not always exist a dose that prevents the spread of both bacterial populations [for details, see 60].

Table 1: **Comparing the life and research of Louis Pasteur and Charles Darwin.** The comparison criteria are in italic.

Charles Darwin (1809-1882)		**Louis Pasteur (1822-1895)**
Intellectual (grandson of medical doctor and philosopher Erasmus Darwin)	*Sociological background*	Worker (grew up near a tanner's shop)
Eventually 'agnostic'	*Religion*	Always very devout
Solitary (will wait years to publish his major book)	*Collaborators*	' Army' (as described by the medical doctors at the time)
Theoretician	*Method*	Empiricist
Field observations	*Data*	Laboratory (disregards results from non-controlled settings)
Fundamental research only	*Applications*	Applied research
Synthesis: making sense from numerous (seemingly) independent observations		
Influence: Legated a sub-field in biology		
Medicine: non-medical doctors with a major impact in medicine		