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Within-host evolution and virulence in microparasites

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Abstract

An important aspect of microparasite biology is the fact that infections are populations of thousands of microbes. As any population, infections are hence subject to two main types of changes: demographic and evolutionary. Here we analyse the consequences of within-host evolutionary changes. We build an epidemiological model where infections are regularly invaded by locally favored mutations affecting various infectious traits (virulence, transmissibility and clearance). Our results are the following. In durable infections, where within-host evolution is an important matter, a drop of transmissibility is only slightly deleterious to the infection, while a reduction of infection lifespan is very costly. In consequence, locally favored mutations reducing transmissibility reach a larger frequency, or even the complete fixation, and the suboptimality accumulated in infections owing to within-host evolution affects more their transmission than their duration. Conversely, taking an infection at random and observing the events of within-host evolution, one is more likely to observe reductions of infection length than reductions of transmissibility, because the mutations affecting transmissibility are often already present in infections. We then discuss the interpretation of these results in terms of deleterious mutations, and we also emphasize that the management of within-host evolution could be used as a novel therapeutic approach to the treatment of infection.

Keywords: Multilevel selection; Deleterious mutations; Suboptimality; Transmissibility

1. Introduction

The evolution of parasite virulence is a central theme in evolutionary biology as well as a potential concrete application of this discipline. Parasite virulence itself—defined as the parasite-induced host's death rate—is not favored by natural selection, since it reduces the expected duration of infections. Virulence is, however, maintained owing to obligate relationships with other favorable traits (e.g., transmissibility, resistance to immunity).

An important aspect of microparasitic infections in this regard is the fact that they are populations of thousands of microbes. As any population, infections are hence subject to two main types of changes: demographic and evolutionary, both having important consequences for pathogenesis. First, within-host parasite demography is at the core of the interaction between microbes and host immune system, and partly determines the pathologic consequences of infections (see models by Antia et al., 1994; Ganusov et al., 2002; Gilchrist and Sasaki, 2002; André et al., 2003). Second, within-host parasite evolution—referred to as "short-sighted" evolution by Levin and Bull (1994)—can lead to significant changes of infection pathology in the course of infections. The objective of the present article is to analyse its potential consequences.

There is no a priori reason to suppose that within-host evolution should always lead to a particular development of pathogenesis. It should lead to some form of suboptimality with regard to the overall host exploitation, but this suboptimality might be of various kind: reduction of infection transmissibility, increase of virulence and/or faster clearance. On empirical grounds, both increases and reductions of the intensity of pathogenesis have been observed owing to within-host evolution. For instance, in the case of *Neisseria meningitides*, the intense disease

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sometimes caused by the presence of bacteria in the cerebrospinal fluid is likely to be the consequence of the evolution of the bacterial population inside the host (Levin and Bull, 1994; Richardson et al., 2002; Meyers et al., 2003). Inverse effects are of course more difficult to observe since a decline of the intensity of pathogenesis is less striking than an increase. However, De Vos et al. (2001) have followed the course of lung infections by the bacterium *Pseudomonas aeruginosa* in cystic fibrosis patients and observed an accumulation of mutants unable to secrete siderophores. Because siderophores are important components of pathogenesis, this observation could be an indication of a decline of pathogenesis owing to within-host evolution (see West and Buckling, 2003).

On conceptual grounds as well, both increases and reductions of the intensity of pathogenesis seem possible. Within-host evolution is the rise, inside the host body, of parasite strains replicating faster than others. The larger replication rate of certain strains may have too distinct causes. It may be due to the ability of these strains to extract more rapidly resources from their host (or to extract resources from previously unexploited niches, e.g., the cerebrospinal fluid). If it is the case, then within-host evolution leads to an aggravation of pathogenesis. However, faster replication rate may also be due to a less straightforward mechanism. Host exploitation often relies on collective mechanism (Turner and Chao, 1999; Brown, 1999; Chao et al., 2000; Williams et al., 2000; Brown, 2001; Brown and Johnstone, 2001; Crespi, 2001; De Vos et al., 2001; Brown et al., 2002; West and Buckling, 2003). One illustrative example is the bacterial secretion of exoproteins manipulating host physiology for the benefit of the infection (e.g., secreted proteins make iron available to bacteria). In this context, faster replicating strains may well be free riders that benefit from the collective mechanism without paying the cost of its expression. If it is the case, then within-host evolution leads to the loss of host exploitation mechanisms, and therefore to a reduction of the intensity of pathogenesis.

In the present paper, our objective is to obtain general predictions on the nature of suboptimality generated by within-host evolution, as well as on the effects of the intra-host evolutionary events likely to occur in the course of infections. In this aim, we build an epidemiological model where infections are regularly invaded by de novo mutations, with variable effects on infection phenotype. We then measure the outcome of within-host evolution, from the observation of this system at stationary equilibrium. This approach is similar to previous works by Bonhoeffer and Nowak (1994a, b). However these authors were specifically interested in the case where within-host evolution leads to a loss of resistance to immunity (Bonhoeffer and Nowak, 1994a) or to larger virulence (Bonhoeffer and Nowak, 1994b), hence they did not aim at predicting its average effect. More recently, Day and Proulx (2004) have built a general theoretical framework able to integrate the occurrence and rise of de novo mutations with great flexibility on their effects. However, the average effect of these mutations must be fixed in this model as an a priori parameter. Our aim here is to show that the average effect of within-host evolution can be somehow predicted in a general model.

2. Methods

Our model is based upon Bonhoeffer and Nowak (1994b). Infections are founded by colonization of uninfected hosts and collapse due to stochastic clearance or host death. Migration between infected hosts is not considered, i.e., there is no super-infection (this hypothesis will be discussed later on).

We consider l different loci designated by lower-case letters, $i = 1 - l$, with two states each: non-mutated or mutated. By definition, mutations at each of the loci are favored by within-host competition. They are hence referred to as "locally favored mutations" in the following. Each locus has a particular effect at the scale of the whole infection. The fixation of a locally favored mutation at a given locus may yield an increase of virulence, while for another locus virulence is decreased, or unaffected. In the same way, locally favored mutations may affect transmissibility and/or clearance of infections in both directions.

A given parasite genotype is designated by a capital letter I and is characterized by a set of loci $\{i \in I\}$ that are already mutated. Genotype I can be invaded by any mutation occurring among its not-yet-mutated loci $\{j \notin I\}$. When such a mutation appears, genotype segregation within the host is ignored and the fixation of the mutation is assumed instantaneous: this is a "transition". Every locally favored mutation i is hence characterized by the rate at which it appears and fixes within infections. This transition rate at locus i is called π_i. We assume no epistasis on the within-host effect of mutations: a given mutation has the same transition rate from any genotype I (every π_i may be different, but each of them is a fixed constant, see Fig. 1). An important peculiarity of within-host evolution is to be unidirectional, because backward mutations are counter-selected when they appear inside the host and thus they do not invade. As a result, the reverse transition rates at each locus are assumed nil.

Infected hosts are characterized by three parameters depending on the genotype I of the parasites they carry. Let β_I be the transmissibility of their infection (the rate at which it generates new infections of same genotype by colonization of uninfected hosts), m_I the total extinction rate of their infection (including both the death rate plus the recovery rate of an infected host) and π_I^T their total susceptibility to within-host evolution. π_I^T is the sum of the transition rates of all the locally favored mutations that can appear in genotype I. As noted above, novel locally favored mutations can only occur at not-yet-mutated loci, and hence $\pi_I^T = \sum_{i \notin I} \pi_i$. In Bonhoeffer and Nowak (1994b), π_I^T is a constant independent of parasite genotype I. This is valid if the number of loci is assumed infinitely large. Here

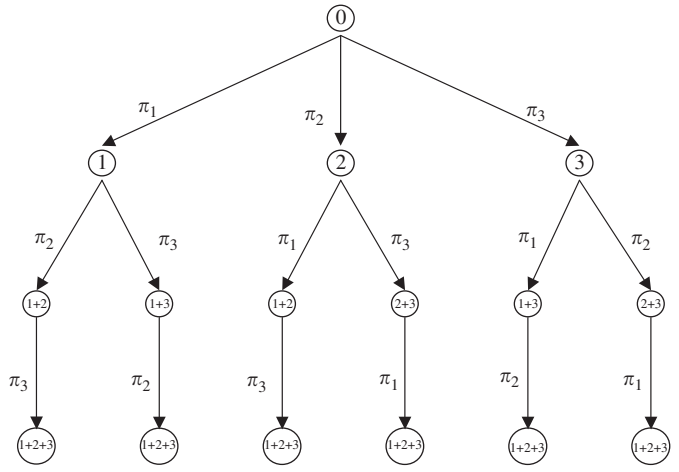

Fig. 1. Cloud of genotypes composing the parasite population. Here we show a scheme for a situation with $L = 3$ different loci, subject to locally favored mutations fixing at rates π_i within infections. The infection with zero mutation can fix three different mutations (total susceptibility to within-host evolution $\pi^T = \pi_1 + \pi_2 + \pi_3$); infections with one locus mutated can fix two mutations; infections with two mutations can fix one more; and infections with all three mutations are not susceptible anymore to within-host evolution ($\pi^T = 0$). Genotypes bearing few locally favored mutations therefore have an intrinsic dynamic disadvantage as they "leak" toward downstream genotypes.

we take into account the fact that a genotype bearing a large number of locally favored mutations has a lower probability to fix one more, because fewer loci remain to mutate.

An infinite host population is considered. A differential equation is derived, giving the dynamics of genotype I. The equation is adapted from Bonhoeffer and Nowak (1994b):

$$\mathrm{d}Y_I/\mathrm{d}t = (\beta_I X - m_I - \pi_I^T)Y_I + g_I(\vec{Y}), \quad (1)$$

where $g_I(\vec{Y}) > 0$ is the rate at which I is generated from all other genotypes by the within-host fixation of one locally favored mutation, and depends on the vector $\vec{Y}$ of the densities of all genotypes. X is the density of uninfected hosts.

3. Results

3.1. Equilibrium frequency of locally favored mutations

At stationary equilibrium, the population of infections is polymorphic, i.e., it is made of a cloud of genotypes (Fig. 1). In Appendix A, we derive the general properties of this system at equilibrium. Each parasite genotype I is characterized by its reproductive number per susceptible host available

$$B_I = \beta_I/(\mu + \chi_I + \alpha_I + \pi_I^T), \quad (2)$$

which represents the total number of infections of type I generated by an infection of type I through its entire course (per susceptible host available). B_I is the product of transmissibility (β_I) by the expected duration of infections ($1/(\mu + \chi_I + \alpha_I + \pi_I^T)$).

At stationary equilibrium, the whole population contains a genotype, arbitrarily called 0, with both the maximum reproductive number over all ($B_0 = \max\{B_I, \forall I\}$) and the least number of locally favored mutations (see Appendix A for a formal proof). The equilibrium density of susceptible hosts X_{eq} is controlled only by genotype 0 ($X_{eq} = \beta_0/(\mu + \alpha_0 + \chi_0 + \pi_0^T)$). Along with genotype 0, the population also contains all the genotypes that are generated from 0 by within-host evolution (see Fig. 1). By definition of equilibrium, all these genotypes must have a lower reproductive number than genotype 0 ($B_I < B_0$).

Therefore, at stationary equilibrium, all the mutations that are favored in within-host competition have a cost on the whole infection (i.e., they reduce the reproductive number of the infections when they fix). Otherwise, these mutations are both favored locally (by within-host selection) and also favored (or neutral) globally, hence they get fixed in the parasite population as a whole (i.e., they are already present in genotype 0).

Let us then consider a given locally favored mutation appearing at a rate π, reducing transmissibility by $\delta\beta$ and increasing either the virulence and/or the recovery rate (i.e., the overall death rate of the infection) by $\delta\alpha$; the mutation has the same additive effect in all genotypes. Each of the two parameters ($\delta\alpha$ and $\delta\beta$) can a priori be positive or negative. In Appendix A, we show that this locally favored mutation reaches an equilibrium frequency at mutation/selection balance given by

$$p = \frac{\pi}{\delta\alpha + \delta\beta X_{eq}}, \quad (3)$$

where we recall that $X_{eq} = (\mu + \alpha_0 + \chi_0 + \pi_0^T)/\beta_0$ is the equilibrium density of susceptible hosts. If $\delta\alpha + \delta\beta X_{eq} \leq \pi$, then the mutation is entirely fixed in the parasite population, i.e., it is carried even by genotype 0. This last condition is analogous to Eq. (10) of Bonhoeffer and Nowak (1994a), and is also related to the criterion of stability of cooperation obtained in Eq. (12) of Michod (1997).

Consider two contrasted types of mutations: (i) mutations affecting only infection duration ($\delta\alpha > 0$, $\delta\beta = 0$) and (ii) mutations affecting only transmissibility ($\delta\alpha = 0$, $\delta\beta > 0$). Duration-mutations are identically counter-selected (i.e., reach the same mutation/selection balance) regardless of the characteristics of the parasite species, because the cost of infection extinction is identical in all cases. In contrast, the strength of selection against transmission-mutations depends on the equilibrium density of susceptible hosts X_{eq}, because the cost of having a reduced transmissibility depends on the actual opportunities for transmission. In the general case, the equilibrium frequency of each locally favored mutation can be represented in a two-dimensional space (Fig. 2), as a function of the absolute effects of the mutation on transmissibility ($-\delta\beta$) and death rate ($\delta\alpha$). Recall then that

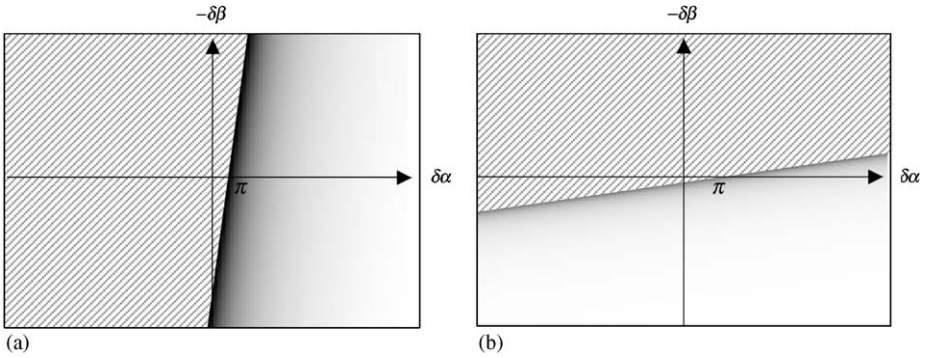

Fig. 2. Equilibrium frequency of locally favored mutations. The parameter space of the effect of locally favored mutations is partitioned as follows. In the dashed regions, the mutations are entirely fixed in the parasite population; in the other regions, the gray intensity gives the frequency of the mutations. π is the rate of appearance of each locally favored mutation. In (a), the equilibrium density of susceptible hosts is low (high transmissibility β_0 and/or low interruption rate $\mu+\alpha_0+\chi_0+\pi_0^T$). In (b), the density of susceptible hosts is high (low transmissibility and/or high interruption rate).

the equilibrium density of susceptible hosts, X_{eq}, depends on two traits of the considered parasite species: the transmissibility of the fittest genotype (β_0) and the overall extinction rate of infections ($m_0=\mu+\alpha_0+\chi_0+\pi_0^T$), through the relationship $X_{eq}=m_0/\beta_0$.

3.2. Suboptimality in virulence and transmission

Let us consider a pathogen with original infections' transmission and extinction rates β and m, and consider an ensemble of locally favored mutations that can arise in this species at a rate π, with various additive effects $\delta\alpha_i$ and $-\delta\beta_i$ on infections' death and transmission rates. The reasoning is quite artificial because the emergence of locally favored mutations does not really occur after β and m have evolved independently, but it can help understanding. First, let us consider duration-mutations. Any weak-effect mutation increasing only infection death rate by an absolute amount $\delta\alpha_i \leq \pi$ reaches complete fixation. This does not affect the ulterior fixation of other duration-mutations, and hence the ultimate pool of fixed duration-mutations is predictable, such as the equilibrium frequency of non-fixed mutations (Eq. (3)). Note that the fixation of duration-mutations reduces the overall extinction rate of infections (because π^T is cut by an amount $\pi \geq \delta\alpha$), which henceforth facilitate the fixation of ulterior transmission-mutations. Second, let us consider transmission-mutations. Any mutation reducing infection transmissibility by an amount $\delta\beta_i \leq \pi\beta/m$ reaches complete fixation. Each of these fixations reduces transmissibility β and thus restricts the range of ulterior transmission-mutations that can fix or become frequent. As a result, the collection of transmission-mutations that are ultimately fixed is impossible to predict, because it depends on their order of fixation. However, overall, the important point is that the collection of transmission-mutations that fix or become frequent depends on the original properties of the pathogen, through the equilibrium density of susceptible hosts $X_{eq}=m/\beta$. In the following, we then contrast various types of pathogens with regard to the fate of locally favored mutations.

Let us first contrast well transmitted vs. poorly transmitted diseases. A large β leaves less susceptible hosts present at equilibrium (lower X_{eq}) and thus facilitates the fixation of transmission-mutations. However, in most cases, transmission is constrained by physiology and/or ecology (e.g., propagule secretion mechanism, external propagule survival, frequency of host-to-host contact). Let us then write pathogen transmissibility as a product of two terms, $\beta=b\tau$, where b represents propagule productivity and depends on the pathogen, while τ represents environment transmission ability and is independent of the pathogen. A mutation reducing propagule productivity by δb reduces transmissibility by $\delta\beta=\delta b\tau$, and reaches an equilibrium frequency $p=\pi b/(\delta bm)$, which is independent of environment transmission ability (τ). Therefore, contrasting diseases with distinct transmissibility owing to environmental and not pathogen differences, which is the most interesting contrast to make, should not reveal any pattern pertaining to the degree of suboptimality in propagule production.

Let us now contrast pathogens with durable vs. brief infections. Just like a large transmissibility, a moderate infection death rate m (and thus large duration $l=1/m$) reduces the equilibrium density of susceptible hosts (X_{eq}) and thus lowers selection against transmission-mutations, leaving unaffected duration-mutations. In other words, locally favored mutations reducing transmissibility reach larger frequency in durable than in brief infections, whereas mutations reducing infection length are unaffected. However, as already mentioned, this last statement is not verified in relative terms. For the same absolute effect on infection extinction rate ($\delta\alpha$), and thus the same equilibrium frequency (Eq. (3)), the relative effect of a mutation is larger if the basic extinction rate is low. Therefore, in durable infections, mutations can have a large relative effect on extinction rate and still reach fixation or a large frequency, but this is only due to the fact that their absolute effect on extinction rate is then very low.

In conclusion, clearly the equilibrium degree of suboptimality in terms of infection length and transmissibility depends in a large manner on the effects of available mutations on both traits. However, apart from this largely unknown parameter, several general predictions can be derived. First, suboptimality should generally be more important in durable infections, because locally favored mutations then have more time to fix. Less obvious is that, in this case, suboptimality should mostly affect parasite transmission rate and only marginally virulence and clearance rates. In fact, when background host mortality, virulence, and clearance are all very low (i.e., infections are durable), then virulence and clearance rates can be significantly increased due to suboptimality but only in a relative term, which cannot represent dramatic increases when these parameters are low.

3.3. Within-host evolution in durable infections

Let us now employ a backward reasoning. Consider a disease with durable infections, and a range of potential locally favorable mutations. Some of these mutations, mostly those reducing transmissibility, get fixed or very frequent in the whole parasite population (Fig. 2a). If one now picks an infected individual at random and follows the course of its infection afterward, the locally favored mutations that one will observe arising are by definition mutations that are originally absent from the infection. They are thus likely to be mutations whose global frequency is low. Therefore, in durable infections, the events of within-host evolution observed in the course of infections should mostly consist in upsurges of infection extinction rate (virulence and/or clearance) (see light gray areas in Fig. 2a). In brief infections, intra-host evolutionary events should affect equally transmission and extinction rates, but these events are less of a matter in brief infections.

4. Discussion

One important peculiarity of microparasitic infections is to be constituted of large populations of microbes. As a result, infections are subject to evolutionary events within their host. The consequences of these events on important infectious traits, such as virulence, transmissibility or clearance rate are difficult to predict a priori.

In order to obtain general predictions on the effects of within-host evolution, we modeled the epidemiology of a microparasite species whose infections can be invaded by de novo mutations appearing at various loci. Our model shows that for certain loci, the allele favored by local selection is entirely fixed in the parasite population at equilibrium, because its cost on the whole infection is not strong enough to compensate for its local advantage. In practice, these loci are not subject to within-host evolution: locally favored alleles are simply carried by every infection. For other loci, both alleles co-exist at equilibrium. The locally favored allele rises repeatedly in the course of infections but is counter-selected, once fixed within a host, because its deleterious effect is strong on the whole infection. These loci are hence currently subject to within-host evolution.

More interestingly, deleterious effects have two components. Within-host evolution can either reduce the lifespan of infections—by increasing their virulence or clearance rate—and/or it can reduce the transmissibility of infections. The relative importance of both components depends on parasite ecology. When infection length is not limited by any external factor (i.e., the host has a long life expectancy and clearance rate is low), then a drop of transmissibility is only slightly deleterious to the infection while a reduction of infection lifespan is very costly. In consequence, locally favored mutations that reduce infection transmissibility reach a large frequency or even the complete fixation. Suboptimality accumulated in infections owing to within-host evolution is thus likely to affect more their transmission than their duration. Conversely, taking an infection at random and observing the events of within-host evolution, one is more likely to observe reductions of infection length than reductions of transmissibility, because the mutations affecting transmissibility are often already present in infections. In the opposite case where infection length is restricted by a parasite-independent mechanism (e.g., the host has a short life expectancy and/or a strong immune system), then a reduction of infection transmissibility is deleterious to the whole infection as well. In this case, all types of locally favored mutations are strongly counter-selected.

Unfortunately, the significance of these results regarding the development of pathogenesis is unclear. In durable infections, where within-host evolution is an important matter, mutations reducing infection lifespan will be observed rising in the course of infections. However, this may occur either through an increase of virulence and thus a reinforced pathogenesis, or an increase of clearance rate and thus a reduced pathogenesis. Therefore, our model does not yield any definitive result regarding the effect of within-host evolution on pathogenesis per se.

4.1. Within-host evolution and super-infection

In the present work, locally favored variants are exclusively generated by de novo mutations. However, they could also come from the immigration of parasites from another infected host, which is often known as super-infection (Nowak and May, 1994). Interestingly, the outcome of super-infection is also a form of within-host evolution as it involves the potential rise of microbial variants favored within the host. Furthermore, the antagonistic effects of within- and between-host selective pressures have also been shown to yield stable polymorphism under super-infection (Nowak and May, 1994). The main difference between the effects of both mechanisms is the following. Among all the possible genotypes that are

favored within infections, de novo mutations are taken at random in proportion of their respective probability of appearance (see Levin and Bull, 1994; Bonhoeffer and Nowak, 1994b). In contrast, among the locally favored genotypes, super-infecting strains are more likely than average to be the relatively well-transmitted and not too virulent ones. Therefore, the effect of *de novo* mutation is most likely to be strongly deleterious on infection fitness (see Fig. 2), whereas the effect of super-infection is most likely to be moderately deleterious. Apart from the fact that super-infection might often be less likely to occur than de novo mutation, we thus argue that most of the drastic changes in pathogenesis observed in the course of infections are likely to be due to the rise of de novo mutations rather than to super-infection. However, of course, such a verbal argument may be misleading. A model including both mechanisms would be necessary to measure properly their respective effects.

4.2. Within-host evolution and deleterious mutations

A simple way to envisage our results is to consider infections as individuals that reproduce, die and mutate. In this respect, the term "mutation" is in fact embracing two distinct mechanisms: first the generation of a mutation in an individual microbe, and second the selection and fixation of this mutation inside the infection (see also André and Day, 2005). Therefore, locally favored mutations can be simply considered as deleterious mutations at the scale of entire infections. The criterion of fixation of locally favored mutations can then be simply interpreted as an error threshold (Nowak and Schuster, 1989): deleterious mutations reach complete fixation when their cost is insufficient to compensate for their rate of appearance. The novelty of the present work is then to point out that the error threshold is not expressed in the same way for a deleterious mutation affecting virulence than transmissibility. We believe that the same type of mechanism could be at work in non-parasitic organisms as well, where deleterious mutations may affect fecundity and/or survival. Beside, extending this model to finite host population sizes would then add another source of suboptimality: the stochastic fixation of deleterious mutations called Muller's ratchet (Haigh, 1978). The speed of the ratchet on a particular locus should be largely determined by the deterministic frequency of the deleterious mutation (Eq. (3)). Therefore, even though the corresponding model has not been realized, we can predict that the Muller's ratchet should reinforce the patterns of suboptimality observed in the deterministic model. Note finally that, in parasites, mutations deleterious both for individual microbes and entire infections are an alternative type of mutations. However, they are likely to be less influential because they do not fix within hosts, except if strong transmission bottlenecks generate important genetic drift (e.g., Bergstrom et al., 1999).

4.3. Within-host virulence management

Virulence management aims at defining public health strategies driving parasite evolution toward low virulence. It is a long-term and large-scale domestication; moreover, the long-term community welfare due to virulence reduction is often in conflict with the individual need for treatments (van Baalen, 2002). We believe that managing the within-host evolution of parasites, instead of their between-host evolution, is an easier application of evolutionary biology. In this perspective, finding virulence traits that are susceptible to within-host evolution is an appealing program. Treatments could be developed that accelerate the rise of relevant locally favored mutations. For instance, impeding bacterial communication (quorum sensing) is feasible and prevents bacteria to express their cooperative virulence factors (Balaban et al., 1998; Williams et al., 2000; André and Godelle, 2005). Resistance to such treatments is favored by between-host selection and disfavored by within-host selection. It should hence be slower to emerge than resistance to conventional antibacterial agents.

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Appendix A

A.1. General properties of the system at equilibrium

Here we derive general properties of the cloud of genotypes (Fig. 1) at equilibrium. The within-host fixation of a locally favored mutation (thereafter called a transition) can only increase the number of mutations among the l loci. Hence, among all the present genotypes, the ones that have the lowest number of locally favored mutations cannot be produced by any other genotype. These genotypes are hence said "non-generated" in the following. We call J any of the non-generated genotypes (with $g_J(\vec{Y}) = 0$). From Eq. (1), for any J we have

$$\mathrm{d}Y_J/\mathrm{d}t = (\beta_J X - m_J - \pi_J^T)Y_J, \tag{4}$$

where we recall that X is the density of susceptible hosts, β_J is the transmissibility, m_J the extinction rate and π_J^T the susceptibility to within-host evolution of genotype J. For any J the equilibrium condition of Eq. (4) is $X = X_J^{eq} = (m_J + \pi_J^T)/\beta_J$, where X is a common parameter for all J. Hence, the non-generated genotype that maintains X the lowest displaces all others, and it is the one with lowest X_J^{eq} and therefore highest $B_J \equiv 1/X_J^{eq}$ with

$$B_J = \beta_J/(m_J + \pi_J^T). \tag{5}$$

We therefore show that the population at equilibrium contains only one non-generated genotype that we arbitrarily call genotype 0. The density of uninfected hosts at equilibrium is controlled only by genotype 0 and is given by $X_0^{eq} = X_{eq} = (m_0 + \pi_0^T)/\beta_0 = 1/B_0$.

By definition, all other genotypes, $I \neq 0$, that are present at equilibrium are generated and hence they have $g_I(\vec{Y}) > 0$ (see Eq. (1)). If such a genotype K has $B_K \geq B_0$, then its dynamics are given by $\mathrm{d}Y_K/\mathrm{d}t = (\beta_K X_{eq} - m_K - \pi_K^T)Y_K + g_K(\vec{Y})$, which is strictly positive, unless $g_K(\vec{Y})$ becomes nil. The necessary stability condition of the system would hence be that K is the single non-generated genotype ($g_K(\vec{Y}) = 0$).

In conclusion, at equilibrium the population of infections contains one genotype, arbitrarily called 0, which has two properties: (i) it bears the lowest number of locally favored mutations and (ii) it has the largest reproductive number. All the other genotypes present at equilibrium are generated from 0 by the within-host fixation of one or more locally favored mutation(s).

A.2. Equilibrium frequency of locally favored mutations

Locally favored mutations arise at l loci from genotype 0. A given mutation i has (i) a constant rate of appearance π_i from any genotype lacking it and (ii) constant additive effects, $\delta\alpha_i$ and $-\delta\beta_i$, respectively, on extinction rate and parasite transmissibility (π_i is positive; $\delta\alpha_i$ and $\delta\beta_i$ are not a priori restricted). There is no epistasis between mutations. A genotype I is characterized by a set of mutated loci $\{i \in I\}$. Y_I is the equilibrium density of genotype I, and X_{eq} the equilibrium density of susceptible hosts, controlled by genotype 0.

From Eq. (1), the equilibrium density of genotype $0 + i$ (with only mutation i) is $Y_{0+i} = \pi_i Y_0/(\delta\alpha_i + \delta\beta_i X_{eq} - \pi_i)$. The equilibrium density of genotype I is found by recurrence as

$$Y_I = Y_0 \prod_{i \in I} \pi_i/(\delta\alpha_i + \delta\beta_i X_{eq} - \pi_i), \tag{6}$$

where $X_{eq} = (\mu + \alpha_0 + \chi_0 + \pi_0^T)/\beta_0$ is the equilibrium density of susceptible hosts as controlled by genotype 0. μ, α_0 and β_0 are, respectively, the host natural death rate, and the virulence, recovery rate and transmissibility of genotype 0.

A given mutation i is present in various genotypes with 0 to $L-1$ other mutations. The equilibrium total density of genotypes carrying i can be written as $Y_i^T = \pi_i Y_{\bar{i}}^T/(\delta\alpha_i + \delta\beta_i X_{eq} - \pi_i)$, where $Y_{\bar{i}}^T$ is the total density of genotypes lacking i. The total density of infected hosts being $Y^T = Y_i^T + Y_{\bar{i}}^T$, we derive the total frequency of genotypes carrying i as

$$p_i = Y_i^T / Y^T = \pi_i/(\delta\alpha_i + \delta\beta_i X_{eq}), \tag{7}$$

which is Eq. (3) of text.

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