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Is selection relevant in the evolutionary emergence of drug resistance?

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The emergence of drug-resistant pathogens is often considered a canonical case of evolution by natural selection. Here we argue that the strength of selection can be a poor predictor of the rate of resistance emergence. It is possible for a resistant strain to be under negative selection and still emerge in an infection or spread in a population. Measuring the right parameters is a necessary first step toward the development of evidence-based resistance-management strategies. We argue that it is the absolute fitness of the resistant strains that matters most and that a primary determinant of the absolute fitness of a resistant strain is the ecological context in which it finds itself.

Evolutionary emergence of resistance

When an infected patient is treated with antimicrobial chemotherapy, the population of microbes within the patient begins to decline. During this process of population decline, genotypes resistant to the antimicrobial drug can appear through mutation or horizontal gene transfer. Resistant microbes also might have been present at the start of treatment. If this population of rare resistant genotypes then grows sufficiently in size to cause symptoms or to be transmitted, we say that a drug-resistant infection has been established. We refer to this process as the evolutionary emergence of drug resistance.

Different chemotherapeutic protocols (e.g., combination therapy versus monotherapy [1], synergistic versus antagonistic drug combinations [2–4], high versus low drug concentrations [5–9]) result in different likelihoods of resistance emergence. This is because such protocols affect the likelihood of resistant genotypes appearing through mutation (or horizontal gene transfer) as well as the fitness of resistant and wild type genotypes once they have appeared. An important research objective is therefore to compare the impact of different protocols on the probability and rate of resistance emergence. Such information makes it possible to design protocols that simultaneously maximize treatment efficacy while managing resistance [5]. Our goal here is to help progress this enterprise by considering the effect of different treatment protocols on the fitness of resistant and wild type microbes within a patient once they are present.

For the most part, studies of the factors influencing resistance emergence have focused on the selective advantage or disadvantage of drug-resistant strains in treated and untreated patients (e.g., [1,10–13]). Here we suggest that, instead, it is often more appropriate to focus on the absolute fitness of resistant strains in treated and untreated patients rather than their performance relative to sensitive strains (see Glossary).

We make this argument in two parts. First, we suggest that the selective advantage of resistance is not the most important indicator of resistance emergence within treated

Glossary

Absolute abundance: the number of pathogens at some point in time.
Absolute fitness: the fitness of a pathogen clone independent of the fitness of any other clone; often involves some measure of change in absolute abundance such as *per capita* growth rate.
Competitive release: the increase in absolute fitness of a resistant clone that occurs when the wild type is removed by chemotherapy; this increase in absolute fitness arises through the increased resource abundance and/or decreased immune response that occurs on the removal of the wild type.
Competitive suppression: the decrease in absolute fitness of a resistant clone as a result of the wild type consuming shared resources and/or stimulating a crossreactive immune response.
Drug resistance: a heritable reduction in the drug sensitivity of a microbe.
Fitness: a term that refers to the reproductive success of a pathogen and involves both reproduction and survival. It is measured in terms of genetic representation in the next generation.
Growth rate (*per capita*): the rate of change of abundance per individual microbe.
Natural selection: any process by which the forms (variants) of organisms in a population that are best adapted to a particular environment increase in relative frequency compared with less well-adapted forms over several generations [37].
Negative selection: when the selection coefficient is negative; in this case the resistant clone will decrease in frequency.
Positive selection: when the selection coefficient is positive; in this case the resistant clone will increase in frequency.
Relative abundance: a synonym for frequency.
Relative fitness: the fitness of a pathogen clone relative to the fitness of another clone; usually involves some measure of change in relative abundance (e.g., frequency).
Resistance emergence: when a population of rare resistant microbes within a patient increases sufficiently in size to cause symptoms or to be transmitted.
Selection coefficient: a measure of relative fitness, often the absolute fitness of the resistant strain minus the absolute fitness of the wild type.

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patients. This is because, by definition, a focus on selection is a focus on the relative fitness of resistant and wild type microbes. However, relative fitness tell us little about the extent to which the size of the resistant population is changing as a result of treatment. A focus on the absolute fitness of the resistant strain is usually more relevant to resistance emergence, because resistance emerges when the absolute abundance of resistant microbes gets sufficiently high. The abundance of resistant microbes relative to that of sensitive microbes is often irrelevant (e.g., when both are very rare).

Second, we ask how different treatment regimens affect absolute fitness. We suggest that different treatment regimens result in different fitnesses of resistant strains by engendering different degrees of competitive release [14], a term borrowed from the ecological literature. Competitive release (defined below) amplifies the numbers of resistant microbes, thus increasing the probability and rate of resistance emergence. We suggest that recognition of the distinction between selection and competitive release will better guide future work on resistance management.

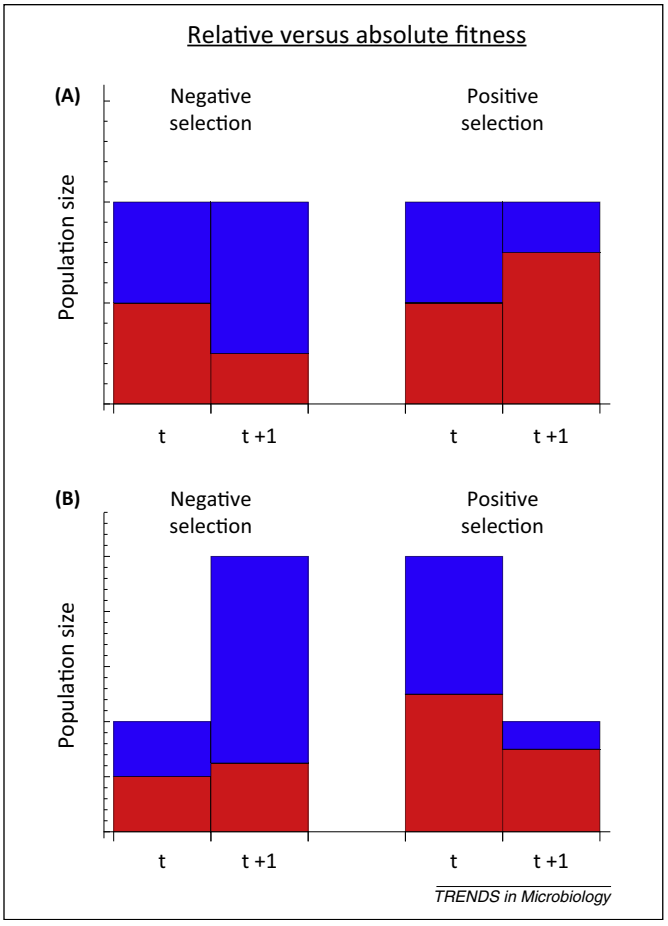

Figure 1. The distinction between relative and absolute fitness. Height of bars indicates total population size. Colors indicates the fractions of the population comprising resistant (red) and wild type (blue) strains. **(A)** Between time *t* and *t* + 1, the population on the left has undergone negative selection and thus resistant strains constitute a smaller fraction of the population. The opposite is true for the population on the right. **(B)** Between time *t* and *t* + 1, the left and right populations have again undergone negative and positive selection, respectively, but the absolute size of the resistant population has nevertheless increased in the case of negative selection and decreased in the case of positive selection.

Absolute versus relative fitness

The first part of our argument is the simplest and rests on the important distinction between absolute and relative fitness. Evolution is a change in the genetic composition of a population. From the standpoint of evolution, all that matters is the fitness of one type relative to another. The difference in fitness between the resistant and wild type strain is referred to as the selection coefficient [15]. If the resistant strain has a higher fitness than the wild type, the selection coefficient will be positive and the resistant strain will come to constitute a greater fraction of the population (termed positive selection). Conversely, if the selection coefficient is negative the resistant strain will come to constitute a smaller fraction of the population (termed negative selection; Figure 1A).

However, the probability of resistance emergence is a function of the absolute fitness of resistant microbes, not their fitness relative to that of the wild type. What matters from the standpoint of resistance emergence (in terms of the potential for resistant microbes to cause symptoms or transmit to other hosts) is the abundance of the resistant strain within a patient. The selection coefficient can tell us little about the predicted change in the population size over time. Figure 1B illustrates this point by showing how a resistant population can be under negative selection and nevertheless increase in size, as well as how it can be under positive selection and decrease in size. A similar point has recently been made in the context of adaptation to environmental change [16].

The hypothetical scenario illustrated in Figure 1 is extremely simple, but analogous outcomes occur in real disease systems. For example, Box 1 presents data from experimental infections in mice with the malarial parasite *Plasmodium chabaudi*. It shows clear instances in which the drug-resistant clone is under positive selection but is nevertheless decreasing in abundance, as well as instances in which the resistant clone is under negative selection but is increasing in abundance to the point where it has high transmission potential. To summarize, then, it is the absolute fitness of the resistant microbes that determines emergence, not their fitness relative to wild type microbes.

Competitive release versus selection

Since it is absolute fitness that matters for resistance emergence, we must consider how different treatment regimens affect the absolute fitness of resistant microbes. To focus our argument, we consider the contentious question of how the extent of drug pressure affects the probability of resistance emergence [5–9,17]. The term 'drug pressure' refers to various factors including the time course of drug concentration during treatment (i.e., the pharmacokinetics). However, for simplicity we refer only to drug concentration. Also, for convenience, in what follows we use the terms fitness and (*per capita*) growth rate interchangeably. We stress, however, that our arguments hold for any reasonable measure of fitness and any reasonable measure of drug pressure.

To begin, it is first helpful to review the main conceptual framework that is used for thinking about the effect of drug concentration on the emergence of resistance. This is the mutant selection window (MSW) hypothesis [18–22].

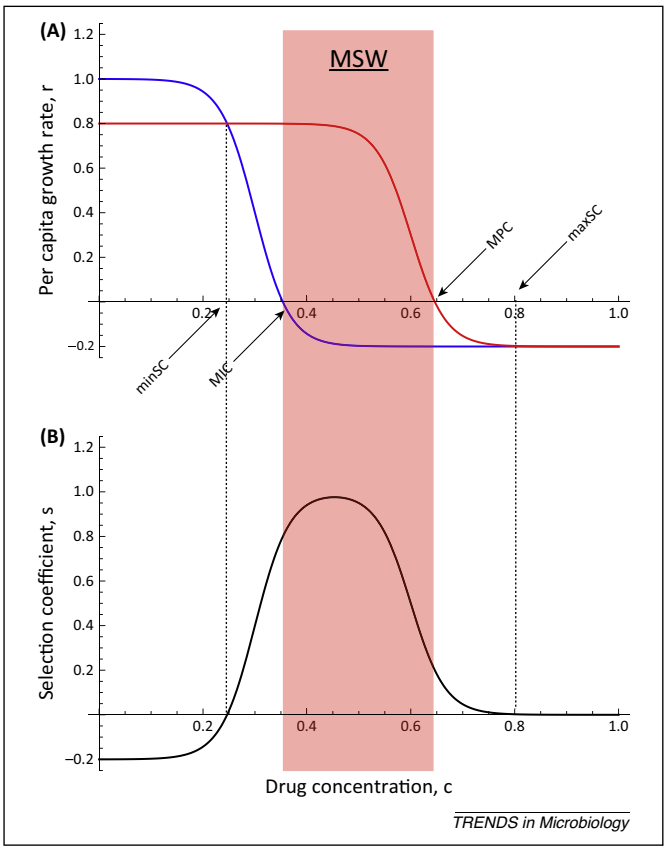

Figure 2. The mutant selection window hypothesis. **(A)** *Per capita* growth rates of mutant (red) and wild type (blue) as a function of drug concentration. Many instances of this model in the literature have the growth-rate curves asymptoting to zero (as opposed to becoming negative). Strictly speaking, they must cross the horizontal axis somewhere and become negative if there exists a drug concentration at which their growth rates are zero. Also labeled are the minimum inhibitory concentration (MIC), the mutant prevention concentration (MPC), the minimum selective concentration (minSC; the smallest concentration for which selection is positive), and the maximum selective concentration (maxCS; the largest concentration for which selection is positive). The shaded red window indicates the 'mutant selection window' (MSW) as defined in the literature. **(B)** The selection coefficient of the mutant is the difference in growth rate between it and the wild type. The mutant will be selectively favored whenever $s > 0$. The mutant can be selectively advantageous for drug concentrations lying outside the MSW.

Drug resistance and the MSW hypothesis

The MSW hypothesis was developed to predict the drug concentrations under which resistance will emerge. It is based on a plot of the *per capita* growth rate of the wild type and mutant strains as a function of drug concentration, c [20–22]. Figure 2A illustrates a hypothetical example displaying several features that are typical of such plots. First, there is a cost to resistance in the absence of the drug, reflected by the resistant strain growth rate being lower than that of the wild type at $c = 0$. Second, by virtue of the resistant strain being able to withstand higher concentrations of the drug, its growth-rate curve will eventually cross that of the wild type at some value of c [the minimum selective concentration (labeled 'minSC' in Figure 2A)]. Third, the growth-rate curves converge again at very high values of c once the growth rate of both types is negative.

The drug concentration at which the wild type has zero growth rate is called the minimum inhibitory concentration (MIC) (Figure 2A). The drug concentration at which the mutant has zero growth rate is called the mutant prevention concentration (MPC) (Figure 2A). The MSW is then defined as the range of drug concentrations between the MIC and the MPC. The reasoning is that, within this window of drug concentrations, the mutant will be 'selectively enriched' whenever it appears [18–20].

The MSW model has been extremely influential and has been subjected to numerous empirical tests, particularly in bacteria [23–33]. It is worth noting, however, that although this terminology is ingrained in the literature it is technically incorrect. In terms of selection it is not the window between the MIC and the MPC that matters. The mutant has a selective advantage at any drug concentration for which its growth rate lies above that of the wild type (i.e., where the selection coefficient is positive; Figure 2B). Thus, the window of selection occurs between the lowest concentration at which the wild type and mutant have the same growth rate (the minSC in Figure 2A) and the concentration at which the two growth rates again converge as the concentration increases [the maximum selective concentration (labeled 'maxSC' in Figure 2A); in practice, the value of maxSC might be reached only asymptotically]. The distinction with respect to the minSC has been indicated clearly before [22] but it also applies to the maxSC.

The misidentification of the MIC and MPC as the boundaries of the window of selective drug concentrations stems from a lack of distinction between relative and absolute fitness. From Figure 2, one can see that the lower boundary of the MSW is classically (and erroneously) defined as the drug concentration at which the wild type's absolute fitness is zero, whereas the upper boundary is the concentration at which the resistant strain's absolute fitness is zero. However, these bounds are unrelated to selection *per se* (cf. Figure 2B).

Although the MSW hypothesis gets the terminology incorrect, it does correctly identify the MPC as the upper boundary of the window of drug concentrations that allows the emergence of resistance. However, the lower boundary is incorrect because it focuses on the absolute fitness of the wild type rather than that of the resistant strain, and it is for this reason that experiments have been able to show that resistance can emerge at concentrations below the MIC [34–36].

If we wish to identify the lowest drug concentration at which the resistant strain has a positive growth rate (i.e., positive absolute fitness), Figure 2 reveals a problem. It suggests that the resistant strain has a positive growth rate for all drug concentrations below the MPC. We know from empirical studies, however, that resistance does not emerge for all such concentrations (for instance, it typically does not emerge in the absence of drugs). How can we resolve this apparent contradiction? The answer lies in the fact that graphs like that in Figure 2 portray the relationship between drug concentration and absolute fitness as fixed. In reality, these relationships are specific to the conditions under which they are measured.

As a microbial population grows, it causes a 'deterioration' in its environment that will ultimately halt its own growth (i.e., make its absolute fitness decline to zero). For example, the depletion of resources and/or the stimulation of an immune response produces density dependence that eventually causes the *per capita* growth rate of the microbe

to fall to zero. The data in Box 1 illustrate this point in the case of *P. chabaudi*, where the growth rate declines to zero (and indeed becomes negative) after 2 weeks or more. This means that growth-rate curves like those in Figure 2 are not fixed but instead are functions of within-patient variables like resource levels and the immune response. Often, curves like those in Figure 2 are measured under pristine conditions (i.e., during exponential growth), but it is the change in these curves that arises from changes in the within-patient variables that ultimately halts (and potentially prevents) the growth of wild type and resistant strains (e.g., see Figure I in Box 1). We suggest that quite generally it is the competitive interaction between resistant and wild type strains through such within-patient state variables that ultimately sets the lower boundary on the range of drug concentrations for which resistance can emerge.

Competitive suppression and competitive release

To illustrate our argument, we begin by considering a process we will refer to as competitive suppression. Box 2 provides a specific hypothetical example. Suppose an infection is initiated and no drug treatment is being used. Then, the growth of the wild type population will degrade the

Box 1. Experimental infections with *Plasmodium chabaudi*

Experimental work with rodent malaria parasite *P. chabaudi* in laboratory mice illustrates the important difference between absolute and relative fitness.

Figure I shows the complex relationships between selection on resistance, measured by the selection coefficient, and the absolute fitness of resistant parasites, measured in terms of either parasite density (top panels) or transmission to mosquitoes (bottom panels). For example, in an untreated mouse, A, resistance is under very strong positive selection between days 13 and 18 post-infection, although the abundance of the resistant strain is decreasing. This is because the sensitive strain is decreasing in abundance even faster. The same occurs following drug treatment in mouse B. In both cases, resistance is not emerging despite strong positive selection. By contrast, following treatment of mouse C, the strong positive selection on resistance declines to zero although the absolute fitness of the resistant strain is increasing following competitive release. That is because the sensitive strain is also relapsing. In this case, resistance is clearly emerging, although the strength of selection is declining to zero. Thus, the selection coefficient is a poor guide to the rate or probability of resistance emergence.

Data from the same experimental system also demonstrate that competitive suppression and competitive release are real biological phenomena. Figure II shows the kinetics of drug-resistant parasites of *P. chabaudi* in nine laboratory mice (red line). When they are alone in an infection (top panels), the resistant parasite population rapidly expands to high densities. However, if drug-sensitive parasites have already proliferated to high densities, the resistant parasite population is unable to expand (middle panels). This is competitive suppression. If the sensitive parasites are removed by drug treatment (lower panels), the resistant parasite population is able to expand. This is competitive release. Thus, the probability of resistance emergence is strongly linked to the extent of competitive release. Other experiments have shown that resistance emergence can be constrained by using treatment regimens that less effectively remove sensitive parasites [7].

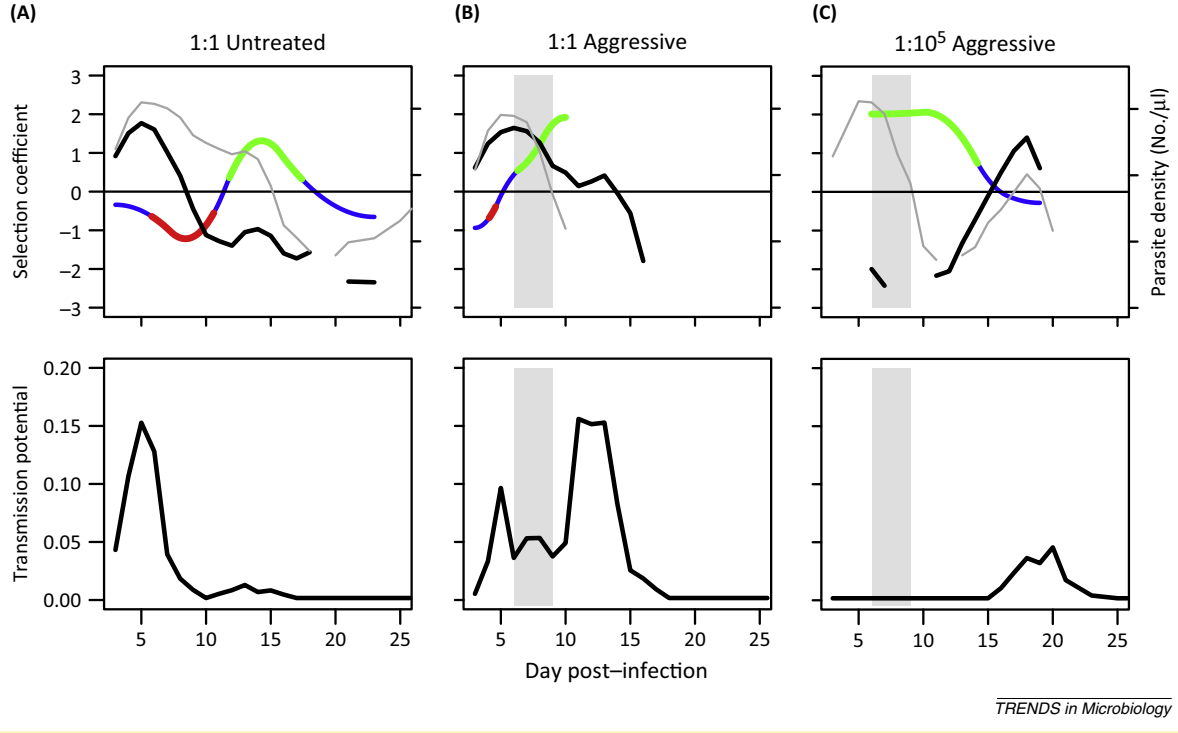

Figure I. Data from three mice infected with pyrimethamine-resistant and -sensitive strains of *Plasmodium chabaudi* at equal densities of 10^6:10^6 **(A,B)** or at a ratio of 1:10^5 **(C)**. Mice were either untreated (A) or treated with 8 mg/kg of pyrimethamine for 4 days (B,C). Top panels show within-host dynamics of resistant (thick black lines) and sensitive (thin black lines) parasites and the selection coefficients (colored lines: green, resistance under positive selection; red, resistance under negative selection; blue, selection coefficient not significantly different from zero). Bottom panels show the predicted proportion of mosquitoes infected by resistant parasites for the corresponding mouse, based on the densities of transmission stages (data not shown). Gray bars indicate the timing and duration of drug treatment. Selection coefficients can be estimated only when both clones are present. Data from [7]; transmission potential estimated as described in [7]; selection coefficients as described in [13].

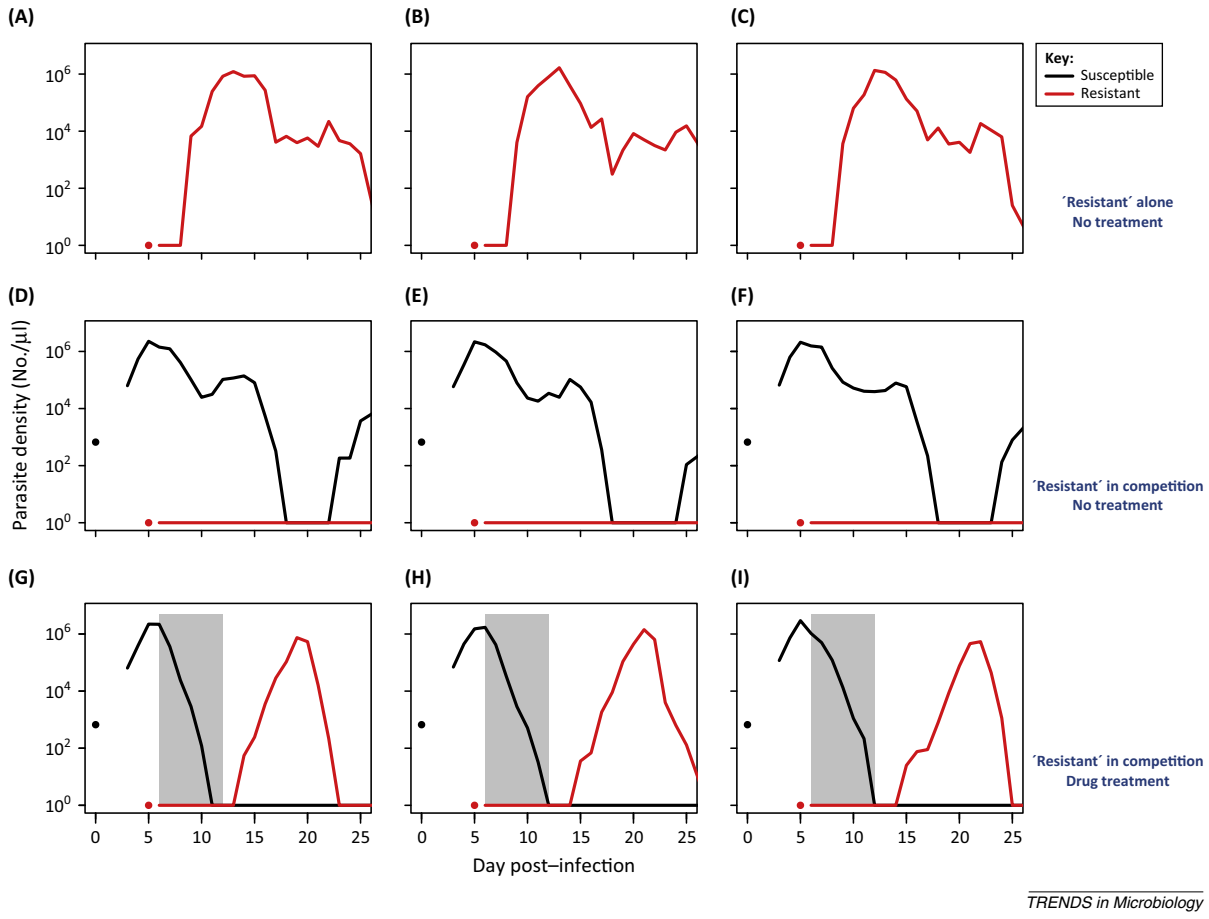

Figure II. (**A–I**) Kinetics of infection in nine mice infected with pyrimethamine-resistant (red) and -sensitive (black) *Plasmodium chabaudi*. All mice were infected with approximately 25 resistant parasites on day 5 (red dots). Mice (D–I) were also infected with 10^6 sensitive parasites 5 days earlier (black dots) and mice (G–I) were treated with 8 mg/kg of pyrimethamine for 7 days to eliminate sensitive parasites. Gray bars show period of drug treatment. Note that, formally, the flat red lines denote times at which densities are below the limit of PCR detection and not necessarily zero densities. Data from [7].

within-patient environment to a point where its fitness reaches zero. In other words, once density dependence (which acts through the within-patient state variables) has become strong enough, the growth-rate curve for the wild type must necessarily cross the horizontal axis at drug concentration $c = 0$. At this point, because of a cost of resistance, the mutant growth-rate curve will lie below that of the wild type and therefore resistance will fail to spread whenever it arises because of competitive suppression (mediated by the within-patient state variable, in this case immunity; see Figure IA in Box 2).

From this hypothetical example we can see that ultimately what matters from the standpoint of the emergence of resistance is the resistant genotype's absolute fitness, r_m, when it appears. If $r_m > 0$, it can emerge. A key observation is that we can have positive selection but nevertheless $r_m < 0$ and therefore emergence is impossible. Likewise, we can have negative selection but $r_m > 0$ and therefore emergence will occur. The latter can occur if, for example, the drug concentration is zero and both the resistant and wild type strains were present at the start of an infection. In this case, the wild type will not yet have caused a change in the within-patient environment (i.e., no immune response or resource depletion will have occurred) and therefore both strains will grow despite the fact that the resistant strain suffers a cost of resistance (e.g., see Figure IA in Box 2). Only once density dependence (competitive suppression) sets in can the cost of resistance reduce the absolute fitness of resistant strains to zero.

With this idea of competitive suppression in hand, we can now define competitive release as the increase in the absolute mutant fitness r_m that comes from removing the wild type with chemotherapy. Competitive release therefore necessarily arises through an ecological interaction between the wild type and the mutant, as mediated through some element of the within-patient environment. Box 3 illustrates the phenomenon of competitive release in the context of the hypothetical example from Box 2 and highlights how it is distinct from selection.

To make this idea more precise, it is helpful to introduce some notation. Suppose x is the within-patient state variable (e.g., density of resources, immune cells) and x_0 is the value of this variable in the absence of infection (i.e., the pristine environment). We use $r(c, x)$ and $r_m(c, x)$ for the growth rates of the wild type and mutant, to indicate that they are functions of both the drug concentration and the within-patient state variable x.

If an infection starts with the wild type and the drug concentration is zero (i.e., $c = 0$), its initial growth rate will be positive; that is, $r(0, x_0) > 0$. As the wild type population

Box 2. Competitive suppression

Consider a population of wild type and resistant microbes with densities p and p_m, respectively. Suppose that density dependence acts solely through a shared immune response, as described by the following model:

$$d\,p/dt = \lambda(c)\,p - a\,l\,p \quad [I]$$

$$d\,p_m/dt = \lambda_m(c)\,p_m - a\,l\,p_m \quad [II]$$

$$dI/dt = f(I, p, p_m) \quad [III]$$

where λ is the *per capita* birth rate of each type as a function of drug concentration and I is the density of a relevant immune molecule. The parameter a scales the effect of the immune response on the growth rate of the microbes. In this example the growth rates of the wild type and mutant are

$$r = \lambda(c) - aI \quad [IV]$$

$$r_m = \lambda_m(c) - aI \quad [V]$$

illustrating how the growth-rate curves (as functions of c) also depend on the within-patient state variable I. The selection coefficient is

$$s = r_m - r \quad [VI]$$

$$= \lambda_m(c) - \lambda(c) \quad [VII]$$

illustrating that, in this example, the selection coefficient is independent of the within-patient variable I.

Figure I illustrates what this means in terms of growth-rate functions like those in Figure 2. We assume that the drug concentration is held at $c = 0$ and the infection starts with only wild type individuals. Figure IA is the pristine environment before any immune response has developed. Figure IB, C shows the growth curves for an increasing immune response, I. As the wild type grows, it stimulates an increasing immune response. This is the deterioration of the within-patient environment (from the standpoint of microbial growth). The mutant curve shows the growth rate that a mutant would have if it appeared under the various conditions. The growth curves are eventually pushed downward until the growth rate of the wild type at $c = 0$ is zero. At this point, any mutant that appears will have a negative growth rate because of the cost of resistance. In other words, it will be competitively suppressed through the within-patient variable I and thus will not spread, although it would have a positive growth rate if it caused an infection on its own.

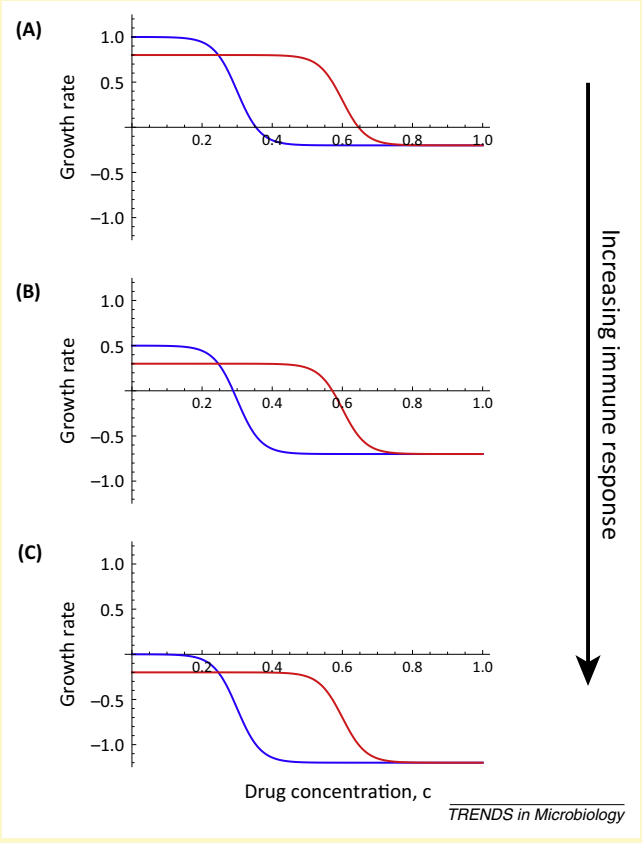

Figure I. Theoretical growth-rate curves for wild type (blue) and resistant (red) genotypes. **(A)** Curves at the beginning of an infection, **(B)** curves as the infection develops, and **(C)** curves once density dependence through the immune response is strong enough to halt wild type growth.

grows, x changes, eventually reaching a value (denoted by x^*) at which $r(0, x^*) = 0$. This corresponds to Figure IA in Box 3. Now suppose we introduce drug treatment at concentration c. The wild type growth rate will be $r(c, x^*)$ and this will be negative, meaning that the wild type will now decrease in abundance. This corresponds to Figure IB in Box 3. As the wild type decreases, the within-patient variable x will rebound toward its pristine value. Competitive release is defined as the difference $r_m(c, x) - r_m(c, x^*)$. This is the change in mutant growth rate that results from the within-patient environment rebounding from x^* to x when the drug concentration is c (see Figure IC in Box 3). By contrast, the selection coefficient is $r_m(c, x) - r(c, x)$, which is the difference between mutant and wild type growth rates when the environment is at x and the drug concentration is c.

Although we have discussed the concepts of competitive suppression and competitive release in abstract terms, they are biologically very real phenomena (Box 1). Resistant strains grow well on their own (see Figure II in Box 1, top panels). When the susceptible strain is already present and has degraded the environment within a mouse, the resistant strain can no longer grow (competitive suppression; see Figure II in Box 1, middle panels). Removing

Box 3. Competitive release versus selection

We continue with the example from Box 2. Suppose that the infection initially contains only the wild type and the drug concentration is $c = 0$. The wild type microbe then grows to the point where a large enough immune response is stimulated to stop wild type growth. In this case the wild type growth curve passes through the horizontal axis at $c = 0$, the growth rate of any mutant that appears is negative, and the selection coefficient is negative (Figure IA; also see Figure IC in Box 2, which illustrates the case where the resistant strain is competitively suppressed).

Now suppose that the drug is administered in way that achieves a constant concentration of $c = 0.35$ (Figure IB). Immediately, the selective coefficient becomes positive, but any mutant that appears will still have a negative growth rate and therefore will not spread. The wild type also now has a negative growth rate, however, and therefore its population will decline. As it does so, the within-patient environmental state will rebound (in this example, I from Box 2 decays), eventually lifting the competitive suppression of the mutant and allowing it to have a positive growth rate (i.e., it experiences competitive release; Figure IC). Thus, in this example, the selection coefficient changes from Figure IA to Figure IB but not from Figure IB to Figure IC. However, the mutant growth rate changes from Figure IB to Figure IC through competitive release and it is this release that allows the mutant to spread.

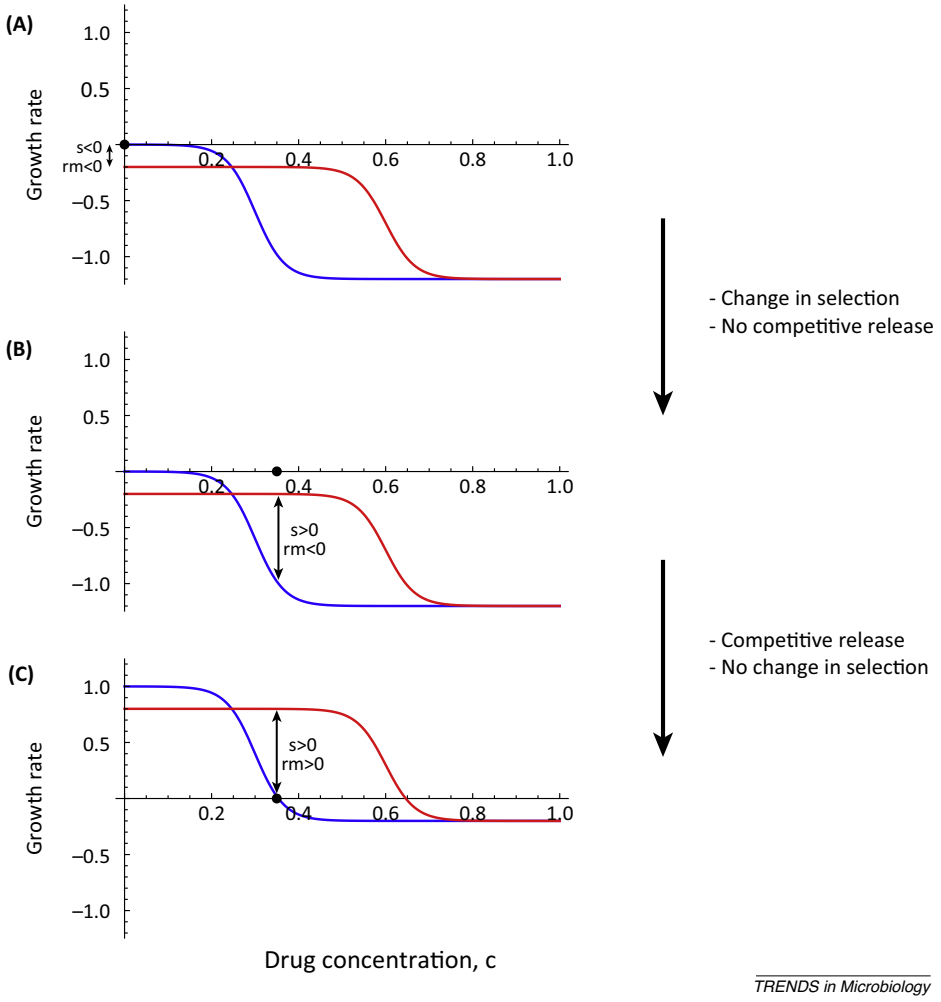

Figure I. Theoretical growth-rate curves for wild type (blue) and resistant (red) genotypes. **(A)** Curves once density dependence through the immune response is strong enough to halt wild type growth but before treatment begins, **(B)** curves immediately after treatment begins, and **(C)** curves once treatment has caused competitive release.

sensitive parasites with drugs allows the environment within a mouse to support the growth of resistant parasites (competitive release; see Figure II in Box 1, bottom panels).

Concluding remarks

It is now commonplace to view the spread of drug resistance through the lens of evolutionary biology, with the goal of using advances in this area of fundamental science to help address the important applied problems that resistance poses. Here we have, in essence, argued that there is a critical ecological process that underlies the emergence of resistance; namely, competitive release. Understanding, and potentially controlling, the initial emergence of resistance therefore requires that we understand how competition works and how contrasting treatment strategies affect this process of competitive release (Box 4). While we have focused attention on the problem of drug resistance and infectious diseases, it is also worth noting that similar issues arise in other instances of adaptation to novel environments. These range from the emergence of resistance in cancer chemotherapy to invasive species biology and adaptation to climate change [16].

Box 4. Outstanding questions

- What drug treatment regimens best reduce the absolute fitness of resistant microbes?
- How common is competitive release?
- What are the mechanisms of competitive suppression and competitive release?
- How do host responses contribute to competitive suppression (e.g., through strain-transcending immunity)?
- Is resistance emergence more or less likely in acute, self-resolving infections?

Disclaimer statement

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