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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.

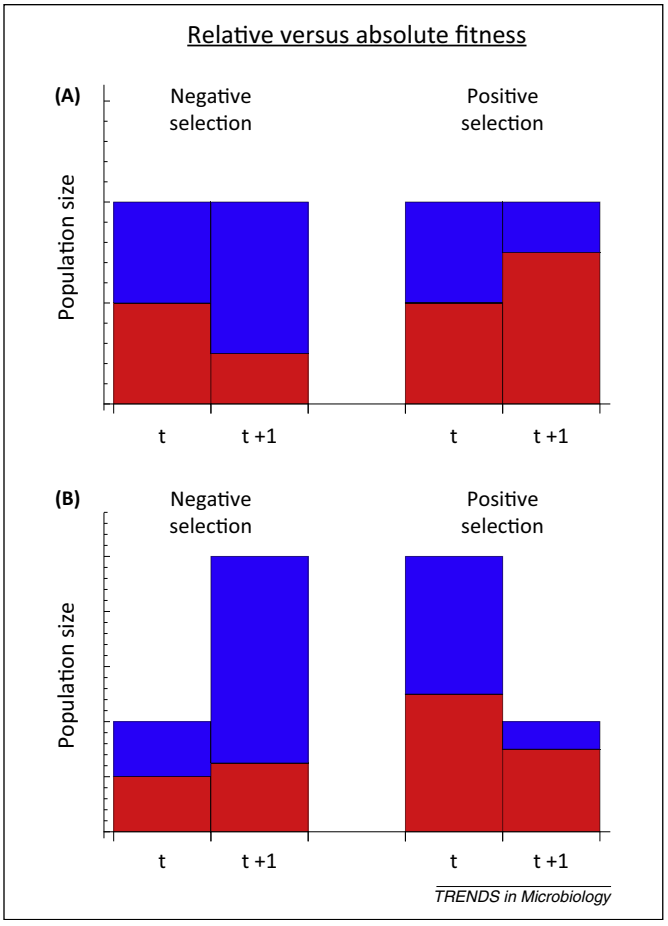

Figure 1. The distinction between relative and absolute fitness. Height of bars indicates total population size. Colors indicates the fractions of the population comprising resistant (red) and wild type (blue) strains. **(A)** Between time *t* and *t* + 1, the population on the left has undergone negative 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].