The effects of individual non-heritable variation on fitness estimation and coexistence

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Abstract

Demographic theory and data have emphasized that non-heritable variation in individual frailty enables selection within cohorts, affecting the dynamics of a population while being invisible to its evolution. Here we include the component of individual variation in longevity or viability which is non-heritable in simple bacterial growth models and explore its ecological and evolutionary impacts. First, we find that this variation produces consistent trends in longevity differences between bacterial genotypes when measured across stress gradients. Given that direct measurements of longevity are inevitably biased due to the presence of this variation and ongoing selection, we propose the use of the trend itself for obtaining more exact inferences of genotypic fitness. Second, we show how species or strain coexistence can be enabled by non-heritable variation in longevity or viability. These general conclusions are likely to extend beyond bacterial systems.

1 INTRODUCTION

Niche theories in ecology (Tilman 1982; Grant 1986; Chesson 2000) and adaptive theories in evolutionary biology (McDonald & Kreitman 1991) emphasize mean differences between species or genotypes, respectively, as key to their coexistence or fixation. Neutral theories in ecology (Caswell 1976; Bell 2000; Hubbell 2001) contend that levels of biodiversity conform to models where individuals have equal fitness and random processes make community compositions inherently unstable while maintaining overall levels of diversity. Neutral theories in evolution (Kimura 1983) also focus on the role of a random process (genetic drift) in maintaining variation instead of niche-based fitness variation across genotypes. Both neutral and niche/adaptive theories tend to overlook the significance of variation at the individual level despite its role in early theory (MacArthur & Levins 1967).

Recently, there has been a resurgence of interest in the impact of intraspecific variation in ecology (Violle *et al.* 2012; Lichstein *et al.* 2007; Hart *et al.* 2016; Des Roches *et al.* 2018), and non-heritable intragenotypic variation in evolution (Steiner & Tuljapurkar 2012; Shen *et al.* 2012). There is substantial evidence for non-heritable variation in traits driven particularly by inherently stochastic variation in life history components including individual variation in longevity (Steiner &Tuljapurkar 2012; Kiviet *et al.* 2014; Hashimoto *et al.* 2016). This type of variation lends itself to selection within cohorts (Kendall & Fox 2002; Hartemink & Caswell 2018) and is likely to contribute substantially to phenomena such as the low heritability of fitness and the high diversity of genotypes and species. It may also result in spurious trends when fitness effects are measured across environments and give false indications of topical mechanisms such as adaptive phenotypic plasticity, bet-hedging and epistasis (Graves & Weinreich 2017).

However, the implications of this type of variation for many ecological and evolutionary processes has not yet been explored.

Here we explore the ecological and evolutionary impacts of individual non-heritable variation in longevity or viability adopting bacterial systems. Section 2 introduces two basic models of bacterial growth which will be used to build study systems in subsequent sections. Section 3 describes the performance of the first model when an antibiotic stress is introduced and confirms its capability to reproduce realistic survival curves (e.g. as in Balaban *et al.* 2014). Section 4 presents the central result that individual non-heritable variation in longevity produces consistent trends when relative fitness between bacterial genotypes is measured across stress gradients. Finally, this lead to a hypothesis that this variation might stabilise coexistence, which is confirmed in Section 5 for the two model systems. These results are discussed more generally in Section 6.

2 BASIC MODELS

Supported by evidence from bacterial systems (Balaban *et al.* 2004; Levin 2004; Kiviet *et al.* 2014; Hashimoto *et al.* 2016; Jouvet *et al.* 2018; Cadena *et al.* 2017; Trauer *et al.* 2019; Gomes *et al.* 2019), we build two model suites which in later sections will be used to explore how non-heritable variation in fitness components may affect the response of a population under different levels of stress, bias common measures of relative fitness between genotypes or strains and associated selection coefficients, and affect their ability to coexist when placed in competition for shared resources.

2.1 Bacterial growth models

First we consider bacteria growing under *in vitro* laboratory conditions with non-heritable variation in cell longevity (elapsed time between cell birth and division) (Powell 1958; Kiviet *et al.* 2014; Hashimoto *et al.* 2016; Jouvet *et al.* 2018). To facilitate specific arguments to be made about mother and daughter cells, in our models we separate the process of cell division into death of mother cells and birth of daughter cells. In the simplest instance of a single genotype with unlimited resources, this is written as:

$$\frac{dB_i}{dt} = p_i\beta\sum_{j=1}^{n}\frac{B_j}{\gamma_j} - \frac{\mu}{\gamma_i}B_i, \quad (1)$$

where B_i, for $i = 1, \ldots, n$, denote the concentration of bacteria with longevity factor γ_i, in a fraction p_i of all births, purporting a distribution with mean $\langle\gamma\rangle = \sum_i p_i\gamma_i$, variance $\langle(\gamma - \langle\gamma\rangle)^2\rangle = \sum_i p_i(\gamma_i - \langle\gamma\rangle)^2$, and coefficient of variation $CV = \sqrt{\langle(\gamma - \langle\gamma\rangle)^2\rangle}/\langle\gamma\rangle$ treated as a varying parameter. Parameter μ controls the mean rate of cell division which, to enable fitness comparisons across distributions, we normalize such that $M = \langle\mu/\gamma\rangle = 1$. Considering that cells replicate by binary fission we impose $\beta = 2\mu$ (i.e. cells are born at twice the rate that they cease to exist). Fig. 1 depicts typical growth curves generated by this model, together with mean cell longevity factors which effectively increase from a common initial value ($\langle\gamma\rangle = 1$ in all cases) as longer-lived cells accumulate (selection for higher longevity and reduced growth). Instantaneous growth rates converge to purely exponential, but the asymptotic limits are lower for higher coefficients of variation even though all populations have their growth distributions being constantly reset to the same mean through births (variation in individual longevity is non-heritable). Without variation ($CV = 0$) selection vanishes and the model defaults to exact exponential growth ($dB/dt = \mu B$, where $B = \sum_i B_i$), but any arbitrarily small perturbation that confers non-heritable variation in cell

longevity will induce the phenomenon described here and set the scene for a multitude of outcomes which we describe in subsequent sections.

As noted by Hashimoto *et al.* (2016), non-heritable variation in longevity can also reduce the doubling time of a population in relation to the mean longevity of its constitutive cells. Simple arguments, which attend to the normalization $M = 1$, show that this finding is compatible with our results (not shown).

We note, however, that not all fitness traits lend themselves to this form of selection when variation is non-heritable. Non-heritable variation in fecundity, for example, may also result in reduced growth but, in contrast with the description above, this is due to stochastic effects which become negligible in large populations (Gillespie 1974).

2.2 Host colonization models

Second we resort to models for microbial colonization of a host population to address variation in susceptibility among hosts (Diekmann et al. 2012; Gomes *et al.* 2019) as a natural manifestation of variation in resource suitability (from the bacterial viewpoint):

$$\frac{dS_i}{dt} = p_i\mu - \alpha_i\beta I S_i - \mu S_i \quad (2)$$

$$\frac{dI}{dt} = \sum_{i=1}^{n} \alpha_i\beta I S_i - \mu I, \quad (3)$$

where μ is the host death and birth rate, β is the effective contact rate between infective (colonized) and susceptible hosts, α_i is the susceptibility factor of hosts S_i that enter the system as a fraction p_i of all births, purporting a distribution with mean $\langle\alpha\rangle$, variance $\langle(\alpha - \langle\alpha\rangle)^2\rangle$, and coefficient of variation $CV = \sqrt{\langle(\alpha - \langle\alpha\rangle)^2\rangle}/\langle\alpha\rangle$ treated as a varying parameter. In line with the previous system, also in this case a form of non-heritable variation reduces bacterial growth, now manifested as fewer hosts being colonized (Fig. 2). When four bacterial strains with the same infectivity (i.e. same $\langle\alpha\rangle$) independently

invade a host population, the growth curves for the prevalence of colonized hosts start tangential to the same exponential growth curve and decelerate to reach an equilibrium as susceptible hosts are depleted. One of the strains finds all hosts equality suitable and as a result experiences the minimal deceleration and reaches the highest endemic prevalence. The other strains find some hosts more suitable than others, decelerate more due to the accumulation of less suitable hosts in the susceptible pool and reach endemic equilibria which are lower for higher variances in host susceptibility.

In the treatment below, we build more elaborate systems from the blocks introduced here, always considering that non-heritable variation in fitness is affected by selection even though measurements of selection coefficients typically focus on only heritable components of variation (Chevin 2011). Analyses are presented incrementally, with various results being highlighted along the way, concluding with an exposition of how coexistence of bacterial genotypes or strains can be maintained by non-heritable variation in individual fitness components. The mechanisms rely on mean-variance trade-offs which can be arbitrarily small leading us to note the fragility of strict neutrality formulations and adding to already expressed concerns about their merits as null hypotheses (Gotelli *et al.* 2006).

3 NON-HERITABLE VARIATION UNDER ANTIBIOTIC STRESS

Populations of genetically identical bacteria placed under selective antibiotic pressure typically exhibit a decline over time in their rates of mortality (Balaban *et al.* 2004; Levin 2004). When observed in time frames that are not long enough to reflect increases in the frequency of heritable mutations, this pattern has been attributed to non-heritable variation in sensitivity of individual cells to the antibiotic, which in turn has been linked to variation in rates of cell growth and division. The notion that individual bacterial

cells of the same genotype vary in their rates of cell division is supported by independent studies that used microfluidic techniques to track thousands of bacterial cells to determine their individual lifespan and map division events (Hashimoto *et al.* 2016; Jouvet *et al.* 2018). Jouvet *et al.* (2018) concluded that 90% of the variability is non-heritable, presumably corresponding to the characteristics of each individual cell being molded by its own sequence of stochastic events throughout life.

To explore the impact of this non-heritable variation on the population response to antibiotics, we modify established mathematical formalisms representing bacterial population dynamics (Stewart & Levin 1973; Hsu *et al.* 1977; Smith 1981) to include individual variation in rates of cell division, similarly to how frailty variation has been treated in demography (Vaupel *et al.* 1979; Vaupel & Yashin 1985). More specifically, we adopt model (1) and introduce an antibiotic that reduces the viability of newborn cells by a factor σ_a:

$$\frac{dB_i}{dt} = p_i\beta(1-\sigma_a)\sum_{j=1}^{n}\frac{B_j}{\gamma_j} - \frac{\mu}{\gamma_i}B_i. \quad (4)$$

Balaban *et al.* (2004) investigated the persistence of inherently sensitive cells when a population of genetically identical bacteria is exposed to an antibiotic stress, a phenomenon first observed in the early days of penicillin use (Bigger 1944). The authors described mathematically the dynamics of surviving cells by switching mechanisms between a majority of rapidly growing (normal) cells and a minority of slowly growing (persister) cells. Coupling model (4) to a system of continuous resource provision we obtain:

$$\frac{dB_i}{dt} = \phi(R)p_i\beta(1-\sigma_a)\sum_{j=1}^{n}\frac{B_j}{\gamma_j} - \frac{\mu}{\gamma_i}B_i - \rho B_i \quad (5)$$

$$\frac{dR}{dt} = \rho(c-R) - \phi(R)\beta(1-\sigma_a)\sum_{j=1}^{n}\frac{B_j}{\gamma_j}, \quad (6)$$

where R is the concentration of resources in the chemostat, c its concentration in the input flow, ρ the rate at which medium enters and leaves the chemostat, and $\phi(R) = R/(1+R)$ is a nonnegative increasing function between 0 and 1 describing the viability of newly born cells as a function of resource availability. This parsimonious model is capable of reproducing the results of Balaban *et al.* (2004) without invoking phenotypic switches (Fig. 3) and will be used for multiple purposes throughout this paper.

The model was solved numerically without antibiotic ($\sigma_a = 0$) until reaching a stationary state, at which stage the antibiotic was introduced ($\sigma_a = 0.9$). In the absence of variation in cell longevity, the antibiotic causes an exponential decay in cell density (solid black line in Fig. 3a). The slightest variation in longevity induces a form of selection that results in decelerated population decay (illustrated by the dashed black line in Fig. 3a generated with a coefficient of variation of 0.05). The greater the variation, the greater the deceleration (magenta line in Fig. 3a generated with the coefficient of variation set to 3). Fig. 3b shows the action of selection on cell longevity. As time under antibiotic increases, the faster dividing cells become rarer in the population (i.e. the fraction of persisters increases). The original distribution of cell longevity factors is continuously being reset through new births, but viability is generally low due to antibiotic pressure leading to an accumulation of long-lived cells.

The same phenomenon occurs regardless of whether the population is structured into two discrete groups or shows a more continuous distribution of longevity factors (Fig. 3d, e). Indeed, different types of survival curves, as reported by Balaban *et al.* (2004), can be obtained by concordantly setting the distribution of longevity factors without needing additional switches or other processes (Gefen *et al.* 2008; Rotem *et al.* 2010; Johnson & Levin 2013). These ideas apply to growing cell populations more generally

and may be extended to describe failure of treatments in cancer patients (Mizrahi *et al.* 2016), as well as a wide variety of bet-hedging strategies in nature (Philippi & Seger 1989).

In contrast with adaptive phenotypic plasticity theory (Pigliucci 2001; Chevin *et al.* 2010; Coulson *et al.* 2017), our model populations are essentially the same irrespective of what stresses they may experience, but the non-heritable variation in a trait that affects fitness produces survival profiles dependent on environmental specificities. As a result, a population is never completely represented by those individuals who are alive at any one time and the exact misrepresentation depends on the nature and strength of environmental stresses.

4 RELATIVE FITNESS DEFINED ALONG STRESS GRADIENTS

Fundamental to the results above is a notion of genotype fitness that is wider than that commonly used. By accommodating explicitly for individual non-heritable variation in longevity, the measurable genotype fitness becomes dependent on the strength of selection which may vary between environments. We consider different intensities of environmental stresses which either act to reduce cell viability at birth (σ_a in model (4)) (Fig. 4a, b) or, alternatively, reduce survival at any age (σ_b):

$$\frac{dB_i}{dt} = p_i\beta\sum_{j=1}^{n}\frac{B_j}{\gamma_j} - \frac{\mu(1+\sigma_b)}{\gamma_i}B_i \qquad (7)$$

(Fig. 4c, d), or even act as a favorable factor reducing the need for cells to divide and thereby slowing down the rate of cell division (σ_c):

$$\frac{dB_i}{dt} = \sigma_c\left(p_i\beta\sum_{j=1}^{n}\frac{B_j}{\gamma_j} - \frac{\mu}{\gamma_i}B_i\right) \qquad (8)$$

(Fig 4e, f). Considering mutants derived from ancestral genotypes, we describe the possible patterns which may occur when fitness ratios are measured (r_m/r_a, where r_a and r_m denote ancestor and mutant growth rates, respectively, as given by the right-hand side of the respective model equations at some time point during exponential phase [illustrated at 6 hours in the figure but the results are not specific to this particular choice]). First, we assume that the phenotypic variance of the ancestor is negligible compared to the mutant and find this to result in measurable fitness ratios (solid colored lines in Fig. 4a, c, e) that are consistently lower than those that would have resulted from the same mean effects if mutant and ancestor had the same variance (dashed lines), a discrepancy that increases with stress. This trend is common in data (Kraemer *et al.* 2016), but the reverse has also been observed (Kishony & Leibler 2003) and occurs in our framework when mutants are less variable than their ancestors (Fig. 4b, d, f). The level of non-heritable variation therefore defines the relative fitness of a genotype across a gradient.

Genetic stresses can induce similar phenomena on new mutations and affect measurements of epistasis (Agrawal & Whitlock 2010). Any mutation with an effect on fitness sets a differential in stress levels between ancestral and mutant genotypes, introducing a bias in the assessment of the effects of additional mutations. If an initial mutation has increased fitness variance, for instance, a second mutation may appear less deleterious without necessarily involving epistasis between the mutations. More generally, these trends may impact the estimation of distributions of fitness effects of mutations (Perfeito *et al.* 2007; Eyre-Walker & Keightley 2007; Robert *et al.* 2018).

In light of these issues, we argue that when non-heritable variation in individual fitness exists, unbiased fitness ratios between genotypes and corresponding selection

coefficients ($1 - r_m/r_a$) cannot be measured directly from population-level observations but can be estimated by fitting a curve to measurements taken across stress gradients. The performance of a genotype is expected to vary along the gradient in response to the level of non-heritable variation present generally and specific to that genotype.

Common procedures for measuring fitness and associated quantities do not accommodate the phenomena described above. This is strikingly conveyed by Fig. 4 where fitness curves of two genotypes measured across a stress gradient effectively cross at some critical stress value (solid blue and black curves in Fig. 4a, c, and green and black curves in Fig. 4b, d) where the selection coefficient appears to be zero. The populations differ, however, in their fitness distributions and the crossing is due to the action of selection on non-heritable fitness components. This suggests that unaccounted non-heritable phenotypic variation within genotypes is capable of promoting coexistence or at least persistence of multiple genotypes and unexpectedly affect patterns of genetic variation (Johnson & Barton 2005).

These considerations have implications for genetic variation within populations. Firstly, genetic diversity of fitness traits could be present but be difficult to detect, even though these traits typically have low heritability (Fisher 1930; Merilä & Sheldon 1999). Secondly, the effects of genetic drift on fitness traits may be slowed relative to models that account for fluctuations of selection over time (due to random environmental conditions) but no individual variation (Gillespie 1973). Individual variation in longevity, as considered in our models, may also increase the persistence time of finite populations independently of environmental stochasticity (Kendall & Fox 2002), with implications for the extent of adaptive evolution inferred from observations.

5 NON-HERITABLE VARIATION PROMOTES STABLE COEXISTENCE

5.1 Bacterial growth models

A classic debate in community ecology concerns whether the high diversity of species able to coexist in competition for the same resources is attributed to "equalizing" (neutral theory) or "stabilizing" (niche theory) mechanisms (Chesson 2000). The neutral theory (Hubbell 2001) posits that individuals, irrespective of species, are basically identical in their fitness and their interactions, and community dynamics are driven by demographic stochasticity and speciation. The niche theory, by contrast, proposes that species differ in their niches (Tilman 1982; Grant 1986) and that the negative effects of intraspecific individual interactions are larger than those due to interspecific interactions. This dichotomy has also been presented as a contention between stochasticity and determinism (Chave 2004). More recently, these arguments have relaxed considerably, mainly due to the increasing recognition of the significance of individual variation (Violle *et al.* 2012; Lichstein *et al.* 2007; Hart *et al.* 2016; Des Roches *et al.* 2018) to species coexistence.

To add to this issue we extend the models used above to accommodate two bacterial species (A homogeneous and B with variation in longevity) and introduce a $24h$ oscillation in the concentration of the single resource entering the system:

$$\frac{dA}{dt} = \phi(R)\beta_A A - \mu_A A - \rho A \tag{9}$$

$$\frac{dB_i}{dt} = \phi(R)p_i\beta_B \sum_{j=1}^{n} \frac{B_j}{\gamma_{Bj}} - \frac{\mu_B}{\gamma_{Bi}} B_i - \rho B_i \tag{10}$$

$$\frac{dR}{dt} = \rho[c(t) - R] - \phi(R)\left(\beta_A A + \beta_B \sum_{j=1}^{n} \frac{B_j}{\gamma_{Bj}}\right), \tag{11}$$

where $c(t) = c_0[1 + \cos(2\pi t/24)]$. Fig. 5 shows a tongue-shaped region (in yellow) outlining stable coexistence of the two species. Previous studies have described coexistence in similar systems (Stewart & Levin 1973; Hsu *et al.* 1977; Smith 1981), but relied on different species having different viability functions $\phi(R)$, thus complying strictly with the niche theory. In contrast, the mechanism we describe here relies on selection acting on individual variation in longevity under oscillating resources, and would appear neutral if framed within traditional theories which are essentially blind to intraspecific individual variation. When resources are low, high-longevity cells are at an advantage and so are species exhibiting higher variance, whereas under abundant resources species with lower variance have the advantage because they effectively grow faster, generating a pattern that can be interpreted as negative frequency-dependent selection when no such dependence has been imposed. It has previously been noted that a similar mean-variance trade-off mechanism could stabilize coexistence in a plant system (Lichstein *et al.* 2007), although the effect was weak as it lacked the oscillation in resource availability.

Extending the model to three species this mechanism does not appear to sustain coexistence of more than two species in our numerical explorations (Fig. 5c), but fitness is typically governed by many traits and variation in other processes may conceivably extend possibilities for coexistence.

5.2 Host colonization models

Shifting from longevity to resource accessibility, and its effect on bacterial cell viability, we now build on model (2)-(3) for the colonization of a host population by multiple microbial strains, each affected by an independent distribution of host suitabilities (susceptibilities from the host viewpoint). The model for 3 strains (A, B and

C) circulating in a host population, with n_A, n_B and n_C susceptibility groups, respectively, to each species is written as:

$$\frac{dS_{i_A i_B i_C}}{dt} = p_{Ai_A} p_{Bi_B} p_{Ci_C} \mu - \left(\alpha_{Ai_A}\beta_A I_A + \alpha_{Bi_B}\beta_B I_B + \alpha_{Ci_C}\beta_C I_C\right) S_{i_A i_B i_C} - \mu S_{i_A i_B i_C} \quad (12)$$

$$\frac{dI_A}{dt} = \sum_{i_A=1}^{n_A} \alpha_{Ai_A}\beta_A I_A \sum_{i_B=1}^{n_B}\sum_{i_C=1}^{n_C} S_{i_A i_B i_C} - \mu I_A \quad (13)$$

$$\frac{dI_B}{dt} = \sum_{i_B=1}^{n_B} \alpha_{Bi_B}\beta_B I_B \sum_{i_A=1}^{n_A}\sum_{i_C=1}^{n_C} S_{i_A i_B i_C} - \mu I_B \quad (14)$$

$$\frac{dI_C}{dt} = \sum_{i_C=1}^{n_C} \alpha_{Ci_C}\beta_C I_C \sum_{i_A=1}^{n_A}\sum_{i_B=1}^{n_B} S_{i_A i_B i_C} - \mu I_C, \quad (15)$$

where β_X, for $X = A, B, C$, is the effective contact rate between hosts infective with strain X and susceptible hosts, α_{Xi_X}, for $i_X = 1, \ldots, n_X$, are the susceptibility factors of hosts $S_{\ldots i_X \ldots}$, who enter the system as fractions p_{Xi_X} of all births, purporting distributions with mean $\langle \alpha_X \rangle = \sum_{i_X} p_{Xi_X}\alpha_{Xi_X} = 1$, variance $\langle (\alpha_X - 1)^2 \rangle = \sum_{i_X} p_{Xi_X}\left(\alpha_{Xi_X} - 1\right)^2$, and coefficients of variation $CV_X = \sqrt{\langle (\alpha_X - 1)^2 \rangle}$ treated as varying parameters. The strain-specific basic reproduction numbers are $R_{0X} = \beta_X/\mu$.

In the special case where the host population is homogeneously susceptible to A ($n_A = 1$), heterogeneous to B with two susceptibility groups ($n_B = 2$), and C is absent ($n_C = 0$), coexistence occurs when $R_{0A}, R_{0B} > 1$ and

$$\frac{-2\alpha_{B1}\alpha_{B2}R_{0B}}{-\alpha_{B1}\alpha_{B2}R_{0B} - \alpha_{B1} - \alpha_{B2} + \sqrt{(\alpha_{B1} + \alpha_{B2} - \alpha_{B1}\alpha_{B2}R_{0B})^2 - 4\alpha_{B1}\alpha_{B2}(1 - R_{0B})}} < R_{0A} < R_{0B}.$$

These conditions were used to delineate the 2-strain coexistence tongues in Fig. 6a, b. In the case of 3 strains ($n_C = 2$), the conditions for coexistence among the various pairs are analogous and were used to partially generate Fig. 6c. To complete the figure, the 3-strain coexistence region, which exists for $R_{0A}, R_{0B}, R_{0C} > 1$, is bounded by the straight

lines $R_{0B} = R_{0A}$ and $R_{0C} = R_{0A}$ (where strains B and C, respectively, become absent), and by a third line, where strain A becomes absent. This line can be obtained analytically by assuming 3-strain coexistence and then setting the equilibrium abundance of strain A equal to zero.

The extension of the model to N strains is straightforward although the notation becomes dense:

$$\frac{dS_{i_1 i_2 \cdots i_N}}{dt} = \prod_{X=1}^{N} p_{X i_X} \mu - \sum_{X=1}^{N} \alpha_{X i_X} \beta_X I_X S_{i_1 i_2 \cdots i_N} - \mu S_{i_1 i_2 \cdots i_N} \quad (16)$$

$$\frac{dI_X}{dt} = \sum_{i_X=1}^{n} \alpha_{X i_X} \beta_X I_X \sum_{i_1=1}^{n} \cdots \sum_{i_{X-1}=1}^{n} \sum_{i_{X+1}=1}^{n} \cdots \sum_{i_N=1}^{n} S_{i_1 i_2 \cdots i_N} - \mu I_X \quad (17)$$

where β_X, for $X = 1, \ldots, N$ is the effective contact rate between hosts infective with strain X and susceptible hosts and all the remaining parameters are as before. In the special case where the host population is homogeneously susceptible to strain 1, we find an N-strain coexistence region with all $R_{0X} > 1$. This region has a simple geometry in the R_{0X} space that generalizes the 3-strain coexistence. It is bounded by the hyperplanes $R_{0X} = R_{01}$, for $X = 2, \ldots, N$, and by a hypersurface that can be obtained as before by setting to zero the coexistence abundance of strain 1. This coexistence region persists when we allow for heterogeneous susceptibility to strain 1 as well.

Simpler versions of heterogeneous systems such as these have been shown to provide more accurate descriptions of infectious disease dynamics than their homogeneous analogues (Dwyer *et al.* 1997; Langwig *et al.* 2017; King *et al.* 2018; Gomes *et al.* 2019). Here we demonstrate their capacity to support coexistence of multiple strains in a scenario where competition mediated by host immunity is maximal, as shown for 2 and 3 strains in Fig. 6 and generated inductively for any natural number N.

Until now stabilizing mechanisms that sustain coexistence have been tied to species or strains as homogeneous static entities (Chesson 2000; Lipsitch *et al.* 2008). We challenge this paradigm by showing how unmeasured variation in individual fitness can stabilize coexistence across environmental conditions. Strictly neutral models are singular in the sense that their outputs are not robust to unspecified forms of individual variation. Their use as null hypothesis should therefore be considered with care (Gotelli & McGill 2006). Our arguments pertain to the interpretation of stable coexistence as evidence in support of specific niche mechanisms (Enquist *et al.* 2002; Lipsitch *et al.* 2008), but the rationale may be more general. Null theories should incorporate individual variation.

6 DISCUSSION

According to neutral theories of diversity at genetic (Kimura 1983) and species (Hubbell 2001) levels, the heritable variation that continually arises through mutation and migration is subject to stochastic processes that allow transient and therefore unstable coexistence of multiple genotypes or species. Stabilization of coexistence, on the other hand, can arise from specialization of genotypes or species in separate fitness peaks and ecological niches, respectively. Here we demonstrate with two examples from bacterial systems – bacterial population growth under laboratory conditions and colonization of a host population – that non-heritable variation among individuals can stabilize coexistence in models that would otherwise be neutral. The mechanisms rely on a form of selection operating on variation in individual abilities to remain within cohorts: variation in bacterial longevity (Vaupel *et al.* 1979; 1985; Kendall & Fox 2002; Hartemink & Caswell 2018), pertaining to time elapsed between cell birth and division; or variation in host susceptibility (Langwig *et al.* 2017; King *et al.* 2018; Gomes *et al.* 2019), referring to time since a susceptible host is born until it acquires infection. These

384 cause cohort compositions to change in response to varying strengths of selection,
385 providing a buffer that decreases or even hinders the effects of selection between
386 genotypes or species and promotes coexistence.

387 In recent studies, intragenotypic variation has been shown to contribute to phenotypic
388 variance to a large degree (Steiner and Tuljapurkar, 2012; Shen *et al.* 2012; Kiviet *et al.*
389 2014; Hashimoto *et al.* 2016; Jouvet *et al.* 2018), although the significance of these
390 findings to the performance of neutral or adaptive theories of evolution has not been
391 explored. While evidence is accumulating for intraspecific variation and its ecological
392 significance (Violle *et al.* 2012; Des Roches *et al.* 2018), the literature has so far only
393 indicated that coexistence may be weakly facilitated (Lichstein *et al.* 2007) or further
394 destabilized (Hart *et al.* 2016) by intraspecific variation. The coexistence mechanism we
395 describe in the context of bacterial systems is in contrast with Hart *et al.* (2016) in that
396 selection is operating in our case, and differs from Lichstein *et al.* (2007) in that
397 selection is dynamic.

398 We also describe how individual variation in non-heritable fitness components is
399 expected to bias direct measures of relative fitness between genotypes, an effect that
400 increases with stress, resulting in inconsistent selection coefficients. We therefore
401 propose that traditional measures of relative fitness are generated (experimentally or
402 observationally) for several conditions across a stress gradient and a model accounting
403 for individual variation is fitted to enable the simultaneous inference of within-genotype
404 variances and unbiased between-genotype relative fitness. We consider three alternative
405 ways to incorporate stress and obtain similar trends, although the exact formalisms
406 should be submitted to experimental tests which are feasible in bacterial system given
407 current technologies for high-throughput imaging.

In summary, the importance of non-heritable variation in fitness components is now being increasingly recognized, but there are opportunities to further incorporate it into theoretical treatments and empirical tests in ecology and evolution. We illustrate some of these applications through showing impacts on genotypic fitness estimation, host use and clonal coexistence.

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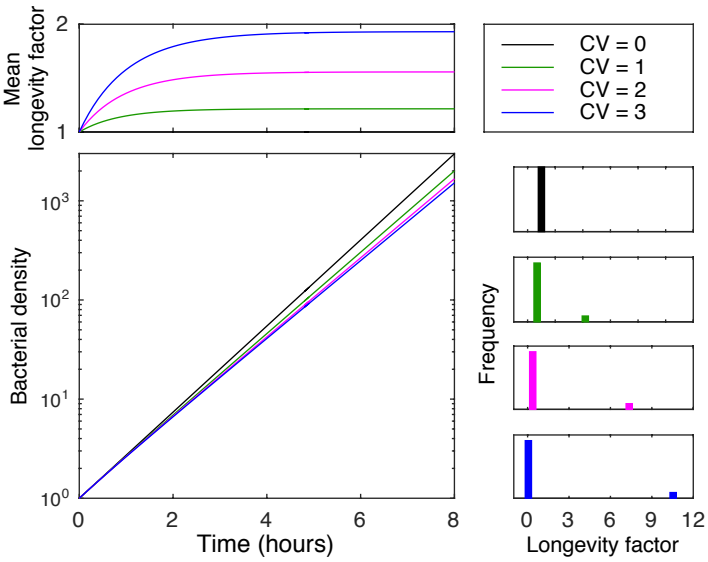

Figure 1: Bacterial growth with non-heritable variation in cell longevity. Solutions of model (1) with distributed longevity factors, γ, with mean $\langle\gamma\rangle = 1$. The fraction of cell births entering the high-longevity group was set to 0.09. Three distinct coefficients of variation are represented: $CV = 0$ (black), $CV = 1$ (green), $CV = 2$ (magenta), and $CV = 3$ (blue). Mean growth rates at birth are set to $M = 1$ in all cases. This condition is also imposed at the beginning of all trajectories by setting initiation conditions accordingly: $B_i(0) = p_i$, for $i = 1,2$. Growth curves bend due to the accumulation of long-lived cells and this effect increases with CV.

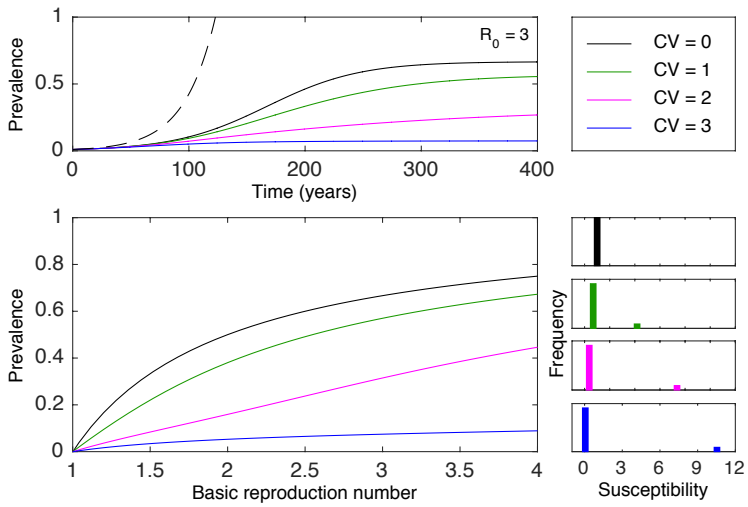

Figure 2: Prevalence of a bacterial strain facing variation in individual host susceptibility. Solutions of model (2)-(3) with distributed susceptibility factors, α, as a function of the basic reproduction number $R_0 = \langle\alpha\rangle\,\beta/\mu$, assuming mean $\langle\alpha\rangle = 1$. The fraction of high-susceptibility hosts was set to 0.09. Four distinct coefficients of variation are represented: $CV = 0$ (black), $CV = 1$ (green), $CV = 2$ (magenta), and $CV = 3$ (blue). The dashed curve represents exact exponential growth with unlimited uniform hosts ($dI/dt = \beta I$) for comparison. Other parameters: $\mu = 1/80$ per year. Limited resources limit growth, naturally, and variance in host susceptibility leads to lower colonization prevalence due to the accumulation of less suitable hosts in the susceptible pool.

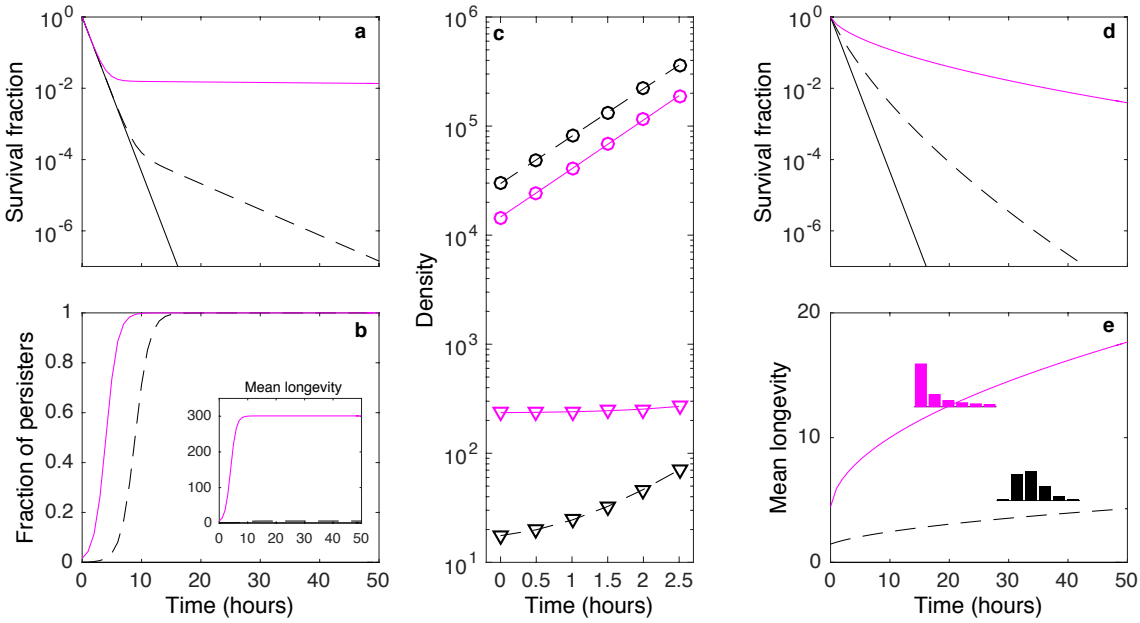

Figure 3: Bacterial persistence to antibiotic treatments. a, **b**, **c**, Solutions of model (5)-(6) with two-group distributed longevity factors, γ (Methods). The fraction of cell births entering the high-longevity group was set to 0.0001. Two distinct coefficients of variation are represented: $CV = 0.05$ (dashed black), and $CV = 3$ (magenta). The solid black curve represents a homogeneous population: $CV = 0$. A pre-antibiotic phase ($\sigma_a = 0$) was simulated with $c = 2$ and $\rho = 0.003$, until a stationary phase was established. Stationary phase solutions were used to simulate: **a**, **b**, antibiotic introduction by setting $\sigma_a = 0.9$ and turning off the chemostat flow ($\rho = 0$); and **c**, growth without antibiotic by keeping $\sigma_a = 0$ and setting $R(0) = 10^6$. Curves punctuated by circles represent total populations, whereas triangles refer to persistent fractions. **d**, **e**, Solutions of model (4)-(5) with gamma distributed longevity factors and two distinct coefficients of variation: $CV = 0.5$ (dashed black), and $CV = 2$ (magenta). Other parameters: $M = 1$, $\phi(R) = R/(1 + R)$.

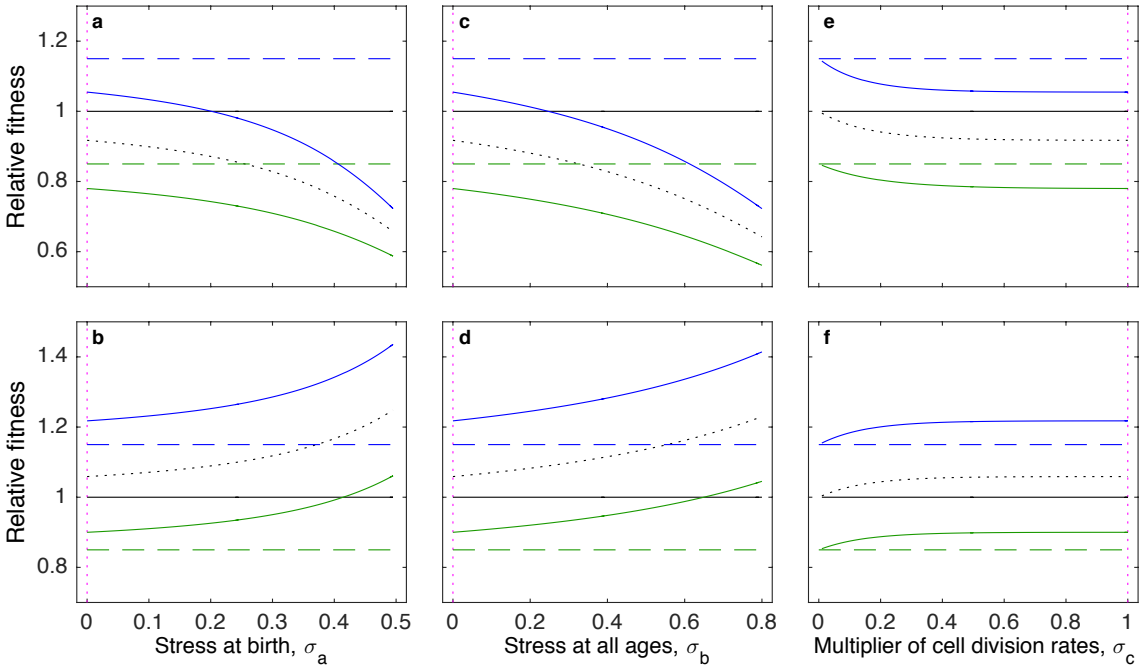

Figure 4: Relative fitness across stress gradients. Relative growth rates between mutant and ancestral genotypes calculated at time $t = 6h$ with: (**a**, **c**, **e**) $M = 1$ and $CV = 0$ (black, ancestral genotype), $M = 0.85$ and $CV = 3$ (green, mutant), $M = 1.15$ and $CV = 3$ (blue, mutant), and $M = 1$ and $CV = 3$ (black, dotted); (**c**, **d**, **f**) $M = 1$ and $CV = 1$ (black), $M = 0.85$ and $CV = 0$ (green), $M = 1.15$ and $CV = 0$ (blue), and $M = 1$ and $CV = 0$ (black, dotted). Stress was implemented in three ways: (**a**, **b**) reduction in cell viability at birth (parameter σ_a in model (4)); (**c**, **d**) increase in cell mortality at all ages (parameter σ_b in model (7)); or (**e**, **f**) factor affecting the rate of cell division (parameter σ_c in model (8)). Vertical dotted lines (magenta) indicate where the three axes (σ_a, σ_b, σ_c) intersect. The fraction of cell births entering high-longevity groups is set to 0.09.

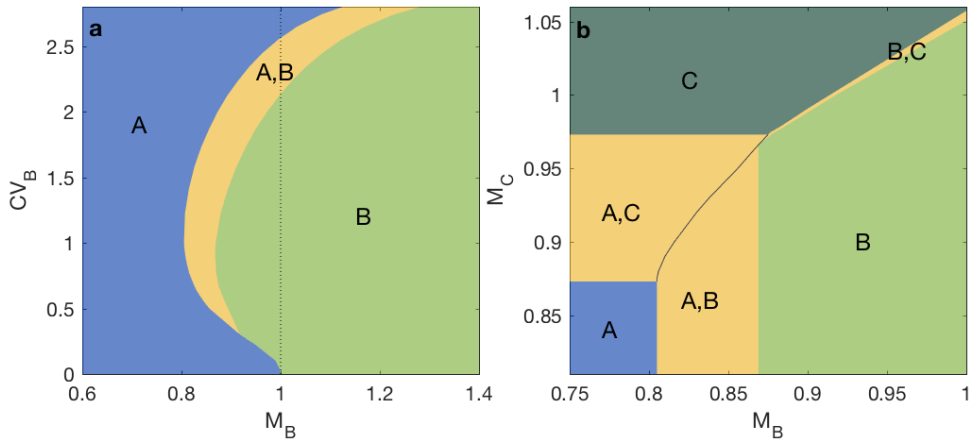

Figure 5: Stable coexistence of microbial species in an oscillating chemostat. Model (9)-(12) was solved numerically with two (**a**) and three (**b**) species (Methods). Yellow tongues represent regions of stable coexistence among the indicated species. All species have the same cell viability function $\phi(R) = R/(1+R)$, the chemostat flow is set to $\rho = 0.1$, and the concentration of resources in the input flow oscillates as $c(t) = 3[1+\cos(2\pi t/24)]$. Other parameters: (a, b) $CV_A = 0$ and $M_A = 1$; (b) $CV_B = 1$ and $CV_C = 2$.

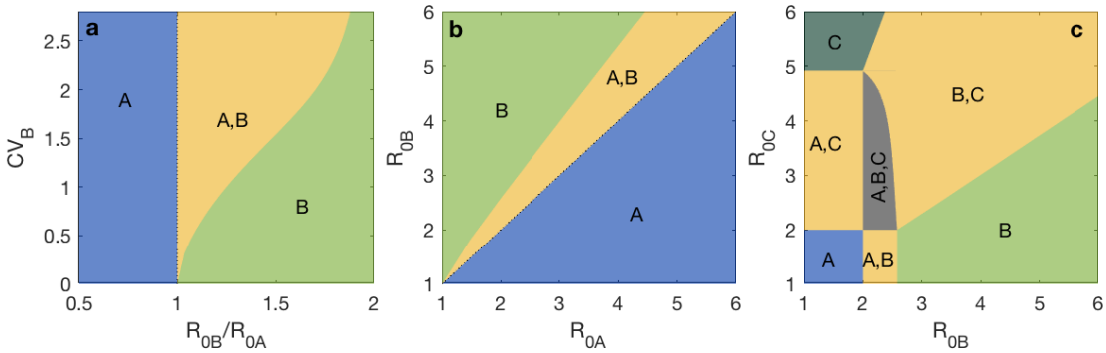

Figure 6: Stable coexistence of microbial species colonizing a host population. Model (13)-(16) was solved analytically with two (**a**, **b**) and three (**c**) species (Methods). Yellow regions represent conditions for 2-species stable coexistence as indicated, while 3-species coexistence is found in the gray zone (c). Other parameters: (a, b, c) $CV_A = 0$; (b, c) $CV_B = 1$; (c) $CV_C = 2$ and $R_{0A} = 2$.