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Metabolic trade-offs and the maintenance of the fittest and the flattest

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How is diversity maintained? While environmental heterogeneity is considered important[1], diversity in seemingly homogeneous environments is nonetheless observed[2]. This, it is assumed, must either be owing to weak selection, mutational input or a fitness advantage to genotypes when rare[1]. Here we demonstrate the possibility of a new general mechanism of stable diversity maintenance, one that stems from metabolic and physiological trade-offs[3]. The model requires that such trade-offs translate into a fitness landscape in which the most fit has unfit near-mutational neighbours, while a lower fitness peak exists that is more mutationally robust. The "survival of the fittest" applies at low mutation rates, giving way to "survival of the flattest[4,5,6]" at high mutation rates. However, as a consequence of quasispecies-level negative frequency-dependent selection and differences in mutational robustness we observe a transition zone in which both fittest and flattest co-exist. While diversity maintenance is possible for simple organisms in simple environments, the more trade-offs the wider the maintenance zone. The principle may be applied to lineages within a species or species within a community, potentially explaining why competitive exclusion need not be observed in homogeneous environments. This principle predicts the enigmatic richness of metabolic strategies in clonal bacteria[7] and questions the safety of lethal mutagenesis[8,9] as an antimicrobial treatment.

Stimulated by the enigmatic recovery of many ecotypes, defined[2] as clusters with respect to global regulation, metabolic strategies, surface properties and nutrient permeability pathways, found in a single-resource chemostat despite an absence of ecological or periodic selection[2], we analyse a theoretical, *n*-'type' chemostat within the framework of mutation-selection models.

With evidence that within-chemostat diversity may not be maintained by frequency-dependent selection[2] and that types differ in fitness[7], it is classically supposed[10,11,12] that diversity must be transitory. Seeking a different explanation, we ask what conditions permit the stable maintenance of a diversity of simple organisms in a simple environment.

Consider n phenotypically distinct heritable types with the dynamics of type frequencies cast as the following mutation-selection chemostat (MSC) model:

$$\begin{aligned}
\frac{d}{dt}\boldsymbol{f} &= \varepsilon(M-I)\boldsymbol{f} + \boldsymbol{G}(S)\boldsymbol{f} - \langle \boldsymbol{G}(S), \boldsymbol{f} \rangle \boldsymbol{f}, \\
\frac{d}{dt}\Delta &= \Delta\left(-d + \langle \boldsymbol{G}(S), \boldsymbol{f} \rangle\right), \\
\frac{d}{dt}S &= d(S_0 - S) - \Delta\langle \boldsymbol{U}(S), \boldsymbol{f} \rangle.
\end{aligned} \qquad [1]$$

Here, $\boldsymbol{f}(t)$ is a vector of frequencies of each type, M is a stochastic mutation matrix, I is the identity (multiplicative unit) matrix, ε the "mutation" rate, t is time, Δ the total density of cells per unit volume, $\boldsymbol{G}(S)$ is a vector of growth rates and $\boldsymbol{U}(S)$ is a vector of resource uptake rates in an environment whose sole carbon source has concentration S. The latter is supplied at concentration S_0 to the chemostat and all matter is lost from the culture vessel at the washout rate d.

To discount factors known to affect diversity, we assume no cross-feeding[13] and that, within a population, all types are subject to the same mutation rate.

Throughout, phenotypes of cell type *j*, the latter label ranging from 1 to *n*, will be a function of *x* where $x = j/n$. The maximal uptake rate of the limiting resource by cell type *j* will be $x \cdot V^{max}$ where V^{max} is a fixed constant and so we term *x* the *normalised* maximal uptake rate. The uptake rate of that resource, $U(x,S)$, is a Monod function proportional to the maximal uptake rate,

$$U(x,S) = V^{max} \cdot \frac{xS}{K(x) + S}, \qquad [2]$$

where *K*, a half-saturation constant, is constrained to the maximal uptake rate and so given as a function of *x*. The growth rate (i.e. absolute fitness) at a given resource concentration *S* is denoted $G(x,S)$ and defined by

$$G(x,S) = c(x) \cdot U(x,S), \qquad [3]$$

where $c(x)$ is cell yield per unit resource (for further details see supplement 1-7).

Equations [2] and [3] can capture two trade-offs. First, growth rate depends on cell yield and so a rate-yield trade-off arises when $c(x)$ decreases with *x*. Second, the growth rate depends on a cell's affinity for resources and so a rate affinity trade-off occurs when $K(x)$ increases with x. Biochemically, the rate-yield trade-off is well-described and is conjectured to be a thermodynamic necessity[14] or evolutionarily optimal strategy[15] (supplement 4).

As temporal fluctuations in resource abundance and ecosystem feedback can maintain diversity, we only consider what happens when resource levels

converge to equilibrium, computing equilibrium loci of [1] for relevant mutation rates with d and S_0 fixed.

For our first computation we set K to be constant, thus abolishing the rate-affinity trade-off and we assume the rate-yield trade-off is convex. The resulting model captures two recognized evolutionary principles (supplement fig 12a). First, at low mutation rates the fittest type and its mutational neighbours dominate; this is classical "survival of the fittest" with limited diversity maintained at mutation-selection equilibrium (cf. [14]). At high mutation rates another type dominates where the fitness landscape is flat and organisms are robust to mutation; this is survival of the flattest[4,5,6]. The transition from survival of the fittest to survival of the flattest, as the mutation rate increases, passes through a region in which both quasispecies are abundant. This we term the principle of the maintenance of the fittest and flattest.

With just one trade-off we find a relatively narrow maintenance region. However, there potentially exist multiple trade-offs even in simple organisms (supplement 4). Consider, for example, what happens in our model if we also permit the rate-affinity trade-off. Even supposing this trade-off to be linear, the region through which the fittest and the flattest are maintained can be broader than the simpler model (supplement fig 12b). Examination of more complex models reveals the more parameters through which trade-offs exist, the broader the span of mutation rates through which diversity is maintained (supplement 6.4, cf. Ref [16]). A quantitative model incorporating empirical rate-yield and rate-affinity data captures the maintenance of the fittest and the flattest (Fig 1,

supplement 6.4.1). The mutation rate required for co-maintenance appears to be realistic (supplement 6.4.2). We also modelled populations using individual-based stochastic simulations and the results are unaffected (methods and supplement 7). We conclude that the region of maintenance is broad enough to be relevant to even metabolically simple organisms and is likely to be especially relevant in metabolically rich microorganisms.

The trade-offs assumed in our model have been observed within species of microorganisms[17,18,19] and within communities[20] (supplement 4). In the simplest model with a single trade-off, the trade-off must have a convex component to enable co-maintenance. A convex component in the rate-yield trade-off is possible for pathways with opposing properties in rate and yield[17] and has been observed multiple times: in a eukaryote (yeast[18]; supplement fig 5), a prokaryote (*E. coli*; supplement fig 4) and in a community (soil microbes[20]; supplement fig 3). A rate-affinity trade-off with a convex component is also observed in yeast (supplement fig 5). With multiple trade-offs, their rich variety can provide sufficiently complex fitness landscapes for the principle to work (supplement 6.4). While here we consider well-described metabolic trade-offs, the same principle may apply to other metabolic or physiological trade-offs, for example the balance between stress protection and nutritional competence can also support the maintenance of diversity (Nilsson, Ferenci, Haynes and Gudelj, unpublished).

Prior explanations for the maintenance of diversity in homogeneous, well-mixed environments (weak selection/neutrality, mutation-selection equilibrium and genotypic negative frequency-dependence) do not account for these findings. If we consider a population with a broad diversity of possible types and let it evolve to steady state but without further mutations, one type, the fittest, predominates (supplement 7.2). The various types are hence not maintained by any weakness of selection.

To exclude mutation-selection equilibrium we tracked lineages using individual-based simulations asking whether mutants derived from one quasispecies can contribute biomass to another. Importantly, we find that the two quasispecies can be mutationally independent (Fig 2). If we force a mutational barrier between the two peaks after formation, their stable maintenance remains and may be enhanced (supplement 7.4). The types are therefore not maintained by mutational input from one quasispecies alone and hence simple mutation-selection equilibrium is not a full explanation. Similarly, introducing two mutationally-distinct populations with no recent common ancestor (i.e. different species) competing for the same resource can also result in maintenance, demonstrating relevance to diversity between-species in a community.

The maintenance of diversity is not owing to classical frequency-dependent selection. If it were, the diagnostic of enforcing competition between two types, one from each quasispecies and in the absence of mutation, would result in stable balance between the two[12]. However, in the absence of mutation here, one

fittest type excludes all others (supplement fig 19a). Considering just two types from each fitness peak in competition, the fittest type from the highest peak excludes the other (supplement fig 19a/b).

While our principle is logically distinct from prior explanations, it does bear some resemblance. Notably, the co-maintenance of quasispecies can be configured as a form of quasispecies-level (not genotype level) negative frequency-dependence (Fig 3a/b). If we perturb the frequencies of the quasispecies, those below their equilibrium value increase in frequency, while those above decrease in frequency (Fig 3b), a property that requires mutational connectivity within quasispecies but not between (supplement 7.4, supplement fig 23). This dynamic is mediated by shifts in resource levels that arise upon perturbation: quasispecies differ in the rate at which they consume resources and raising the frequency of either one changes those resource levels (Fig 3a, supplement 8). Owing to the trade-offs, this in turn affects relative growth rates differently, thus ensuring that the quasispecies and resource levels return to their prior equilibrium (Fig 3a). For example, when the frequency of high-rate 'fit' types is increased, the resource concentration is reduced. The concomitant increased growth of the high-efficiency 'flatter' cells re-sets resource levels.

The principle of the maintenance of the fittest and the flattest requires more than some form of negative-frequency dependence. A toy population-genetical formulation highlights a set of minimal requirements (box 1). Stable diversity is seen in the absence of spatial or temporal heterogeneity and requires neither

direct biotic interactions nor mutation rate heterogeneity. For the principle to hold, as seen in the toy model, there needs to be (1) negative frequency-dependent selection between the quasispecies (arising here through resource competition and metabolic/physiological trade-offs), (2) the quasispecies that is less fit must be more robust to mutations (i.e. flatter) and (3) the type of a parent and its offspring must be positively correlated to ensure continuity of the quasispecies. The same principle can be extended to the maintenance of more than two quasispecies.

Our model is consistent with the unexpected outcome of chemostat experiments. Close examination of the metabolic efficiencies of chemostat-derived bacteria found remarkable metabolic diversity, some with high rates, some with high yield and many rate-yield hybrids[7], as our model predicts. This is contrary to the expectations of alternative models[14].

The principle is not simply a new theoretical formulation to explain diversity found in microbial communities, the results apply in principle to any clonally derived population, such as tumours and their metastases. While tumour cells are typically clonal derivates they are often highly heterogeneous[21]. This may reflect niche differentiation[21]. However, much as with chemostat bacterial populations, cancer cells often show paradoxically[22] inefficient metabolism[23], notably low ATP-yield aerobic glycolysis[23] and our model suggests why inefficient metabolism might be found. The observed metabolic heterogeneity in response to chemotherapy[24] is explicable.

We assumed that all types have the same mutation rate. This need not, however, be a realistic assumption even within species, not least because bacteria can increase their mutation rate when subject to stress[25]. How higher rates of mutation within a population affect diversity and density is relevant to understanding anti-microbial treatments that increase mutation rate (lethal mutagenesis[8]). Importantly, the survival of the fittest and flattest questions the safety of this therapy. While recent models[26] suggest that pathogen densities should decrease linearly as mutation rates increase, our work suggests that increasing mutation rates can increase both density and diversity by pushing the population to a slightly lower but much flatter fitness peak (supplement fig 11).

METHODS SUMMARY

Stochastic simulations: An individual-based stochastic model was constructed consisting of n heritable types, each with a unique phenotype consisting of cell yield, resource affinity and maximal uptake rate. Each heritable type, j, was numbered from 1 to n (noting a clonal population of n types is equivalent to having n alleles at one locus) and the phenotypic state, x_j, of type j is then $x_j = j/n$ and all phenotypes are derived from this number. The environment contains resource molecules that can be adsorbed by each cell and symmetric division occurs only when a given number of molecules is metabolised, a number that defines the efficiency of each cell. Mutations are introduced with a fixed probability of occurrence per unit time that convert a cell of type j to either $j+1$ or $j-1$; the latter is not a necessary assumption but is conservative and prevents peak-jumping through the generation of "hopeful monsters" (supplement 6.5 and 7.5). Relaxation of this assumption can even promote diversity (supplement 7.5).

Analysis of the MSC model: With mutation rate as the bifurcation parameter, equilibrium states that branch transcritically from the trivial solution of [1] (where $\Delta=0$) were computed using the pseudo arc-length computational strategy[27] (with LU decomposition for the Newton solver, 10^{-14} residual, implemented in Matlab 7.9, 2009b) and extended into the region of lowest mutation rates. Global stability of the unique equilibrium of [1] can be proven under restrictions, where proofs are not available eigenvalue computations and

the geometric structure (bifurcations) of solution branches was used to deduce local stability.

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Fig 1 Maintenance of the fittest and flattest with known parameter values. Using a parameterisation of the MSC equation with rate-affinity and rate-yield trade-off data from empirical studies of *S. cerevisiae* (see supplement fig 5), the diagram shows a locus of steady-state densities for different mutation rates. The cited *multimodality index* is the ratio of biomass supported under each quasispecies. Normal distributions have been fitted to each quasispecies to provide a guide.

Fig 2 Maintenance of fittest and flattest is not simple mutation-selection equilibrium. A single type (number 25) seeded a clonal population subject to rate-yield and rate-affinity trade-offs that diverged into two lineages of quasispecies of efficient and inefficient generalists. For the trade-offs see supplement fig 17, for further details see supplement 7.3.

Fig 3 Quasispecies negative frequency-dependent selection. (a) The MSC equation maintains distinct quasispecies though negative frequency-dependent selection mediated by the abiotic environment. Shown are typical responses in resource concentration resulting from changes in the frequency of fit quasispecies (high uptake) and flat quasispecies (high yield): removing 'fit cells' momentarily decreases sugar concentration, removing 'flat cells' increases it. (b) This results in negative frequency dependence at the level of quasispecies. For trade-offs see supplement fig 8b.

Box 1: A toy model explains how two quasispecies may be co-maintained

Consider two clusters of types forming quasispecies '*a*' and '*A*'. The fitness of quasispecies *a* is G_a, the fitness of *A* is G_A and the relative frequency of the totality of all types in quasispecies *A* is f_A. We assume that negative frequency-dependence is exhibited (see Figure 3, supplement 8):

$$\frac{G_A}{G_a} = r_0 - f_A(r_0 - r_1), \qquad \text{[b1]}$$

where $r_0 > r_1 > 1$ are fixed constants and $0 \leq f_A \leq 1$. This implies $G_A > G_a$, meaning *A* is the *fitter* of the two quasispecies.

We now assume three types of mutational event:

ME1: $a \rightarrow a$ or $A \rightarrow A$: intra-quasispecies mutation;

ME2: $a \rightarrow A$ or $A \rightarrow a$: inter-quasispecies mutation;

ME3: $a \rightarrow$ dead or $A \rightarrow$ dead: lethal mutations.

To discount mutation-selection equilibrium, we assume an absence of inter-quasispecies mutations (ME2) and, for simplicity, the fitness effects of intra-quasispecies mutational events (ME1) are ignored.

Now, suppose that the loss of cell types from *a* and *A* due to death (ME3) reduces the overall fitness of *a* and *A*, so

$$w_a = G_a - \mu_a \quad \text{and} \quad w_A = G_A - \mu_A, \qquad \text{[b2]}$$

where $\mu_a > 0$ and $\mu_A > 0$. Thus w_a and w_A are the quasispecies fitnesses in the presence of mutation. Importantly, as *a* is the more mutationally robust (flatter) quasispecies, quasispecies *a* has a lower rate of lethal mutation, hence we assume $\mu_A > \mu_a$ and set $\alpha = \mu_a / \mu_A$ with $0 < \alpha < 1$.

This toy model is consistent with survival of the fittest and the flattest, respectively. *Survival of the fittest* is relevant when $w_A > w_a$ which occurs when $G_a(r_1 - 1)/(1-\alpha) > \mu_A$. S*urvival of the flattest* is relevant when $w_A < w_a$, i.e. when

$G_a(r_0 - 1)/(1-\alpha) < \mu_A$. Co-maintenance of both quasispecies is possible when $w_A = w_a$, this occurs when mutation rates take on intermediate values: $\frac{G_a(r_1 - 1)}{1-\alpha} < \mu_A < \frac{G_a(r_0 - 1)}{1-\alpha}$, (see Fig B1(a)).

This principle requires $G_A > G_a$, $\mu_A > \mu_a$ and that negative frequency-dependent selection acts between the two quasispecies. Were we to assume that mutations in quasispecies *a* had the same probability of being lethal as those in quasispecies *A*, (i.e. $\mu_A = \mu_a$), *A* would be the fitter for all mutation rates. Similarly, in the absence of negative frequency-dependence, $r_0 = r_1$, [b1] would become

$G_A = r_0 G_a$. [b3]

Allowing for $\mu_a = \alpha\mu_A$ with $0 < \alpha < 1$ and substituting [b3] into [b2] we obtain

$$\frac{w_A}{w_a} = \frac{G_a r_0 - \mu_A}{G_a - \alpha\mu_A}. \qquad \text{[b4]}$$

In [b4] there is just one value of μ_A for which a non-robust form of co-maintenance may occur, namely $\mu_A = G_a(r_0 - 1)/(1-\alpha)$ (see Fig B1(b)).

Fig B1 The relative fitness of the *fit* quasispecies A as a function of its frequency, f_A. (a) For increasing mutation rates, given by the direction of the arrow, the survival of the fittest quasispecies (label (1)) gives way to survival of the flattest (label (3)) via a regime of co-maintenance (label (2)). (b) Only a non-robust form of co-maintenance (label (2)) is possible in the absence of negative frequency-dependent selection.