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Brief note

Metastable coexistence of multiple genotypes in a constant environment with a single resource through fixed settings of a multiplication-survival trade-off

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Abstract

The biological complexity of trade-offs has been a major obstacle in understanding bacterial diversity and coexistence. Here we reduce the biological complexity by using isogenic *Escherichia coli* strains differing only in a multiplication-survival trade-off regulated by RpoS. The contribution of trade-off characteristics to fitness in different environments was determined. We then designed an environment with intermediate-stress levels that elicits an equivalent fitness. We found metastable coexistence of three strains in steady-state chemostats until mutations changed the relative fitness of competing strains. Our results help explain the rich intra- and inter-species diversity of bacteria through alternative settings of relatively few trade-offs.

Keywords: Trade-offs; Stress resistance; Resource allocation; Reaction norm; *Escherichia coli*; RpoS sigma factor

1. Introduction

Free-living organisms such as bacteria constantly face fluctuations in their environments, from abiotic factors such as variation in temperature, salinity and nutrient availability to biotic factors such as phages. These environmental changes exert important physiological stresses upon bacteria, and adaptation to one specific environment by optimizing a particular life history trait is often accompanied by a trade-off in different environments [1]. A negative correlation between different life history traits has been increasingly invoked to explain different ecological and evolutionary phenomena, including coexistence of various forms of life both in nature [16,33,35,31] and in controlled experimental settings [2,21,12,4,5]. There are wide ranges of trade-offs that can potentially support species coexistence. Examples include trade-off between competition and colonisation, antibiotic or phage resistance and fitness, and trade-off due to resource specialization [13,34,6,15]. While these studies have shown the importance of trade-offs in maintaining diversity, we do not know yet whether a single trade-off is sufficient to support the coexistence of multiple types. A recent study using a large number of lambda-phage-resistant mutants of *Escherichia coli* suggested that more than one type of trade-off is required to explain the coexistence of different phage-resistant mutants in a single niche [26]. The quantitative contribution of trade-offs is also unknown, but as suggested by Tilman [35], to understand the diversity of species, "the answer may lie in quantifying the trade-offs that organisms face in dealing with the constraints of their environment".

With most organisms and conditions, the contribution of complex ecological interactions is very difficult to disentangle, let alone quantify. Asexual organisms such as bacteria growing in simple environments enable a reduction in the complexity of this problem, but even here, earlier studies provide examples that are inconsistent in demonstrating hypothesised trade-

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offs [29,37,36]. One of the best-studied bacterial trade-offs was the analysis of the costs and benefits of phage resistance of *E. coli* B to T-type bacteriophage with respect to growth fitness [18,4]. Isolated phage-resistant mutants exhibited a range of resistance-growth trade-offs and the fitness of these strains could be tested for coexistence. This trade-off allowed the coexistence of different phage-resistant mutants of *E. coli*. Still unanswered in these studies was whether random spontaneous mutants influenced trade-offs in multiple ways. The lack of molecular and physiological detail of the phage-resistant mutants left open the possibility that coexistence is due to multiple trade-offs, as well as perhaps other ecological interactions such as negative frequency-dependent selection [5].

In this communication, we analyse a multiplication-survival trade-off to test whether a single trade-off is sufficient to elicit the same level of competitive fitness of multiple types in an environment supported by a single carbon and energy source, at least until other mutations perturb this metastable state. We employed a model organism *E. coli*, in which the molecular basis of trade-off involving resource allocation to either multiplication (reproduction) or survival (stress tolerance) is well understood [12,24]. Central to this trade-off is the relative concentration of two key sigma factors of RNA polymerase; RpoS (alias σ38 and σ^S) and RpoD (alias σ70 and σ^D), which determine the relative transcription levels of vegetative and general stress genes in vitro [10,30]. As the concentration of RpoD in cells is relatively constant, it is the variable concentration of RpoS that is the main determinant of transcriptional allocation [17]. However, both expression of *rpoS* and the stability of RpoS are highly stress-affected in *E. coli* species and are usually dependent on environmental signals such as osmotic stress, low pH and slow growth [14]. Thus, accurate quantification of RpoS-dependent trade-offs using environmental isolates of *E. coli* is difficult.

We have recently constructed a set of synthetic strains with fixed, environment-independent RpoS levels [24]. These strains do not have transcriptional, translational and post translational control over RpoS levels, and instead contain an artificial promoter to express fixed levels of *rpoS* in an environment-independent manner, which reduces complexity in trade-off analysis even further. The strain set has previously been used to determine the shape of trade-off curve in multiple environments and employed to predict the outcome of evolutionary divergence [24]. However, we do not yet know how strains with different RpoS levels respond to the varying levels of stress intensity. We also do not know whether the strains can coexist in some environments.

In this study we chose three representative strains as a surrogate means of fixing the stress level in bacteria. This approach enabled us to quantify the contribution of RpoS to fitness in different stress intensities, and to identify the environment where all three RpoS strains showed a similar level of competitive fitness. We then used this information to test how fitness equivalency promotes coexistence of multiple genotypes based on a single trade-off.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Bacterial strains used in this study are listed in Table 1. Bacteria were cultured in Luria Bertani broth or minimal medium A (MMA, [27]) with either 0.2% wt/vol glucose for batch cultures or 0.02% wt/vol glucose for chemostat. When MMA was used as growth medium, culture media were also supplemented with 1 μg/ml thiamine and 1 mM $MgSO_4$. All growth experiments were performed at 37 °C.

To construct the BW5205T, we replaced the *aphA* gene responsible for kanamycin resistance in BW2952 by Tn*10* using the method described in Ref. [39]. The primer set used for construction of *aphA*::Tn*10* was: KanTetF1(5′GGATTA TCAATACCATATTTTTGAAAAA GCCGCAAGAGGGTCA TTATATTTCG-3′) and KanTetR1 (5′-GAGGCCGCGAT-TAAAT TCCAACATGGATGCTGACTCGACATCTTGGTT ACCG-3′). To construct the kanamycin-sensitive derivative of BW5206 (BW5206M), the synthetic *rpoS* leader sequence of BW5206 transferred into the wild-type MC4100 by P1 transduction [27] (see Table 1 for details).

2.2. Estimation of fitness and coexistence assay

Fitness comparisons were made against a tetracycline-resistant derivative of BW2952 (BW3454 carrying *metC*::Tn*10* insertion) in MMA medium supplemented with 0.02% (w/v) glucose and 4 μg/ml methionine. The *metC*:Tn*10* insertion is neutral in glucose-limited chemostats in the presence of 4 μg/ml methionine. Head to head competition in chemostat cultures was as previously described in [38,25]. Briefly, 15-h-old independent chemostat cultures growing at a dilution rate of 0.1 h^{-1} were mixed 50/50 on the basis of optical density (OD) of cultures at 600 nm. Dilutions of the mixed cultures were plated onto L-agar (for a total count of both strains) and L-agar containing tetracycline (12.5 μg/ml, for BW3454 counts). Samples were taken at time intervals of 1, 3, 5, 7, 9 and 24 h. The reported fitness as selection coefficients (S) were based on the equation of Ref. [9] in terms of Malthusian parameters determined from the linear slope of regression $\ln[p(t)/q(t)]$, where $p(t)$ and $q(t)$ represent relative frequency of competing strains in the population at a given time point. At least five independent time points were used for the estimation of selection coefficients.

The three-way competitions or coexistence experiments were initiated by mixing BW5205T, BW5206M and BW5208. Two strains with different drug markers, BW5205T and BW5206M, have indistinguishable fitness compared to BW5205 and BW5206, respectively, in glucose-limited chemostats with or without added stress. Each strain was allowed to acclimatise for 15 h in separate chemostats growing at 0.1 h^{-1} before initiating competitions. The head to head competition was started by mixing bacteria at a ratio of approximately 33/33/33 on the basis of the OD_{600} of the individual cultures. Dilutions of the mixed cultures were plated onto Luria Bertani-agar (LB-agar) plates (for a total count of