Molecular Adaptations Are Rarely Reversible during Bacterial Evolution against Alternative Antibiotics

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***Short Abstract* — The degree to which evolutionary adaptations are reversible is a fundamental property of evolution. An adaptation that has caused significant public health concern is the evolution of extended-spectrum β-lactamases (ESBL), which are enzymes able to inactivate a wide-range of β-lactam antibiotics. In particular, five point mutations in the β-lactamase gene collectively confer high level resistance against the third-generation drug cefotaxime. Here we show that although these adaptations against cefotaxime result in a loss of resistance against a first generation drug with a β-lactamase inhibitor, reverse evolution back to the starting allele is generally not possible.**

***Keywords* — Evolutionary reversibility, β-lactamase, antibiotics.**

I. Background

β-lactam antibiotics, which kill bacteria by inhibiting cell wall synthesis, are both the oldest and most widely-used class of antibiotics. Bacteria gain resistance to these antibiotics by expressing the enzyme β-lactamase, although different versions of the enzyme confer varying levels of resistance against different versions of the drug [1]. Five point mutations in the reference allele of the TEM-family β-lactamase gene have been identified to confer high-level resistance to cefotaxime and the ESBL phenotype: g4205a (in the promoter), A42G, E104K, M182T and G238S [2]. However, epistatic interactions between these mutations make many of the evolutionary paths from the reference allele to the ESBL allele with all five mutations selectively inaccessible, as a given mutation is not favorable in all genetic backgrounds (combinations of the other four mutations) [3].

II. Methods and Results

Although ESBLs are highly resistant to cephalosporins like cefotaxime, they have been reported to be sensitive to β-lactamase inhibitors [4]. Clavulanic acid, one such inhibitor, can transiently or irreversibly inactivate the enzyme [5]. By measuring the degree of antibiotic resistance of bacteria containing the β-lactamase gene with all $2^5 = 32$ possible combinations of the five point mutations, we observed a significant fitness trade-off between fitness in cefotaxime and fitness in another common β-lactam, piperacillin, with clavulanic acid.

In contrast to previous reversibility studies that probe just two alleles of a gene and their intermediates [6], we analyze all 284 pairs of non-adjacent alleles in which there is a trade-off in the two antibiotic environments. To measure the degree of evolutionary reversibility on our experimentally measured fitness landscapes, we asked how many of these 284 pairs are connected by at least one selectively accessible path in each of the two conditions. Using this approach, we find that molecular evolution is reversible for only 28 of these pairs, a fraction that is lower than one would expect even if there were no trade-offs.

Because obvious global features of the fitness landscapes failed to explain the lack of reverse evolution, we wondered whether a local feature might. We analyzed how often gaining a given mutation had opposite effects in the two environments (therefore reversible). On average, there is only a 37.5% chance that a mutation is reversible. So in this sense, evolution is more likely to be irreversible. Four of the mutations more frequently than not have the same effect, whereas the large-scale trade-off in resistance is dominated by the G238S mutation, which opens up the active site of the enzyme and increases the binding affinity to both cefotaxime and the inhibitor. Global trade-offs between two conditions can therefore hide local randomness that limits the reversibility of evolution.

III. Conclusion

We found that global fitness trade-offs do not necessarily lead to frequent reversibility because the trade-offs can be dominated by one or a small number of mutations, leaving the remainder of the landscapes uncorrelated. This situation may occur often in single-protein evolution where different environments require different binding specificities. Thus, to accurately predict the prevalence of reverse evolution between two landscapes, a measure of their correlation that captures local randomness must be used.

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