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The Joys and Terrors of Fast Adaptation: New findings elucidate antibiotic resistance and natural selection

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Abstract

Experiments of Pranting and Andersson demonstrate how bacteria adapt to the growth limitation caused by antibiotic resistance mutations. The process of adaptation relies on gene copy number changes that arise at high rates, including duplications (10^{-4} /cell/generation), amplifications (10^{-2} /cell/generation) and mutant copy loss (10^{-2} /cell/division). Reversible increases in copy number improve growth by small steps and provide more targets for rare sequence alterations (10^{-9} /cell/division) that can stably improve growth. After sequence alteration, selection favors loss of the still mutant gene copies that accelerated adaptation. The results strongly support the amplification-reversion model for fast adaptation and argue against the alternative idea of “stress-induced mutagenesis”.

When conditions limit growth, populations of bacterial cells (and higher organisms) adapt genetically at surprisingly high rates. Adaptation is a joy, when your population adapts to a new environment. It is a terror, when pathogens or malignant cells adapt to you. Thus adaptation is important to both the process of evolution and for many aspects of disease. The speed of adaptation under natural conditions is shocking in the light of our experience with stringent lab selections, which prevent growth of the parent population and detect only pre-existing large-effect mutants (Luria & Delbruck, 1943, Lederberg & Lederberg, 1952). By preventing adaptation, lab selections make bacterial genetics possible, but they do not prepare us to appreciate the speed of natural selection during growth. The work of Pranting and Andersson contributes to an understanding of how this occurs.

Over the past 20 years, a controversy has surrounded attempts to explain the fast adaptation seen in several systems that, unlike standard lab procedures, use non-stringent selective conditions. How does selection work so well? Could selective stress be mutagenic? The new results presented here show that selection acts alone and adaptation does not involve “stress-induced mutagenesis”. In explaining fast adaptation without mutagenesis, Pranting and Andersson show how antibiotic-resistant bacteria escape the fitness costs associated with resistance and regain full growth ability. This process is central to understanding microbial populations and how they respond to our use of antibiotics.

The antibiotic used here is protamine, a lethal anti-microbial peptide (AMP) that resembles those produced by metazoans as part of innate immunity (Pranting & Andersson, 2010, Proctor *et al.*, 2006). Mutants resistant to protamine arise at a rate of 10^{-7} /cell/division. This high rate reflects the large size of the mutational target. Resistance is provided by mutations that reduce proton-motive force (PMF). Since PMF depends on many proteins, there are many sites in the genome at which resistance mutations can arise, including heme biosynthetic genes. Impairment of these essential processes provides resistance to the lethal antibiotic, but compromises the general growth rate. Pranting and Andersson show that

bacteria rapidly circumvent growth limitation because natural selection can detect small-effect mutations that are common under all circumstances.

A growth-compromised protamine-resistant (*hemC*) mutant improves its growth ability in small steps due to increases in the copy number of the partially functional mutant gene. Adaptation is initiated by cells with a *hemC* duplication, which are present in the population at a high steady-state frequency (1/100) before selection (Reams *et al.*, 2010). Cells with extra copies grow slowly and improve further by a series of additional copy number increases, each of which arises at about 10^{-2} /cell/generation. See Figure 1A and B.

Cells with extra mutant *hemC* copies grow until the population has enough mutant alleles to permit a rare sequence change that improves one copy of the growth-limiting gene. Such sequence changes occur at about 10^{-9} – 10^{-10} /cell/generation (Figure 1A and center of Figure 1B). The probability of a sequence change (per cell) is increased slightly by the additional mutational target sites (more copies per cell), but selection accelerates the appearance of revertants primarily by allowing an exponential increase in the number of cells with an amplification (both effects are noted at the top Figure 1B). This process involves no increase in mutation rate (mutation/target).

The added copies of the mutant *hemC* allele improve fitness sufficiently to offset the basic fitness cost of a gene amplification (Reams *et al.*, 2010). This situation changes after mutation generates an improved *hemC* allele. At this point, selection holds the improved allele and counter-selects the costly mutant copies. Thus a gene amplification contributes to the formation of a rare mutation by providing more target copies and growth, but is counter-selected once the improved allele is in place. Ultimately haploid revertant cells appear that carry a single improved *hemC* allele and show no evidence of the gene copy number increase that hastened their formation. (top right of Figure 1B)

The sequence of events demonstrated here was initially suggested (Andersson *et al.*, 1998, Roth *et al.*, 2006) to explain fast adaptation in a system described by Cairns and Foster (Cairns & Foster, 1991). In this system, a partially defective *lac* mutant is plated on lactose. The parent cells cannot (quite) grow, and the revertant colonies that accumulate over 6 days have been widely attributed to stress-induced mutagenesis of non-growing cells (Foster, 2007, Galhardo *et al.*, 2007). In the alternative amplification-selection model (left side of Figure 2), common pre-existing copy number variants grow under selection and initiate colonies that adapt quickly. The multiple steps of the adaptation process have been hard to resolve, because of idiosyncratic features of Cairns-Foster system that accelerate adaptation. Notably, the *lac* allele is located on an F'*lac* plasmid, whose conjugative replication origin produces DNA ends that stimulate amplification. Furthermore, selected *lac* amplification is modestly mutagenic (Rosche & Foster, 1999), due to co-amplification of the nearby *dinB* gene (Slechts *et al.*, 2003), which encodes an error-prone polymerase that is mutagenic when over-expressed (Kim *et al.*, 2001, Wagner & Nohmi, 2000). Because of these features, the system allows pre-existing cells with multiple *lac* copies to initiate colonies that rapidly grow and adapt under selection, producing within stable Lac⁺ revertant cells within developing colonies by amplification, reversion, segregation. The mutagenesis caused by *lac-dinB* amplification is not essential to adaptation (Slechts *et al.*, 2003). The rapid adaptation of pre-existing partially-revertant mutants during growth under selection is easily mistaken for stress-induced appearance of single-step mutants in a non-growing population.

Pränting and Andersson demonstrate all the intermediates of the amplification-selection model. Of particular interest are cells whose array of *hemC* copies includes both the original mutant allele and a mutationally improved allele. This intermediate has not been seen previously, but is revealed here because the adaptation process moves more slowly for the

chromosomal *hemC* system (> 100 generations), than for the Cairns-Foster system with lac on an F'lac plasmid (9 generations). These systems are compared in Figure 2 (left and right sides).

The amplification-selection model has now been shown to operate in four different genetic systems. In Figure 2, three antibiotic systems analyzed by Andersson and coworkers are compared to the Cairns system. In each case, cells overcome growth defects by amplifying a growth-limiting gene. In one case, the drug actinonin inhibits the essential enzyme (Fmt), which formylates methionyl-tRNA and forms the initiator f-Met-tRNA. Resistant mutants (*fmt*) reduce formyl transferase and cause a general fitness loss. Growth is improved by amplifying the tRNA^{F^{Met}} gene (Nilsson *et al.*, 2006). In another case, the antibiotic mupirocin inhibits isoleucyl-tRNA synthetase (*ileS*) and resistant mutations have an altered *ileS* coding sequence that severely limits growth. Growth improves first by amplification of the *ileS* gene and later by secondary mutations that either improve the *ileS* promoter or improve the IleS protein (Paulander *et al.*, 2010). Either change enhances fitness and allows rapid loss of mutant alleles from the amplified array. The earlier systems show amplification-selection at work and demonstrate some steps in the process (Indicated in red in the Figure 2). However, the protamine work described here demonstrates all of the intermediates and the progression from one stage to the next (right side of Figure 2).

These results have enormous relevance for understanding effects of antibiotics on real populations. While protamine is not a clinical antibiotic, it resembles the antimicrobial-peptides (AMP), produced by many metazoans. Some chronic and recurring infections are associated with growth-impaired small-colony variants (SCV), which are likely to be positively selected in the host. These resistant bacteria may persist for long periods, only to expand their populations at a later time. Recurring infections can be dire, even when increased fitness restores antibiotic sensitivity.

Pränting and Andersson demonstrate a general mechanism for fast adaptation (whether a joy or a terror). Natural selection (unlike stringent lab selection) detects small growth improvements provided by mutations that are frequent under all growth conditions. These frequent small-effect mutations are not detected in stringent lab selections, so have been largely missed by bacterial genetics. The work presented here shows that such mutations are important to the high rate of genetic adaptation when selection is imposed on growing cells.

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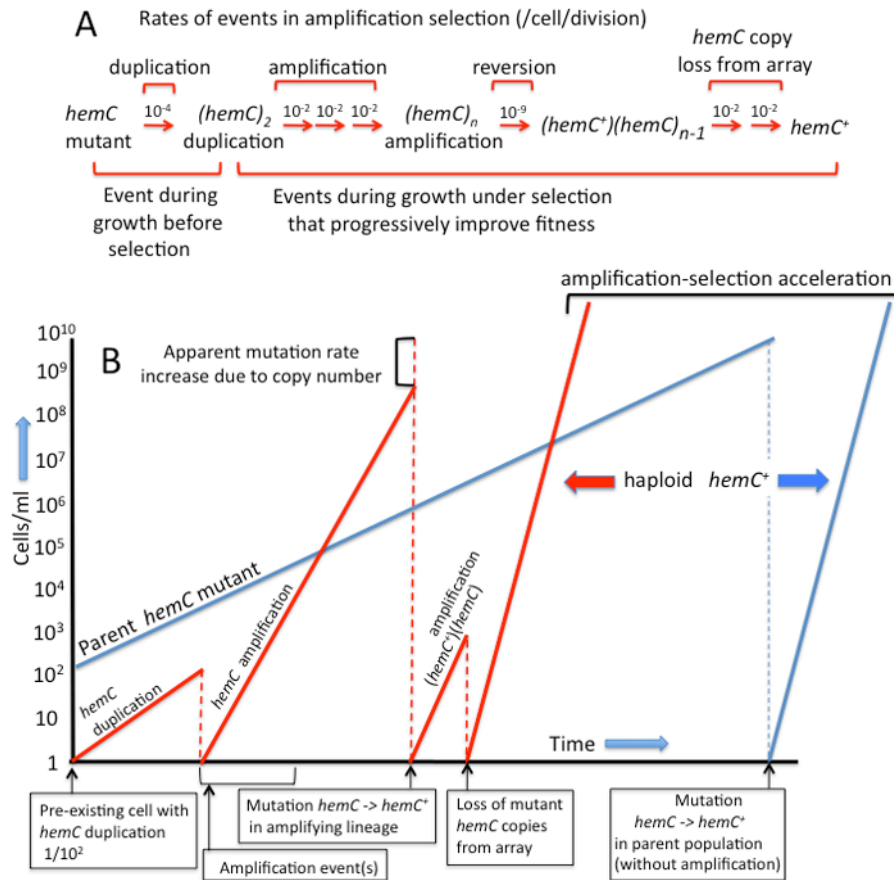


Figure 1. The process of amplification-selection

Part A Duplications form at a high rate (10^{-4} /cell/division) and steps in subsequent copy number change occur even more frequently (10^{-2} /cell/division). The frequency of duplications in an unselected population reaches a steady state of about 10^{-2} . Part B When a *hemC* mutant initiates a culture, 1% of cells have a duplication. Cells with extra copies of *hemC* grow faster than the parent and acquire amplifications that further enhance fitness. The amplification process is likely to involve multiple steps. A sequence change (*hemC* -> *hemC*⁺) occurs at a standard mutation rate when sufficient targets are present. Amplification causes a modest increase in the rate (/cell) of reversion to *hemC*⁺ (top left), but accelerates appearance of full revertants (haploid *hemC*⁺) primarily by allowing exponential growth of the improving lineage (top right). The amplifying lineage reaches a high population density and produces stable fitter progeny sooner than the unamplified parent lineage.

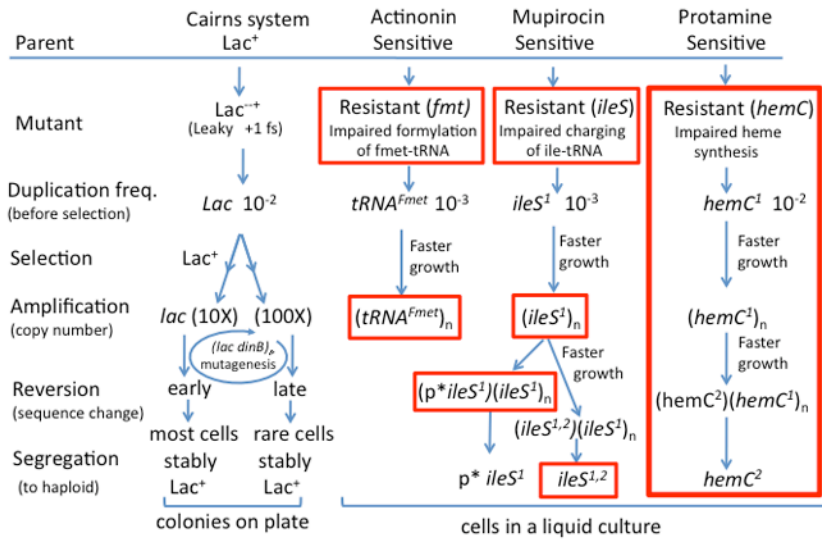


Figure 2. Amplification-selection accounts for fast adaptation in multiple systems
 The amplification-selection model was proposed to explain development of Lac^+ revertant colonies in the Cairns system (at left). The general applicability of this model is shown by its role in three different antibiotic systems. In these cases, selection favors a series of faster-growing cells in liquid culture. Intermediates in red boxes have been demonstrated experimentally. The work presented here on a protamine-resistant *hemC* mutant (diagrammed at right) demonstrates all of the intermediates and steps in the amplification selection process.