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BACTERIAL EVOLUTION

Decoding the bacterial fossil record

> the Cit$^+$ phenotype only re-emerged in populations that were derived from generation 20,000 and beyond

Elucidating the genetic basis for the evolution of novel traits is challenging because intermediate ancestral genotypes are often lacking, which means that the mutational events that generated the evolved trait cannot be reconstructed. By virtue of their rapid growth and the ability to freeze and revive ancestral clones, bacteria are ideal for unravelling the molecular mechanisms of fundamental evolutionary processes. Richard Lenski and colleagues followed the emergence of aerobic citrate utilization in experimentally evolved *Escherichia coli* populations by sequencing the genomes of a number of ancestral clones. Together with evolutionary replay experiments, these sequences have revealed the complex series of molecular events that led to the acquisition of this new function.

More than 24 years ago, Lenski initiated a long-term evolution experiment, which has now seen 12 independent *E. coli* lineages surpass 55,000 generations of growth. Aliquots of the cultures are frozen at regular intervals and provide a detailed history of intermediate genotypes. The bacteria are propagated aerobically in a minimal medium containing glucose as the only available carbon source. Citrate is also present in the medium, but under oxic conditions *E. coli* cannot typically use citrate as an energy source, owing mainly to an inability to transport citrate into the cell. However, a few years ago, researchers in Lenski's laboratory noticed that one of the 12 populations (Ara-3) had evolved the remarkable ability to use citrate for aerobic growth (termed the Cit$^+$ phenotype).

To decipher the genetic basis of the Cit$^+$ phenotype, whole-genome sequencing was carried out on 29 clones that were isolated from various generations of the Ara-3 population, and the phlogenetic history of the lineage was constructed. This analysis revealed that a slow-growing Cit$^+$ clone had emerged by 31,500 generations. The Cit$^+$ phenotype was achieved by a promoter capture event (which the authors term the actualization step) caused by a genetic rearrangement in the citrate fermentation operon. The rearrangement resulted in fusion of the *rnk* gene (encoding a regulator that is unrelated to citrate metabolism) to the *citG* gene, which is immediately upstream of, and in the same cistron as, the citrate transporter (*citT*) gene. Thus, the authors hypothesized that this event would place the expression of not only *citG* but also *citT* under the control of the oxygen-responsive *rnk* promoter, meaning that citrate import could occur in oxic conditions. Consistent with this, insertion of the *rnk* promoter immediately upstream of *citT* in a Cit$^-$ clone enabled weak oxic growth on citrate.

Considering the poor growth of the initial Cit$^+$ clone, the authors went on to identify the additional mutations that allowed Cit$^+$ clones to expand and eventually dominate later generations. They found that the genetic rearrangement was amplified in an array ranging from two to nine copies (termed the refinement step). To confirm that amplification was responsible for refinement of the Cit$^+$ phenotype and that this led to a growth advantage, a Cit$^-$ clone was transformed with a high-copy-number plasmid carrying an *rnk–citT* fusion. As predicted, the transformed clone had a strong Cit$^+$ phenotype and grew rapidly on citrate.

Finally, the authors replayed evolution by reviving clones isolated at various generations to determine whether citrate utilization would re-emerge and whether this was dependent on earlier mutations in the genetic background (termed the potentiation step). Although the nature of the potentiating mutations is currently unclear, there is evidence that they do exist, as the Cit$^+$ phenotype only re-emerged in populations that were derived from generation 20,000 and beyond.

This study demonstrates the power of long-term experimental bacterial evolution in providing a rare snapshot of evolutionary processes at an unprecedented level of detail. Furthermore, the authors define key factors for the evolution of a new function: a potentiating genetic background that facilitates actualization of a mutation conferring a poorly functioning trait which is transformed, through refinement, into a key innovation.

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ORIGINAL RESEARCH PAPER Blount, Z. D. *et al.* Genomic analysis of a key innovation in an experimental *Escherichia coli* population. *Nature* 19 Sep 2012 (doi:10.1038/nature11514)