The role of chance and history in the evolution of a novel trait in *Pseudomonas fluorescens* SBW25

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Abstract

The processes that lead to the evolution of novel adaptive traits are poorly understood. It is not clear how the combination of natural selection and random variation determine evolutionary pathways, specifically how evolutionary history affects the outcome of evolution. In a previous experiment in which populations of the bacterium *Pseudomonas fluorescens* were subjected to repeated evolutionary reversals (the *R*everse-*E*volution *E*xperiment (REE)) stochastically switching genotypes evolved *de novo* in two out of 12 replicate lineages. Whole genome sequencing revealed not only causative mutations in *carB* (referred to as *carB**) and *rpoD* but also the entire evolutionary pathways comprised of eight additional mutations. It was hypothesized that evolutionary history played a major role in switcher evolution.

This was tested by 'reviving' four REE lineages and the performance of 'replay evolution' experiments. Whereas the repeated evolution of switcher genotypes was observed in three of the four lineages, the likelihood of switcher occurrence varied and depended on the genotype. By artificially introducing the original switcher mutation *carB** into genotypes from one lineage, potential fitness benefits of the switcher mutation at each point in evolutionary time were assessed. The introduction of *carB** into the ancestral genotype of SBW25 created a switcher with higher fitness than the ancestor, indicating that evolutionary history is not necessarily required to give rise to an evolutionary successful switcher. This idea was tested further during a real-time evolution experiment using the same genotypes as founder populations. The capacity to evolve a switcher, based on the competitive fitness of *carB**, was only partly reflected in the ability to re-evolve switchers, which is most likely due to the availability of alternative evolutionary pathways, the number of competitors, and the fitness of the founder genotype. In addition, epistasis contributed to an increased capacity to produce a switcher based on *carB**, resulting in an increased likelihood to evolve this novel trait in genotypes from the later evolutionary time points.

Evolutionary history plays an important role in the evolution of switching. Even though switchers evolved repeatedly from different genotypes, the likelihood that this path was taken depended on genetic constraints and ecological factors. These factors and their contribution to the evolutionary outcome were dependent on the genetic composition and changed throughout evolutionary history.

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