

A MODULAR GENOMIC ARCHITECTURE FACILITATES THE REPEATED RE-EVOLUTION OF AN ADAPTIVE PHENOTYPE IN PSEUDOMONAS FLUORESCENS

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Understanding the complex interactions that connect genotype and phenotype as well as the genetic rules that govern the adaptive walk of populations across fitness landscapes is of fundamental interest to modern evolutionary biology. We addressed these issues by using an approach of reverse evolution: Replicate populations of the bacterium *Pseudomonas fluorescens* were allowed to respond to a static environment with the evolution of a new phenotype. Once the new phenotype had reached a detectable frequency, it was transferred to a shaken environment to reverse the newly acquired phenotype. By repeatedly switching genotypes between the two environments, we determined their capacity to respond to repeated environmental reversals with correlated phenotypic changes. An analysis of the mean fitness trajectory of the replicate populations in the shaking environment illustrated that genotypes evolved under static conditions paid a fitness cost, whereas genotypes derived from the shaken environment always restored the fitness back to the ancestral level. The identification of the mutational causes indicated that despite some parallelism among replicate lines, the adaptive phenotypes were realized by taking different genetic routes. However, mutations causing phenotypes adaptive to the static environment and the ones reverting them often occurred pairwise in the same functional module (i.e. gene or operon). Introducing mutations that originated in the static environment into the ancestor and comparing the fitness of the resulting genotype to the genotype in which the mutation arose in the first place, implied that for the vast majority of cases, mutational effects were independent of other, preceding mutations. The evolutionary flexibility observed in these experiments may be a direct consequence of a modular organization of regulatory units that by mutation activate or deactivate niche-specific structural genes.