Recurrence of evolutionary genotypes as an indicator of evolvability.

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Selection-driven evolution of a population is a search through multidimensional sequence space for genotypes that engender phenotypes with a high fitness in a particular environment. Environments or genotypes that could produce high-fitness phenotypes with high probability can be considered those with enhanced evolvability. But how can one measure the likelihood that evolution will generate a specific result unless evolution is allowed to occur multiple times from the same starting point -- to "replay life's tape" (Gould, 1989; *Wonderful Life*; Norton, New York)? For whole organisms, this experiment is not possible, but from a theoretical perspective, there are robust characteristics of evolving systems that make possible the recurrent evolution of certain properties of life (Fontana and Buss, 1994; *PNAS USA* 91:757-761). Recently, it has become possible to test directly evolutionary recurrence by the use of *in vitro* evolution systems of evolving populations of catalytic RNA in the laboratory.

This paper will focus on the assessment of how reproducible the results from an RNA *in vitro* selection experiment can be. If the same starting pool of variants is used to seed multiple parallel selection experiments, one could find that the same sequences are selected in each experiment (evolutionary recurrence), that completely different sequences are selected in each experiment (evolutionary divergence), or that certain mutations or sequences are selected in some experiments but not all. We utilized the continuous evolution protocol developed by Wright and Joyce (*Science* 276:614-617; 1997) to select variants of a ligase ribozyme that were proficient catalysts at progressively lower Mg^{2+} concentrations. A mutant pool of RNA was created by mutagenizing a highly-reactive ligase (the "wildtype") at approximately 8% error per nucleotide position. A distinct 9-error mutant of the wildtype sequence became nearly fixed in the pool after 16 rounds of selection in which the Mg^{2+} concentration of the continuous evolution milieu was lowered by 2.5 mM every third round, starting with 25 mM (Schmitt and Lehman, *Chemistry and Biology* 6:857-869; 1999). It was unclear however, whether the same sequence would come to dominate the evolving population if the experiment were repeated, and if so, if the mutations would appear during the same rounds of selection and in the same order. Thus we repeated the initial experiment eight additional times in parallel, making every effort to maintain identical environmental conditions in each experimental line. The same basic mutant sequence did in fact recur in the eight lines, indicating that these mutations all grant the RNA ligases a distinct fitness advantage in the continuous evolution protocol. Notably, the mutations recurred almost exactly during the same round of evolution, and thus presumably at the same Mg^{2+} concentration. Surprisingly however, some of the same mutations even appeared during control continuous evolution lines, in which the Mg^{2+} concentration was not lowered, suggesting that the initial RNA sequence lies very near a fitness optimum in multidimensional sequence space. Our results demonstrate how recurrence experiments can be used to distinguish mutations that confer a phenotypic advantage to RNA from mutations that increase in frequency during selection *in vitro* as a consequence of chance.

The *in vitro* continuous evolution system is perhaps the most life-like chemical, but abiotic, system developed to date. It remains to be seen whether the inferences drawn from this system with respect to evolutionary recurrence are applicable to higher-level organismal and/or computer life. However, some notable epiphenomena are already apparent in the continuous evolution system that give us confidence that it has utility as a model system. First, the system demonstrates imperfect heritability, in that the phenotype can not be unambiguously predicted from the genotype. The environment intervenes in the manifestation of catalytic RNAs' phenotypic expression from their primary nucleotide sequences. We have determined this to be a consequence of a non-unity probability that any given nucleotide sequence will fold into a catalytically active three dimensional structure (Schmitt and Lehman, *Chemistry and Biology* 6:857-869; 1999). The phenomenon lowers the rate of evolution in a RNA population under selection because a particular genotype may not always generate a high-fitness phenotype. This issue is directly relevant to the concept of evolvability and yet is rarely considered in many artificial life systems. Second, experiments in our lab are now demonstrating the enhanced effect ton evolvability that recombination can have, even in these simple biochemical systems. In addition to a more effective search strategy of sequence space, swapping of portions of RNAs' genomes can potentially counter the accumulation of slightly deleterious mutations (Muller's Ratchet). Experimental studies of both of these phenomena, and their impact on general evolvability, will be presented in this paper.