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**Testing evolution predictability using the aevol software**

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**Abstract**

Motivated by RNA virus' genome biology, we used the aevol software to simulate the evolution of compacted genomes under high mutation rates. 30 independent wild-type (WT) genomes were generated after 300,000 generations of evolution under similar conditions. Then, each of these WTs was cloned 30 times and we let evolution to continue for 30,000 extra generations. By comparing these clones, we aimed to reveal the extent of evolutionary predictability for such compacted genomes. Results show that: (i) WTs are not equivalent in terms of evolutionary potential: some WTs are more prone than the others to increase their fitness during the last 30,000 generations. (ii) Evolution frequently occurs in bursts which implies that the probability to fix a mutation is increased after fixation of another mutation. Moreover these bursts are often initiated by chromosomal rearrangements (mainly duplications) because these rearrangements open new evolutionary pathways in the fitness landscape. Indeed, we quantified the “evolvability potential” of each clone after each mutation and found that the bursts are triggered by a strong increase of evolvability that quickly leads to point substitutions and indels fixation.

**Methods: in silico experimental evolution (ISEE) with the aevol platform**

**Aevol** (www.aevol.fr) is an ISEE platform that models bacteria at the genomic level with explicit mutation and selection processes. ISEE mimics experimental evolution with in silico organisms subjected to variation and selection in a computer.

**Strain generation:**
- 30 WT virus-like genomes have been generated by evolving populations of 4096 individuals up to 200,000 generations under high mutational pressure (10^-5 mutations/site/generation).
- Each WT strain has been cloned 30 times, and evolved for 30,000 further generations.
- The 900 lineages have then been scrutinized to study the dynamics of innovation and seek for evolutionary predictability by analyzing:
  - The evolution of fitness during 25,000 generations.
  - The evolutionary trajectories (fixed mutations).
  - The local curvature of the fitness landscape along the evolutionary trajectory.

**Results (1): Virus-like genomes**

The high mutation rates lead to the evolution of virus-like organisms: small genomes, around 10 coding sequences, few rRNA overlapping genes, and almost no coding base.

**Results (2): Lineage analysis shows that innovation occurs in bursts:**
- In 178/215 innovators 80% of the fitness gain occurs in less than 20% of the time (including 68 clones that gain 80% of the fitness in a single mutational event).
- Fixed mutation are not randomly distributed along time (bursts of mutations).

**Local sampling of the fitness landscape shows that innovation bursts correspond to very specific regions:**
- Areas of low robustness (generally due to genome size increase).
- Areas of very high evolvability: innovations are triggered by mutational events that strongly increase the evolvability of lineages.

**Discussion: the combinatorics of fitness landscapes**

In compacted genomes, like viral ones, the combinatorics of point mutations is quickly exhausted. Yet, the combinatorics of rearrangements is much larger and cannot be fully explored in a reasonable time. Duplications open new paths in the fitness landscape and allows for new favorable mutations to occur, leading to bursts of mutations. Hence, the fitness landscape must be considered as more connected at large scale than at short scale. Moreover, considering only point mutations is likely to miss many important evolutionary phenomena, as large-scale rearrangements allow for evolvability bursts that lead to innovations.

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