In Silico Experimental Evolution Highlights the Influence of Environmental Seasonality on Bacterial Diversification

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Experimental evolution, where fast replicating organisms are evolved in controlled environments for thousands of generations, has shown that microorganisms are able to evolve at an amazing speed: in virtually all experimental frameworks that use bacteria or viruses, important phenotypic innovations have emerged in only a few tens of generations [1], and ecological diversifications are commonly observed [2]. Experimental evolution, by providing a variety of data from genetic mutations to ecological interactions, is an excellent tool to study multilevel evolution. Unfortunately, those experiments remain a long and costly process. As an alternative, computational models of *In Silico* Experimental Evolution (ISEE), where artificial organisms are evolved in a computer for thousands of generations [3], have already explored a lot of theoretical questions [4,5,6]. However, these models usually include only two or three scales (typically the genome, the phenotype and the environment), strongly limiting their possibility to mimic *in vivo* experiments, since evolution of real microorganisms implies the interaction of a wide range of biological structures and levels.

We developed a multiscale framework of ISEE. In this model, bacterial-like organisms own a genome encoding a genetic regulation network and a metabolic network, and evolve on a virtual medium for tens of thousands of generations. By up-taking nutrients and releasing by-products, organisms modify their environment, possibly leading to complex ecosystem evolution. Thus, our individual based model evolves complex genotype-to-phenotype mappings and fitness landscapes. This model allows us to study a large variety of questions raised by experimental evolution, e.g. the evolution of the genome and the genetic regulation network, the evolution of ecological interactions, and so on [3]. A more complete description of the model is available in [7] as well as on the EvoEvo project website (http://www.evoevo.eu).

The Long Term Experimental Evolution (LTEE), the longest bacterial experimental evolution experiment to date [8] has revealed an ecological diversification based on a niche construction associated to a negative frequency-dependent interaction [9]. By performing ISEE experiments with our model, we studied the environmental conditions in which such a diversification could occur. More precisely, we let initial random viable populations evolve during 500,000 time steps ($\sim$40,000 generations) in three different environments:

Seasonal. In this environment, the organisms grow on a unique resource. This resource is periodically provided each ~6-7 generations and the rest of the environment is rinsed at the same time. This environment thus mimics the seasonal serial transfer set-up used in the LTEE.

Continuous. Here, the organisms grow on the same resource, but provided in a continuous flow. The rinse is replaced by a small continuous degradation of the free metabolites (primary resource or metabolites released by the organisms). The continuous environment thus mimics the conditions of a chemostat.

Poisson. This environment is exactly the same as the seasonal one except that the serial passages are no more periodic. The resource is provided (and the environment is rinsed) at random times following a Poisson law.

We evolved 12 independent populations in seasonal and continuous environments, and 15 independent populations in the Poisson environment (Poisson environment is the sole one where populations got extinct during the simulation: among the 15 initial populations only 11 where still alive after 500,000 simulation steps). Importantly, the total amount of resource available is the same in the three environments.

Comparison of the evolutionary outcome in the three experiments show important differences in the structure of the population. Organisms evolving in the seasonal LTEE-like environment often split into two sub-populations that co-evolve for a very long period leading to a phylogenetic tree with two long branches. On the opposite, organisms living in the continuous chemostat-like environment or in the Poisson control environment evolve a single quasi-species. Indeed, the mean distance to the MRCA (Most Recent Common Ancestor) along the 500,000 time steps is equal to 90,198 time steps in the seasonal environment while it is "only" 18,910 and 16,703 time steps in the continuous and Poisson environments respectively. Finally, analyses of the ecological interactions between the two co-evolving sub-populations show that they correspond to two different ecotypes: Ecotype A mainly consumes the primary resource provided by the environment, while ecotype B only consumes secondary resources produced by ecotype A (and possibly by ecotype B themselves). Preliminary analyses of these two ecotypes reveal a negative frequency dependence due to the ecological interaction between A and B and on the regular refreshing of the environment. This negative frequency dependence is similar to what is observed, e.g. in the Ara-2 population of the LTEE [10].

As a conclusion, our results show that the serial transfer and its regular frequency are essential factors of long-term maintenance of the negative frequency-dependent interaction. We are now investigating more thoroughly the ecological interactions between ecotypes A and B as well as their evolutive interactions. Our objective is to investigate whether these two ecotypes could be considered as two different - though interacting - species. A positive answer would constitute a proof of concept that seasonality could trigger adaptive radiation in evolving populations.

Acknowledgement

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