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Parallel Experimental and Computational Evolution of virulence in L. pneumophila

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Biological systems are shaped by Darwinian evolution, a process constrained by both environmental and historical factors. How these factors contribute to the emergence of pathogenic traits and their spread within microbial populations is poorly understood, although it would help us to better understand and fight pathogens.
Legionella pneumophila, the causative agent of Legionnaires' disease, an acute pneumonia, is a Gram-negative facultative intracellular pathogen that thrives in warm aquatic environments. It can infect many phagocytic hosts, including unicellular protozoa, but also the macrophages of the human lung. The contact between the bacteria and humans is enhanced by man-made hot water systems, which can produce contaminated aerosols that are inhaled. However, although the genus Legionella comprises more than 60 species, only a few of them cause disease in humans. One can therefore ask which evolutionary processes have led to the pathogenicity of L. pneumophila. Since it is not transmitted from person to person, mammals are an evolutionary dead-end for the bacterium and virulence may be a side-effect of selection for other traits. It has been speculated that the interaction of L. pneumophila with protozoa generated a pool of virulence traits that allows for infection of human cells. Thus, the emergence of L. pneumophila as a pathogen reflects complex evolutionary processes of general importance, involving historical factors (here, traits selected long ago in the history of the species) and environmental factors (here, the development of new human activities).

We propose to carry out an interdisciplinary approach with both experimental and in silico evolution strategies to understand how L. pneumophila evolved into a human pathogen, and more generally to evaluate the relative contributions of the environmental and historical components of evolution. Experimental evolution uses controlled and replicated experiments to study evolution in action, and to complete the static snapshot given by genome sequencing. We designed experimental evolution strategies with L. pneumophila under conditions where we expect the virulence and/or host spectrum to rapidly evolve. The originality of our project is to combine "wet" approaches (at UMR5163 in Grenoble) and computer simulations (at UMR5205 and 5668 in Lyon), which are complementary. While "wet" experiments allow us to characterize mechanisms on real organisms, it may be difficult to generalize their results to other contexts or species. In contrast, computer simulations of generic processes allow us to extract general rules, but they are of little use if not validated on real organisms.

To directly observe the evolution of Legionella's ability to infect various hosts, the bacteria have been evolved in the absence of hosts: the project will address the question of the mutational robustness of virulence and host spectrum by focusing on whether or not the evolved bacteria will retain their infection ability after hundreds of generations without host contact. Our preliminary experiments have suggested that the ability to infect protozoa could be lost more easily than the ability to infect macrophages. This is surprising because macrophages are an evolutionary dead-end for the bacteria. Our research program will focus on four points:

- confirm this result by testing other amoeba species and mammal cell lines,
- identify the mutations and regulatory changes responsible for the phenotypic changes,

- develop computer simulations mimicking the experimental evolution to investigate several evolutionary scenarios that could explain the data, by using a computer model of bacterial evolution called aevol (artificial evolution) developed at UMR5205 and 5668 in Lyon,
- use the simulations to generalize the results and design future wet experiments.

This project involves a strongly connected interdisciplinary collaboration by a co-design of experimental evolution and in silico models. As far as we know, such an upstream co-design was never carried out in the digital evolution community. Thus it represents a challenging objective of elucidating the mechanisms of host spectrum evolution and the molecular targets of selection in L. pneumophila. Assessing the genericity of these mechanisms with computer simulations will shed light on the interplay between environmental changes and pathogen evolution. In the worldwide context of rapid evolution of lifestyles, these still unanswered questions are of utmost importance.