

Year of CfP: 2007

Project No: 07058 Completed

Project title: Understanding emergence of infectious diseases: Linking genomic evolution to the evolution of virulence of a phytovirus during the course of adaptation to its environments

Unit managing the project: BGPI (Biology and Genetics of Plant/Pathogen Interactions) (INRA, CIRAD, SupAgro)

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Research units from the Foundation's scientific network involved: LEPSE, LGDP, PROTEOMIQUE

Subthematic axes: IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*)

Objectives:

Phenotypic expression of mutations is of particular interest to our understanding of life processes especially when it concerns the evolution of pathogens. In viruses, phenotypic expression of mutations may be manifested not only in symptoms but also in the levels of viral fitness (reproductive capacity of a genotype) and virulence (decrease in host fitness). In the last several years, complete nucleotide sequences, transcriptomes, and proteomes of living organisms have become easier to acquire.

However, these techniques show an instantaneous image of a genome: No indication is given regarding the kinetics of mutations accumulation or the phenotypic expression of mutations in different environments. The study of phenotypic expression is important because natural selection acts on phenotypic expression of mutations in both constant and variable environments.

Our project proposes to fill this gap by focusing on three objectives:

- to characterize the phenotypic expression of adaptive mutations
- to evaluate how the nature of the interactions among mutations is involved in the kinetics of accumulation of adaptive mutations
- to determine how the genotype X environment interactions are involved in fixation of adaptive mutations

We will study the experimental evolution of *Cauliflower mosaic virus* (CaMV, Caulimovirus). CaMV is an appropriate biological model because we have a good knowledge of its biological cycle, and also because it presents a high evolution rate and a large host range. Viral populations will be transmitted from plant to plant during at least 200 generations (\pm 200 days) in either two homogeneous environments (*Arabidopsis thaliana* or *Nicotiana bigelovii*) or a variable environment (alternation of both species). Because CaMV is easy to manipulate (e.g. cloning, sequencing, etc), we will be able to easily determine the kinetics of accumulation of adaptive mutations, and to test their phenotypic expression when alone or in combination in all types of environments. We will work only on mutations that appeared in parallel in independent populations that were subjected to similar directional selective pressures, such as adaptation to the environment. Moreover, we will correlate the phenotypic expression of viral adaptive mutations to modifications of *A. thaliana*'s transcriptome and proteome by comparing the levels of gene and protein expression after inoculation by non-evolved or evolved viral genotypes.

Action carried-out and results obtained:

Since the viral environment is mainly governed by the host, we designed an experimental protocol in which populations of Cauliflower mosaic virus were transmitted from plant to plant in two homogeneous environments (*Arabidopsis thaliana* or *Nicotiana bigelovii*), and also in a variable environment (alternating in both species). One single viral genotype was used to found all viral populations that evolved in parallel for each of these three treatments. During the time of the contract, we designed one technique to quantify viral DNA standardized by host DNA (targeting the number of actin gene). Moreover, we designed a new technique allowing a non-destructive quantification of the deleterious effect of viral infection on the development of the plant (through the measurement of the evolution of leaf area).

After five passages from plant to plant (± 150 viral generations), we established the consensus full-length sequence of each viral populations and detected several fixed mutations in several populations. However, after ten passages, we did not observe these same mutations anymore. Moreover, evaluation of within-host viral load and virulence (i.e. reduction of leaf area due to viral infection) of viral populations that were passages from plant to plant did not allow us to reveal any particular pattern that would be a signature of adaptation (high variance within and among populations). It thus seems that mutations accumulating within viral populations are transitory. Taken all together, these results suggests that in our conditions, the effective size of populations of plant viruses (N_e) is relatively low leading to genetic drift as the predominant force compared to selection. Another hypothesis suggests that the time for accumulation of adaptive mutations is longer than spontaneous mutations and that it would be necessary to continue serial transfers of viruses in order to be able to observe contrasted patterns between the different environments.

Prospects for the future:

Evaluate the effective population size and mutation rate in different hosts

Total Agropolis Fondation funding: € 120,000 (salary for a post-doctoral fellow, running costs)

Funding categorie(s): Agropolis Fondation post-doctoral fellowship

Project duration: 1 March 2008 – 31 May 2011

Keywords: virus – adaptation – mutation – pathogen – *Arabidopsis* – phenotypic – fitness – virulence – evolution – Cauliflower Mosaic Virus