BVET: Bee Viruses Evolution and Tolerance

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Key words
Apis mellifera, Colony losses, Deformed wing virus (DWV), *Varroa destructor*, Virulence, Viruses,

Aim of the study
The aim was to study the evolution of the deformed wing virus (DWV) virulence once vectored by *Varroa destructor*, an ectoparasitic mite of the honey bee which support DWV replication.

Material and methods
The virus was obtained by reactivation of a cryptic infection in Ouessant honey bees through intra-hemocelic injection of a saline solution. Viral particles were concentrated by several centrifugation and ultracentrifugation steps from homogenized bee material before RNA extraction. 10 different part of the genome were amplified by PCR and sequenced using the Sanger method. In parallel attempts were made to amplify the whole genome of the virus in order to create a clonal population. Two strategies were followed: first the viral genome was amplified by conventional PCR to subsequently enable the synthesis of viral RNA copies in vitro; second, the viral genome was cloned and inserted in the pTOPO vector. After repetitive attempts both of these strategies did not fully deliver the expected results, and we eventually opted for the direct chemical synthesis of the viral genome, associated with a tag sequence in the helicase domain of the genome; this work is on-going and has not been finished yet.

Results and significance
We reported the sequence of a DWV isolate found on Varroa-free colonies. This strain is non-pathogenic for the bees while the DWV isolated from mites produce typical clinical signs on adult bees and is involved in colony collapse during winter. This latter might also produce ovary degeneracy in queens. Sequence comparisons between both strains revealed polymorphisms mostly located in the 5'-end of the genome. Additionally data generated from deep sequencing analyses of different bee samples in USA permitted to estimate the structure of global bee virus populations and suggest that the 5'-end region of the genome is a major virulence determinant. In order to demonstrate this, we designed a tool consisting in the production of a tagged clonal DWV population by amplification of the whole genome. Unfortunately these attempts were not completely successful and future works are needed to fully develop the technics since this will open a new area of reverse genetic to understand the evolution of virulence of DWV vectored or not by *Varroa destructor*. Alternatively we also investigated the effects of mix infection of DWV with other pathogens on life expectancy of winter workers by using the poorly known protozoa *Crithidia mellificae* as a model. This work is the first report of *Crithidia* impact on biological trait in honey bees, and it shows that this parasite is a potential pathogen equivalent to its homologous *C. bombi* in the bumble bees. As a whole our efforts in continuation to the PhD thesis of Benjamin Dainat (BVET funding 1.08.1) have largely contributed to the understanding of viral dynamics in honey bee colonies in relation with varroa infestations. The data will help to reduce the detrimental impact of bee losses in Switzerland and worldwide and will form a solid basis for hypothesis-driven research.

Publications, posters and presentations
7 presentations, 4 posters, 9 conference proceedings, 14 publications in Beekeeping journals (in I, F, D), 12 publications and manuscripts aiming at international peer-reviewed journals (including publications of works started under the BVET project 1.08.1).

Project *1.11.02*

Project duration *November 2010 - October 2012*