

The inner life of bees. Individual and systemic consequences of changes in the tripartite virus-microbiome-red mason bee (*Osmia bicornis*) interaction

Pollination is a basic ecosystem service performed primarily by insects in our latitude. Bees are best suited to fulfil this task, with over 2,000 species in Europe and over 480 in Poland. The vast majority of bee species lead a solitary lifestyle. Bees are exposed to many threats resulting in their abundance and diversity losses. **One of the main dangers for bees is the spread of diseases and parasites, especially pathogens that can coexist in different species.** The commercially bred honeybee (*Apis mellifera*) contributes to the increase in the transmission of diseases to other bee species due to its massive presence in the environment. The spill-over of pathogens between individuals of different species occurs during food collection from flowers, where infected individuals leave faeces containing viruses capable of re-infection (virulent). Female solitary bees carry pollen contaminated with viruses to the nest, where eggs and larvae of the next generation come into contact with viruses. Males also use flowers as a food source, but their possible role in virus transmission has not yet been described. **Disease-causing honeybee viruses are detected in other solitary bees. Still, it has not yet been investigated whether the viruses can multiply in another host or the effects of such infections.** It is also unknown whether solitary bees can contribute to spreading virulent viruses in the environment through their faeces. However, insects are not alone in the fight against viruses. **Through simultaneous evolution with the microorganisms living in them, they have developed cooperation with some bacteria inhabiting their digestive systems, which can limit the multiplication of viruses, thus fulfilling a protective function for their host.** Such a relationship has been confirmed in the honeybee, while in solitary bees, it remains unknown.

The goals of the project are [1] to describe how **viral infection affects the survival of male and female solitary bees** both in optimal and stress conditions, [2] to investigate whether **viruses are present in the faeces of solitary bees**, and if so, whether they are virulent and [3] to determine the impact of **virus infection on survival and development of solitary bee larvae with natural and disturbed microbiome composition.** In addition, we will develop a new procedure for determining the number of virus copies in bees using amplicon sequencing. The representative of **solitary bees** and the model organism in this project is the **red mason bee (*Osmia bicornis*)**, which is reported to have viruses pathogenic to honeybees. We will check the effects of infection with **RNA viruses** that are common almost worldwide in honeybee colonies: black queen cell virus (**BQCV**) and deformed wing virus type B (**DWV B**).

First, we will develop a new methodology for amplicon sequencing, which is necessary for further quantifying RNA viruses in this project. The first experiment **involves infecting adult red mason bees with viruses under laboratory conditions.** Bees will be individually infected by food with one of the viruses to simulate natural conditions of virus transmission, and we will keep them in cages. We will quantify the number of virus copies in the following days after infection and the survival rate of adult insects separately for both sexes. The stressor in this experiment will be the limited access of bees to food. In the second experiment, we will **use faeces collected from the bees from the first experiment to determine the number of copies of the virus and then inject the pupae of the original host, the honeybee.** We will record their mortality and the number of virus copies three days after injection. In the third experiment, we will **infect the early larval stages of red mason bees with two viruses to compare their mortality, changes in development** (time between selected larval stages), and the change in the number of virus copies during the development of the bee. In some individuals, we will **disturb the microbiome's composition** by giving them sterilised pollen or an antibiotic. **This will allow us to check whether the number of specific symbiotic bacteria significantly affects the number of virus copies.**

This project's most significant expected result is to understand the course of viral infection, determine the possibility of multiplication of viruses in other hosts and describe its impact on the life expectancy of solitary bees. The current lack of information does not allow us to conclude about the transmission effects of this type of pathogen on wild bees. The increasing popularity of the honeybee carries the risk of more frequent contact with other bee species and, thus, a greater chance of transmitting viruses. Moreover, the impoverishment of plant food sources of wild bees can lead to malnutrition and a change in the microbiome's composition, leading to greater susceptibility to infections. **Knowledge about the course of infection in wild bees is necessary to predict their effects and protect pollinating insects more effectively.** The project will result in new and relevant information on topics of interest in ecology and microbiology.