

Honey bees native to Poland traditionally inhabited forests and woodlands, mainly occupying hollows in old trees, with the number of bee colonies not exceeding one per square kilometer. Modern apiaries, located near cultivated fields, urban areas, and forests, consist of dozens or even hundreds of colonies. Throughout their evolutionary history, honey bees underwent significant natural selection, resulting in the evolution of numerous subspecies adapted to local environmental conditions. Despite attempts at regulatory measures, beekeepers indiscriminately import genetic material from various parts of the world. In combination with the natural insemination of honey bee queens in Polish apiaries, this practice leads to the hybridization of subspecies that would never encounter each other in natural conditions.

Recognizing the potential of native honey bee lines, Polish authorities implemented protective measures in the 1970s. Currently, the genetic resource protection program includes five honey bee lines (four Dark European honey bee lines and one Carniolan honey bee line). However, the assessment of the subspecies affiliation of the protected lines is still based on morphometric analyses and behavioral traits. Meanwhile, modern molecular tools allow us to trace potential historical hybridization incidents.

Currently, the main factor influencing the survival of honey bee colonies is the pressure from pathogens. Honey bees must cope with pathogens and parasites with which they had no chance to coevolve, such as *Nosema ceranae* or *Varroa destructor*. Pathogen pressure leads to significant colony losses each year, and existing methods for their detection rely on manual spore counting or inefficient and costly molecular techniques.

Another significant factor affecting the health of honey bees is their microbiome, in other words, the bacterial composition of their guts. The microbiome of bees has long been described as stable, consisting of nine main bacterial taxa. Nevertheless, recent studies indicate the genetic diversity of symbionts, which can significantly influence the adaptation of bees to local conditions and mitigate pathogen pressure. However, these studies often rely on laboratory conditions, individual bee populations, or single stressors.

In this project, I will use high-throughput sequencing of symbiont and honey bee pathogen marker genes, combined with the characterization of the genetic composition of honey bee families from historical and modern samples, to:

- I) Assess the genetic purity and potential hybridization of honey bee lines included in the genetic resource protection program.
- II) Describe and understand the interplay between microbiome and pathogens quantities and honey bee genetic backgrounds.
- III) Understand the factors influencing the survival of honey bee families across anthropogenic gradients (primeval forests, agricultural areas, cities).

Such an approach has the potential to revolutionize the prevailing paradigm of processes shaping honey bee adaptation in the face of anthropogenic pressures. Additionally, the project results will guide future strategies for protecting native honey bee lines in Poland.