

The GenoDrone project is a collaboration between two research groups from Slovenia and Poland with the goal of advancing our understanding of how Western honeybees (*Apis mellifera*) reproduce. The main focus is on addressing key challenges in honeybee mating biology, such as unique characteristics in the production of sperm, the diversity of a gene called complementary sex determiner (*csd*), and evaluating the success of mating control.

The first specific goal is to investigate the instability of the honeybee genome in sperm cells, especially in the *csd* hypervariable region. State-of-the-art techniques like single-cell sequencing will be used to develop a method for sorting individual drone sperm cells and estimating mutation rates in their genetic material before and after insemination.

The second specific goal is to develop an innovative method for assessing the success of mating control in honeybees, overcoming current limitations. This involves studying the variation in *csd* alleles in selected honeybee colonies, testing the suitability of stored drone sperm DNA in the queen's spermatheca for evaluating mating control, and creating a tool for breeders to assess mating control efficiency in a timely manner.

To achieve these goals, we will apply our expertise in honeybee breeding, selection, instrumental insemination, genetics, and advanced molecular biology methods. The project builds on the experiences of previous collaborative research projects (BeeConSel and SimDrone) from Slovenia and Poland, incorporating recent insights into the functional diversity of the *csd* gene. Using advanced sequencing technologies like next-generation and single-cell sequencing, GenoDrone aims to address important gaps in our knowledge of honeybee reproductive biology.

Apart from honeybee research, the developed techniques may have applications in other *Apis* species and arthropods with sperm storage organs. The project's outcomes can contribute significantly to comparative and population genetics research, addressing challenges associated with gaps in paternal pedigree information.

In summary, the GenoDrone project is a forward-looking initiative with the potential to revolutionize our understanding of honeybee mating biology. It aims to improve coordinated regional efforts for sustainable and effective breeding programs, as well as the conservation of local honeybee populations. The project also aims to enhance cross-border research and scientific excellence through international cooperation between Slovenia and Poland.