

Western honey bees (*Apis mellifera*) have always fascinated humans. Their mysterious nature made them a part of many myths and believes. Along with the development of science honey bees have lost their place in myths and became perfect model organisms for evolutionary studies. Nowadays they became a perfect model organism for epigenetic studies. The solely biology of this highly eusocial insect is a great example of epigenetic in action. From the same larvae could develop two extremely different castes: fertile and long-living queen or unfertile and short-living worker. What decides of fate of larvae is diet – workers larvae are fed with pollen and nectar, while queen-larvae with royal jelly. Royal jelly is a secretion of head gland of nurse bees (young workers in 5-9 day after emerging from the cell) and is rich in essential amino acids, lipids, vitamins, acetylcholine as well as in substrates for methylation activities. One of the scenarios when a new queen is raised is reproductive swarming, which is a complex, multi-step process that leads to division of the colony with an old queen leaving hive with approximately 75% of bees. Typically it occurs in mid-spring when the food source is most abundant. Preparations to such division require cooperation between thousands of individuals. Although many factors such as population size, brood nest congestion, and skewed worker age distribution have been hypothesized to triggering swarming preparations, none of them alone proved to be effective. This results may suggest that there still is an undefined critical cue that triggers swarming and is correlated with all of the mentioned factors. Moreover most of the studies focused solely on behavior, gene expression or age composition of bees initiating exodus or after swarming.

My hypothesis indicates that previously studied factors can be linked to a single factor (royal jelly) leading to change in behavior via DNA methylation. The following chain of events may explain the role of royal jelly in swarm preparations: (i) threshold of population is reached, excess of young nurses and (at the same time) brood nest congestion, leads to over-production of royal jelly; (ii) young bees start to feed each other with royal jelly via trophallaxis (mouth-to-mouth feeding), which leads to changes in DNA methylation; (iii) the queen is forced to limit egg production, therefore next generation of young bees also feed each other with royal jelly; (iv) massive queen rearing occurs.

In my study I am going to examine offspring of artificial inseminated sister queens with semen of drones from the same maternal line. It is necessary to exclude any differences caused by genetic factors. Next I will use epigenetic tools to verify differences in DNA methylation in brains of same-aged young workers during different stages of swarming preparation. The aim of this part is to define pattern of DNA methylation in brains of same-age workers that differed in behavior. After defining reference patterns I am going to check if feeding young workers with royal jelly or synergic influence of different factors (feeding with royal jelly, high concentration of CO<sub>2</sub>, lack of queen pheromones) will result with similar DNA methylation pattern as during swarming preparations.

The results of my project will provide a complex knowledge about potential influence of royal jelly on changes in DNA methylation pattern in brains of workers during swarming preparations. Moreover the results will allow to point out the triggering factor for swarming preparations. The relatively small number of genes, diet-driven phenotypic plasticity and behavioral complexity make honey bees excellent model organisms in epigenetic studies. Results of this study will allow to understand the transition from methylomes to sophisticated behaviors, which, in the future, could be linked to some mechanisms in human brain at the molecular level. Moreover results of my study could be used by beekeepers. If the diet reach in royal jelly is a key factor leading to swarming behavior, than removal of protein supply in proper time from colony could prevent it from swarming.