

Honey bees (*Apis mellifera*), like other Hymenoptera (e.g. wasps and ants) are haplodiploid organisms. This means that the males (drones) develop from unfertilized eggs and have a single set of genetic information (derived solely from their mother), while the females – double set (derived from father and mother). The molecular mechanism by which the organism translates the fact of having a single or a double set of genetic information to the development of the appropriate sex for a long time remained unknown. The matter had been further complicated by the discovery that in rare cases formation of a diploid male (having double number of chromosomes) is also possible. The solution to this puzzle was brought by the discovery of the *csd* gene (complementary sex determiner). This gene is present in multiple forms (so called alleles) which differ from each other in the sequence of a specific fragment (hypervariable region). It turns out that those bees, which have in their genome two different *csd* alleles develop as females, while those that have two identical alleles or only one - as males. The fact of the formation of diploid drones does not remain without consequences for the health of bee colonies because such males (which cannot reproduce), are eliminated by their families at the early stage of development. It should be taken into account that diploid drones are produced “by mistake”, because they develop from fertilized eggs, which, instead of developing into females, develop into organisms which are immediately destroyed. As a result, the number of females in those bee families which tend to form diploid drones is reduced. One should keep in mind that the strength and fitness of the bee colony depends on the number of females, because the vast majority of female bees are worker bees.

It is easy to realize that if very many different *csd* alleles is present in given bee population, the probability of co-occurrence of two identical alleles in a single organism is low and the impact of the formation of diploid drones on the fitness of the colony will be negligible. The probability of co-occurrence of identical *csd* alleles will increase dramatically when factors decreasing the genetic diversity of bee populations will be introduced – those factors, unfortunately, also include breeding procedures introduced by man (e.g. large-scale breeding of queen bees and their artificial insemination). Even a slight decrease in the number of bees and colonies will lead to even greater narrowing of the pool of *csd* alleles, which in turn will lead to increased inbreeding. This positive feedback loop – the “Diploid Male Vortex” – has been proposed and predicted by appropriate simulations by Zayed and Packer as a mechanism responsible for raised vulnerability of haplodiploid organisms to extinction in conditions affecting genetic diversity of the population.

To fully understand these mechanisms and predict the impact they may have on the bee population, attempts should be undertaken to estimate the number of *csd* variants and the mechanisms of the emergence and spread of new *csd* alleles in the population. In our recent work we have shown that the number of *csd* alleles has been greatly underestimated due incorrect assumptions of their distribution in the honey bee population. We have shown that *csd* alleles are distributed nonuniformly and we concluded that the reason for the observed uneven distribution is a very high incidence of mutations that due to specific features of *csd* sequence might occur during mitosis/meiosis and generate new alleles.

Our project is aimed at characterizing some the factors that influence the diversity of *csd* alleles in honey bee population. First, we will try to estimate the rate of generation of new *csd* alleles. We want to use high-throughput sequencing to genotype the eggs and sperm of previously selected and genotyped queen bees and drones, respectively. This method will allow for the detection of possible mutations, which could occur during the gametogenesis of a given single cell. We hope that the number and nature of the detected mutations will determine the rate of the emergence of new *csd* alleles and will give us insight into mechanisms responsible for their generation.

Keeping bees in environments that are populated by small number of bee colonies is likely to cause the decrease of *csd* diversity. We would like to check the severity of this effect by monitoring continuously in several years several of such colonies kept in the city environment. We also plan to analyze the honey bee population located in the area where a large apiary is present in which large-scale breeding and artificial insemination of the queen bees is routinely used. We want to estimate the impact of such procedures not only on the pool of *csd* alleles in such apiary, but also in the population of bees reared in the vicinity of the apiary.

We are also planning to create a computer model that would allow to simulate the survival of bee populations depending on a number of variables that can not always be controlled during the observation of real populations. Such a model will also allow us to predict the long-term effects of the changes introduced to the environment.

We expect that the implementation of our project will determine the mechanisms and rate of the formation of new *csd* alleles and answer questions regarding factors shaping *csd* diversity and their possible role in the survival of honey bee population. This in turn may eventually contribute to the development of more sophisticated strategies for their breeding and protection which is of great importance in the context of the recent worldwide decline of the honey bee population.