

**Sympatric and Longitudinal Comparison of Midgut/Pyloric  
Bacterial Population in European Honey Bee (*Apis mellifera*)  
and Japanese Honey Bee (*Apis cerana japonica*)**

**April 2017**

**Marko STOIC**

**Sympatric and Longitudinal Comparison of Midgut/Pyloric  
Bacterial Population in European Honey Bee (*Apis mellifera*)  
and Japanese Honey Bee (*Apis cerana japonica*)**

A Dissertation Submitted to  
the Graduate School of Life and Environmental Sciences,  
the University of Tsukuba  
in Partial Fulfilment of the Requirements  
for the Degree of Doctor of Philosophy in Agricultural Science  
(Doctoral Program in Biosphere Resource Science and Technology)

**Marko STOIC**

**Sympatric and Longitudinal Comparison of Midgut/Pyloric  
Bacterial Population in European Honey Bee (*Apis mellifera*)  
and Japanese Honey Bee (*Apis cerana japonica*)**

(セイヨウミツバチとニホンミツバチの中腸腸内細菌叢の同所的縦断的比較)

**STOIC Marko**

*Apis mellifera* honey bees are the most valuable pollinators of fruits and crops, giving a significant contribution to world's agriculture. Nonetheless, major increases in food production, alongside bacterial, viral, parasitic, and fungal diseases, have started to inflict weakening and losses in honey bee populations. To resist environmental stressors, honey bees developed mechanical, physiological, and immune defences. Alongside those barriers, antibiotics are still used against bacterial pathogens. Scarce number of studies was performed on honey bee's ability to use food sources as a mean of protection against various pathogens. Further, particular bacterial isolates, which are a part of honey bee's gut microbial community, have exhibited antibacterial activity through certain experiments. Due to those properties, gut bacteria recently gained large interest in honey bee research. Nowadays, gut bacteria are mainly surveyed in European honey bee. There is still no research of a possible sympatric effect of two bee species, kept in the same apiary, on their microbial population. Therefore, amongst previously mentioned disease threats and assorted defence mechanisms, the objective of this research was explained by focusing on antibacterial potency of chestnut honey as a food source, and defining the status of microbial population in two bee species, in the beginning and in the end of foraging season.

Four chestnut honey samples from Japan and four from Croatia, along with two non-chestnut honey samples, were tested for their antibacterial property against *Paenibacillus larvae*

bacteria, the causative agent of a highly contagious American foulbrood disease (AFB). Samples which possessed the highest amounts of chestnut pollen were characterised as a high purity chestnut honey, and those samples expressed strong, significant inhibitory properties. Within the chestnut group, certain honey samples showed noticeably better activity than the others. After comparing analysed measurements of inhibition zones caused by Japanese and Croatian honey samples, I concluded that the Croatian group inhibited the growth of *P. larvae* more effectively than the Japanese group. The results showed that antimicrobial potency of raw chestnut honey depended on its purity. Various levels of antibacterial activity were identified and varied from samples obtained from different locations. By using artificial honey, a substance made with the same ratio of sugars as in real honey, it was shown that sugar content did not influence the sizes of inhibition zones. Additionally, non-chestnut honey samples were also less effective against bacterial growth. Although pH level did influence honey's activity, the purity, based on the amount of chestnut pollen, was a significantly stronger factor in suppressing bacterial growth. Results provided in this experiment could benefit as a first step in finding an alternative approach in fighting highly pathogenic diseases like AFB.

To establish the status of symbiotic bacteria between two sympatric honey bee species, samples of worker bees from April and September were analyzed by using metagenomic analysis of 16S-rRNA gene. Only apiaries which contained European honey bee (*A. mellifera*) and Japanese honey bee (*A. cerana japonica*) were used for sampling. The part of digestive system used for this analysis was midgut and pyloric section, due to their highly dynamic environment. Metagenomic analysis of V1 and V3 variable regions of 16S rRNA gene analysis identified that in the group of non-dominant bacterial species the most abundant were *Pasteurella pneumotropica* with 31.4%, followed by *Actinobacillus pleuropneumoniae* (25.2%), *Lactobacillus apis* (8.4%) and *Chromobacterium subtsugae* (6%) With the usage of BLAST (Basic Local Alignment Search Tool) to obtain more thorough results of particular bacterial

families showed that the most dominant bacterial family was *Pasteurellaceae* (34%), with its most abundant species *Gilliamella apicola* (49%), which remained dominant in both bee species and depended on location. *Snodgrassella alvi* was the third most abundant bacteria (4%), a member of third most abundant family (*Neisseriaceae*). Its abundance as a core gut member was low in both samplings. *Flavobacteriaceae* were the second most abundant family (12%) with its member *Apibacter adventoris* (31%), mostly present in *A. cerana japonica*. Similar, but opposite situation was noticed with bacteria *Frischella perrara* (12%), a member of *Pasteurellaceae* family, which occurred mostly in *A. mellifera*. *Lactobacillus* family was present in less than 3.5% and depended only on the factor of location. All bacteria were equally abundant in the beginning and in the end of foraging season. Results of this bacterial research show that even though midgut and pyloric sections of honey bee digestive system present somewhat dynamic medium, particular specificities may be detected. Presence of *P. pneumotropica*, *A. pleuropneumoniae* and *C. subtsuage* was documented as a result of their frequent occurrence. They were not the most dominant species, yet the most detectible, non-core microbiota. Fluctuations of gut microbiota are to be expected regarding location since bees are subjected to various living conditions. Establishing certain patterns in their change may prove to be beneficial in developing certain approaches in disease prevention and immunity enhancement.

The importance of environment regarding honey bee food provision and its effect on microbial population has already been discussed. It remains to be established if foraging location could provide bees with food sources, like chestnut flowers in spring, which could help them inhibit the growth of *P. larvae*. The importance of pollen source and diversity still remains an important factor. Although the precise reason for the insignificance of season with gut microbiota still remains unclear, a possibility of constant temperature between two sample periods remains as a feasible explanation. The results of midgut and pyloric analysis showed

that *G. apicola*, bacteria connected with pollen digestion, remained the most abundant species from two sampling in both bee species. Same apiaries have expressed different trends in microbial population. Results from this survey were closer to previous results of other researchers, who found no significant shifts in observed bacteria. In this survey, only *A. adventoris* and *F. perrara* depended on their sympatric bee hosts as influential factors.

It was concluded that honey properties and bacterial abundance of different members may diverge in various samples due to location and traits in species. Chestnut honey was established effective in inhibiting the growth of a pathogen closely connected to honey bee development. Nevertheless, further actions should be continued in elucidating the exact factors, which could contribute in establishing chestnut honey in bee diet as a valid antibacterial product. This dissertation presented location as a factor that can condition changes. Various locations may contain various food sources, which can consequently influence symbiotic bacteria. Further studies should definitely confirm what particular aspects of a location contribute to the abundance of symbionts and what steps can be taken in improving the honey bee's food sources.