

A SCIENTIFIC NOTE ON THE EVOLUTIONARY RELATIONSHIPS BETWEEN HONEY BEES AND THEIR ENEMIES

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ABSTRACT. Honey bees are used in intensive way in agriculture due to their vital role in pollination of crops. Moreover, there are many valuable products from the bee colonies. Unfortunately, there are many enemies to honey bees. These enemies belong to various taxonomic ranks, including birds, insects and mites. Serious damages can be caused to honey bee colonies by these enemies. The sophisticated evolutionary relationships between honey bees and their enemies are not well investigated. In this study, phylogenetic trees between honey bees and their enemies were constructed based on the mtDNA and the COX1. The constructed trees reflected the evolutionary relationships according to behavior and taxonomical characters based on mtDNA and COX1, respectively. Predators, cavity-nesting bees, and parasites were separated than each other based on the first 1000 bases of the mtDNA. Insects were separated than mites and birds, in line with the classification of each organism based on the COX1.

Keywords: phylogeny; honey bees; hornets; Varroa; wax moths.

INTRODUCTION

Honey bees, *Apis mellifera*, are very valuable social insects to the agricultural sector. Beekeeping is currently existed in everywhere. The risk of losing bees due to many biotic and abiotic factors has been increased during the last few years. The colony collapse disorder (CCD) is the name given to the phenomena of mass losing of bee colonies. Many factors are anticipated to cause the CCD (vanEngelsdorp *et al.*, 2009; Neumann & Carreck, 2010), and so far there is no specific reason for it. In fact, honey bees are a good target to many enemies due to the presence of many individuals in a single colony beside stored honey and pollens. These enemies include species belong to birds, hornets, moths and mites.

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The bee-eater bird, *Merops* spp., is a serious enemy to honey bees (Galeotti & Inglis, 2001). This bird can impact the foraging activity of honey bees negatively. Also, this bird eats large number of bee workers, beside virgin bee queens and drones during their mating flight. Hornets are another enemy to honey bees including the oriental hornets, *Vespa orientalis*, in the Middle East and Asian countries (Khodairy & Awad, 2013; Taha, 2014, and Islam *et al.*, 2015). These hornets attack honey bee colonies and can destroy them. They eat large number of bee workers and invade colonies to feed on stored food. Similarly to bee-eater bird, they may attack virgin queens and drones during mating flight. Based on some observations, bee-eaters can attack oriental hornets to feed on them. This makes the relationships between bees, hornets and bee-eaters are very sophisticated.

Wax moths are another serious enemy to bee colonies (Ellis *et al.*, 2013). There are two species of these moths: greater wax moth *Galleria mellonella* (L.) and lesser wax moth *Achoria grisella* (F.). These moths do not feed on mature or immature stages of honey bees, but they feed on beeswax. They can destroy weak honey bee colonies, and may cause colonies absconding (Tsegaye *et al.*, 2014). They are also able to complete their development on nests of paper wasps (Abou-Shaara, 2017). Moreover, they can feed on beeswax of wild honey bees (Swamy *et al.*, 2009). The relationships between

these moths and honey bees are very complex. Currently, *Varroa* mites (*Varroa destructor*) are the major threat to honey bees worldwide. These mites feed on hemolymph of immature and mature bees (Rosenkranz *et al.*, 2010). They can also transmit diseases to honey bees and impact them passively, which cause loss and weakness of bee colonies (Chen *et al.*, 2004, Shen *et al.*, 2005 and Çakmak *et al.*, 2011). Understanding the evolutionary relationships using genetics between honey bees and their enemies can help understanding the sophisticated interactions between them. Two methods were used to construct the phylogenetic trees between them. The resulted trees were then compared to better understanding of the evolutionary relationships between honey bees and their enemies.

METHODS

Phylogenetic tree based on 1000 bases of the mitochondrial DNA

The phylogenetic tree was constructed using Jalview 2.10.3, based on the first 1000 bases of the mitochondrial DNA. The sequences were obtained from NCBI database for Western honey bees (*Apis mellifera*), Asian honey bees (*Apis cerana*) and dwarf honey bees (*Apis florea*) and their enemies: *Varroa* mites (*Varroa destructor*), greater wax moth (*Galleria mellonella*), oriental hornet (*Vespa orientalis*), and bee-eater bird (*Merops viridis*). Clustal alignment was used to align the sequences, and then the percentage identity (PID) in the first 1000 bases for the investigated species

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was used to construct the phylogenetic tree.

Phylogenetic tree based on COX1

The sequences of the cytochrome c oxidase subunit I (COX1) for the above mentioned species were obtained from the NCBI database. Then, clustal alignment was used to align the sequences, followed by constructing the phylogenetic tree by Jalview 2.10.3 using PID. The trees obtained from this and the previous methods were compared in light of the known taxonomic rank of each organism.

RESULTS AND DISCUSSION

Phylogenetic tree based on 1000 bases of the mitochondrial DNA

The constructed tree (*Fig. 1*) shows that Western honey bees and Asian honey bees are more close to each other. Wax moths, followed by dwarf honey bees and *Varroa* mites, were somewhat close to the Western and Asian honey bees. Bees and wasps (Hymenoptera) are basal to beetles (Coleoptera) and moths (Lepidoptera) (Savard *et al.*, 2006). This is in line with the present study, especially the relationship between bees and moths. Each of bee-eater and Oriental hornets were not close to the other organisms. It is clear those predators (bee-eater and hornets) were separated than the other species. Western and Asian bees are cavity-nesting bees unlike the dwarf bees. Wax moths invade hives of cavity-nesting bees to feed on beeswax. These moths were placed close to cavity-nesting bees. Dwarf bees are wild bees and do not live in cavities,

but construct nests exposed to light, and were placed away from the other two bee species. Accordingly, Western and Asian honey bees were placed in group of cavity-nesting bees, while *A. florea* in group of dwarf bees, based on sequences of two genomic regions (ND2 mitochondrial gene and EF1- α intron) (Arias & Sheppard, 2005). Also, Raffiudin & Crozier (2007) separated dwarf honey bees than cavity-nesting bees using sequences from nuclear gene (*itpr*) and three mitochondrial genes (*rrnL*, *cox2*, *nad2*). Moreover, *A. mellifera* and *A. cerana* are close to each other and were separated than *A. florea* using the complete sequence of the mitochondrial cytochrome oxidase II (COII) gene (Willis *et al.*, 1992).

Finally, *Varroa* mites were placed away from the other organisms may be due to their feeding behavior as ectoparasites on bees. It seems that the first 1000 bases of the mitochondrial DNA reflected the general behavior of the studied species. Eusocial species of Hymenoptera are classified into two major groups (one contains Apoidea and ants, the other one contains vespid wasps), based on behavior in feeding immature stages and nest construction (Johnson *et al.*, 2013). This supports the present study on the basis that species can be separated rely on their behavior. The relationships presented here is different than the classification (*Table 1*) and relationships presented by the second method (*Fig. 2*).

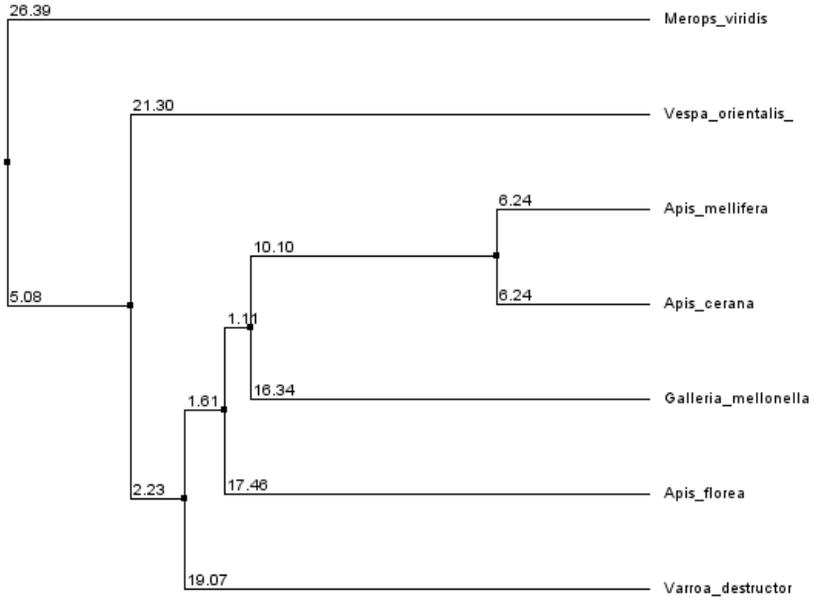


Figure 1 - Phylogenetic tree based on the first 1000 bases of the mtDNA

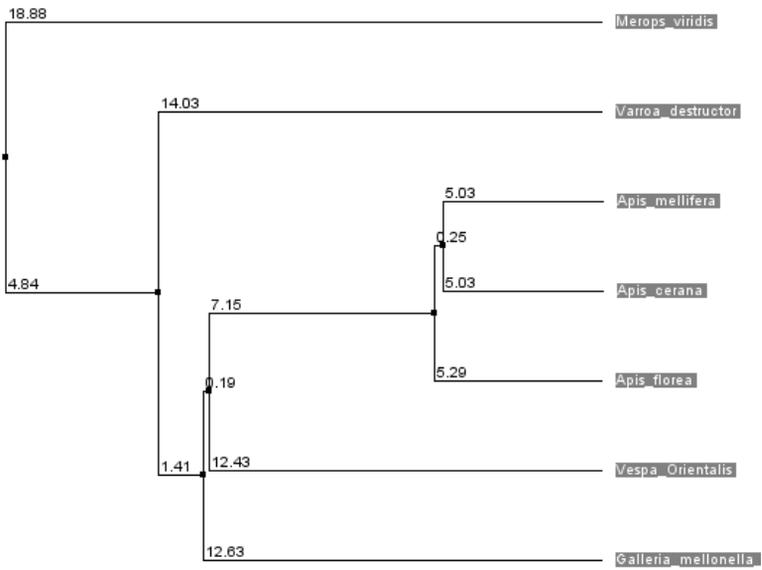


Figure 2 - Phylogenetic tree based on COX1

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Table 1 - Classification of the investigated species

Common name	Classification
Western honey bees	Class: Insecta Order: Hymenoptera Family: Apidae Species: <i>Apis mellifera</i>
Asian honey bees	Class: Insecta Order: Hymenoptera Family: Apidae Species: <i>Apis cerana</i>
Dwarf honey bees	Class: Insecta Order: Hymenoptera Family: Apidae Species: <i>Apis florea</i>
Eastern hornet	Class: Insecta Order: Hymenoptera Family: Vespidae Species: <i>Vespa orientalis</i>
Greater wax moth	Class: Insecta Order: Lepidoptera Family: Pyralidae Species: <i>Galleria mellonella</i>
<i>Varroa</i> mite	Class: Arachnida Order: Parasitiformes Family: Varroidae Species: <i>Varroa destructor</i>
Blue-throated bee-eater	Class: Aves Order: Coraciiformes Family: Meropidae Species: <i>Merops viridis</i>

Phylogenetic tree based on COX1

The tree constructed by COX1 (Fig. 2) is in line with the classification (Table 1) of studied species. Western, Asian and Dwarf bees are belong to the same order and family, and they were placed close to each other. Western and Asian bees were more close to each other than the Dwarf bees. This is in line with the previous tree, based on the first 1000 bases of mtDNA. Oriental hornet was placed close to the bees and all of them belong to the same order.

Finally, wax moths were placed somewhat close to bees and hornets, and all of them belong to the same class (Insecta). Each of *Varroa* mites and bee-eater bird were placed far away from the insects, and they belong to different classes (Arachnida and Aves, respectively). It is clear that using COX1 to construct the phylogenetic tree reflected the classification of the studied organisms.

CONCLUSION

The study presented the evolutionary relationships between honey bees and their enemies based on their behavior and classification. In light of this study, the first 1000 bases of the mtDNA were used to separate species based on their behavior into predators, cavity-nesting bees, wild bees and parasites. The sequences of the COX1 were used to build phylogenetic tree, which reflected taxonomy of the studied organisms.

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