PREVALENCE OF PATHOGENS AND OTHER ASSOCIATED MICROORGANISMS IN TURKISH HONEY BEE SUBSPECIES AND DIFFERENTIAL RESPONSES TO *NOSEMA CERANAE* INFECTION

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ABSTRACT

PREVALENCE OF PATHOGENS AND OTHER ASSOCIATED MICROORGANISMS IN TURKISH HONEYBEE SUBSPECIES AND DIFFERENTIAL RESPONSES TO *NOSEMA CERANAE* INFECTION

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Honey bees face numerous biotic threats from viruses to bacteria, fungi, protists, and mites. Here we describe a thorough analysis of microbes harbored by worker honey bees collected from field colonies in geographically distinct regions of Turkey. Turkey is one of the World's most important centers of apiculture, harboring 5 subspecies of *Apis mellifera* L., approximately 20% of the honey bee subspecies in the world. We use deep ILLUMINA-based RNA sequencing to capture RNA species for the honey bee and a sampling of all non-endogenous species carried by bees. After trimming and mapping these reads to the honey bee genome, approximately 10% of the sequences (9-10 million reads per library) remained. These were then mapped to a curated set of public sequences containing ca. 60 megabase-pairs of sequence representing known microbial species associated with honey bees. Levels of key honey bee pathogens were confirmed using quantitative PCR screens.

We contrast microbial matches across different sites in Turkey, showing new country recordings of Lake Sinai virus, two *Spiroplasma* bacterium species, symbionts *Candidatus Schmidhempelia bombi*, *Frischella perrara, Snodgrassella alvi*, *Gilliamella apicola, Lactobacillus spp*.), neogregarines, and a trypanosome species. By using metagenomic analysis, this study also reveals deep molecular evidence for the presence of bacterial pathogens (*Melissococcus plutonius*, *Paenibacillus larvae*), *Varroa destructor*-1 virus, Sacbrood virus, *Apis* filamentous virus and fungi. Despite this effort we did not detect KBV, SBPV, Tobacco ringspot virus, VdMLV (Varroa Macula like virus), *Acarapis spp*., *Tropilaeleps spp.* and *Apocephalus* (phorid fly). We discuss possible impacts of management practices and honey bee subspecies on microbial retinues. The described workflow and curated microbial database will be generally useful for microbial surveys of healthy and declining honey bees. Additionally we addressed the immune defence reactions, locomotor activity differences of five honey bee subspecies upon *N. ceranae* infection and confirmed the phylogenetic relationship between these subspecies. It was observed that honey bee immune system quickly activated defence mechanisms for all of five the subspecies after infection with *N .ceranae*, which includes the increase in the expression of genes encoding antimicrobial peptides.

Key Words: *Apis mellifera*, pollination, Colony Collapse Disorder, RNA Sequencing, bioinformatics, honey bee viruses, trypanosomes

ÖZ

TÜRKİYE' DEKİ ARI IRKLARINDA PATOJEN VE İLGİLİ MİKROORGANİZMALARIN YAYGINLIĞI VE *NOSEMA CERANAE* ENFEKSİYONUNA KARŞI GÖSTERDİKLERI FARKLI TEPKİLER

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Bal arıları virus, mantar, bakteri, protista ve akar gibi pek cok biyolojik tehdit etkisi altındadır. Bu çalışmada Türkiye'nin farklı bölgelerindeki kolonilere ait işçi arı örneklerinin patojen analizleri yapılmıştır. Türkiye dünyanın en önemli arıcılık merkezlerinden biridir. Dünya'daki tüm arı ırklarının 20% sini oluşturan *Apis mellifera* L'nin 5 farklı alt türü Türkiye'de bulunmaktadır. Arı örneklerinin taşıdığı RNA türlerini saptamak için RNA sekanslama tekniği kullanılmıştır. Bu okumaları düzenleyip arı genomu üzerinde haritalandırdıktan sonra geriye sekansın 10%'u kalmıştır. Kalanlar da 60 megabaz uzunluğundaki arılarla ilişkili mikrop türlerini oluşturan datalar kullanılarak haritalandırılmıştır. Arı patojen düzeyleri kantitatif PCR ile doğrulanmıştır. Sonuç olarak Türkiye'de ilk kez Lake Sinai virüsü, *Spiroplasma* bakteri türleri, *Candidatus Schmidhempelia bombi* simbiyontu, *Frischella*, *Snodgrassella alvi*, *Gilliamella apicola*, *Lactobacillus spp*., neogregarin, ve trypanasom turleri saptanmıştır. Metagenomik analizle *Melissococcus plutonius*, *Paenibacillus larvae* gibi bazı bakteriler, *Varroa destructor*-1 virüsü, Sacbrood virüsü, *Apis* filamentous virüsü ve bazı mantar türleri de saptanmıştır. Ancak KBV, SBPV, Tobacco ringspot virüsü, VdMLV (Varroa Macula like virus), *Acarapis spp*., *Tropilaeleps spp.* and *Apocephalus* türlerine rastlanmamıştır.

Bazı arı bakım-yönetim uygulamaları arı ırklarının içerdiği mikrop türleri üzerindeki etkileri de tartışılmıştır. Çalışmada açıklanan iş planı ve mikrobiyal datalar, sağlıklı veya koloni kaybı yaşayan arıların mikrobiyal haritalamasının oluşturulması için faydalı olacaktır. Ayrıca bu calışmaya ek olarak, 5 arı ırkının *N. ceranae* enfeksiyonu sonrası bağışıklık cevapları, lokomotor aktivitelerindeki farklar ve filogenetik ilişkileri belirlenmiştir. Tüm arı ırklarının *N .ceranae* enfeksiyonundan sonra bağışıklık sistemlerinin hızlı bir şekilde aktive olduğu ve antimikrobiyal peptitleri kodlayan genlerinin ekspresyonlarında artış gerçekleştiği gözlenmiştir.

Anahtar kelimeler: *Apis mellifera*, polinasyon, koloni çöküş sendromu, RNA sekanslama, bioinformatik, bal arısı virüsleri, tripanazom

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to my parents and my beloved friend Papish…

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LIST OF ABBREVIATIONS

ABPV Acute Bee Paralysis Virus

AFB American Foulbrood

ANOVA Analysis of variance

AmFV *Apis mellifera* filamentous virus

AMP Antimicrobial Peptides

BQCV Black Queen Cell Virus

bp Base-pair

cc Cubic Centimeters

CBPV Chronic Bee Paralysis Virus

CCD Colony Collapse Disorder

CCD Colony Depopulation Syndrome

cDNA Complementary DNA

Ct Cycle Threshold

DAM Drosophila Activity Monitoring

Df Degrees of Freedom

DNA Deoxyribonucleic Acid

DWV Deformed Wing Virus

EB Elution Buffer

EFB European Foulbrood

FDR False Discovery Rate

g Gravity

IAPV Israel Acute Bee Paralysis Virus

KBV Kashmir Bee Virus

LS Least Squares

LSV Lake Snai Virus

min Minute

ml Milliliter

mM Milimolar

mRNA Messenger Ribonucleic Acid

n Sample Size

p Probability

PCR Polymerase Chain Reaction

rDNA Ribosomal DNA

RFLP Restriction Fragment Length Polymorphism

RNA Ribonucleic Acid

RMSE Root Mean Square Error

Rsq Coefficient of Multiple Determination

RT-qPCR Real Time Quantitative Polymerase Chain Reaction

SBV Sacbrood Virus

SBPV Slow Bee Paralysis Virus

Sec Second

TRSV Tobacco Ringspot Virus

ul Microliter

µl Microliter

VdMLV Varroa Macula Like Virus

Vg Vitellogenin

CHAPTER 1

INTRODUCTION

1.1. Honey bee subspecies of Middle East

Natural distribution *of A. mellifera* L. includes Central Asia, Europe, Near East and sub-Saharan Africa and also introduced to East and Southeast Asia, Australia and the Americas (Ruttner, 1988). mtDNA variation provides important prehension into the phylogeny and biogeography of honeybees because the same mitochondrial DNA (mtDNA) are shared by the workers and drones as the queen in a colony on the basis of the information that mitochondria are inherited only from the mother. At least 29 subspecies of *A. mellifera* have been recognized and divided into four major lineages according to morphometric, genetic, ecological, physiological, and behavioral characteristics (Han et al., 2012).

(1) mtDNA group A includes *A. m. intermissa, A. m. scutellata , A. m. litorea, A. m. sahariensis, A. m. monticola,* and *A. m. capensis,* which are subspecies of African populations north and south of the Sahara (2) group M involves basicly *A. m. mellifera* and some *A. m. iberiensis* which are subspecies of western and northern European populations (3) group C consists of Turkish *A. m. anatoliaca,* Turkish and Georgian *A. m. caucasica, A. m. ligustica* and *A. m. carnica,* which are southeastern European plus northern and eastern Mediterranean populations (4) subspecies from Turkey and the Middle East composed of *A. m. syriaca,* and others belongs to the last group, group O. Within each lineage, some of the subspecies indicates variation (Ruttner et al., 1978; Ruttner, 1988; Franck et al., 2000; Arias and Sheppard, 1996). The groups C and O couldn't be categorized well and were named as C (Cornuet and Garnery 1991; Garnery et al., 1992). Mitochondrial DNA (mtDNA) and microsatellites analysis were used to confirm group O (Franck et al., 2000).

Central Iran and Caspian coast to Black Sea area, Caucasian Alpine region and Anatolia harbours seven Middle Eastern subspecies; *A. m. adami, A. m. anatoliaca, A. m. armeniaca, A. m. caucasica, A. m. meda, A. m. cypria,* and *A. m. syriaca* according to the morphometric analysis (Ruttner, 1988). *A. m. carnica, A. m. anatoliaca, A. m. caucasica, A. m. syriaca,* and *A. m. meda* are the five subspecies of *A. mellifera;* has been found in Turkey (Kandemir et al., 2000; Tunca, 2009). Most of the geographical regions of Turkey is constituted by *A. m. anatoliaca*, involving European Turkey, except for the northeastern part where *A. m. caucasica* is observed and *A. m. meda* exists in the southeastern part of the country. Thrace region of European Turkey is occupied by *A. m. carnica* (Palmer et al., 2000; Tunca, 2009; Tunca and Kence, 2011), while *A. m. syriaca* take part in south regions near Hatay (Kandemir et al., 2006).

The Carniolan honey bee (*Apis mellifera carnica*, Pollmann) is distributed from Turkey to southern Austria, Slovenia parts of Croatia, Bosnia and Herzegovina, Serbia, Hungary, Romania, and Bulgaria. Thrace region includes Carnica honeybees in Turkey (Kandemir et al., 2000). They share the same size with the Italian bee but they differ from them by the short haired and dusky brown-grey colored bodies with lighter brown stripes and 6.5 to 6.7 mm long tongue which is well adjusted for clover. Lighter colored or brown colored rings and dots can be found on their dark chitin. After the Italian bee, Carniolan honey bee is the second most popular subspecies among beekeepers for several reasons, such as resistance to some disease and parasites, gentle behaviours, ability to adjust the worker bee population to nectar availability, good spring and summer honey production (http://www.melitabees.com/carniolan.html).

A. mellifera anatoliaca inhabits across Anatolia from north to south and east to west with locally adapted ecotypes like Muğla, Giresun and Yığılca (Bouga, 2011). Their bodies are generally yellow colored but soiled orange or brown rings can be seen on their abdomen. Broader abdomens and tarsi, short legs and wings and yellow bodies with soiled orange or brown rings are the morphologic characteristics of this subspecies. They have poor ability to collect nectar but they show high adaptation to various extreme climatic conditions with high power of reproduction (Ruttner, 1988).

A. mellifera caucasica originates from Central Caucasus, Georgia and also distributed in Turkey, Armenia and Azerbaijan. In Turkey, this subspecies can be found in northeastern Anatolia, near the Georgian border, especially in Ardahan and Artvin (Kandemir et al., 2000). Chitin is dark involving brown spots and leading to grey hair. Their size and shape is the same with *A.m. carnica*. They have the longest proboscis (up to 7.2 mm) among all *A. mellifera* species (Ruttner, 1988).

A. mellifera syriaca is distributed in southeastern Anatolia, Israel, Lebanon, Jordan and Syria. They are found in Hatay in Turkey (Kandemir et al., 2000). They are the smallest honey bee subspecies in Middle East by a pointed, slender, short-haired abdomen and yellow coloration of the first three dorsal segments and a bright yellow scutellum. Although their nectar collection is very good, management of these colonies of bees is difficult owing to their high defensiveness (Ruttner, 1988). It is much better adapted to survive extreme summer temperatures in dry-hot regions without any honey flow (Al Ghzawi et al., 2001a).

Mugla and Yigilca bees are ecotypes that are less well identified and need additional investigation. Mugla bee is an ecotype of *A. m. anatoliaca* with some different traits. Because it is adapted to foraging on the scale insect *Marchalleina hellenica* on pine trees, it persists to produce brood to build up a large population in the autumn while Anatolian bees in other regions halt producing brood to prepare for winter (Doğaroğlu, 2009). Yıgılca bees are also considered to be ecotypes of *A. m. anatoliaca*, with a larger size.

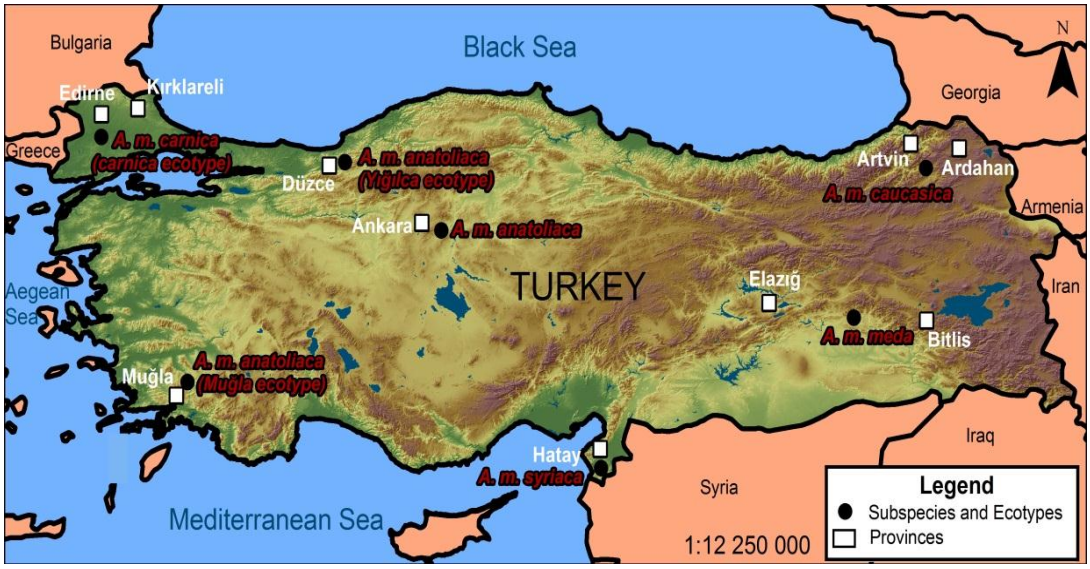

Figure 1 The provinces and the distribution of honey bee subspecies in this study (Tozkar et al., 2015)

1.2. Nosemosis

Nosemosis has been reported as an important honeybee disease that is common in most of the countries with beekeeping activities (Bailey and Ball, 1991). The phylum *Microsporidia*; are highly specialized fungi with 160 genera that consists of 1,300 species including *Nosema spp*. (Becnel and Andreadis, 1999; Weber et al. 1994). *Nosema apis* and *Nosema ceranae* are the parasites that belong to genus of *Nosema* and are known as causative agents of Nosemosis. *Nosema apis* Zander which was firstly found in *Apis mellifera* L. (Zander, 1909) was accepted as the main agent of Nosemosis. Recent studies indicated that *N. ceranae*, a species of *Nosema* originally found in the Eastern honey bees, *Apis cerana* (Fries et al., 1996), is now highly pathogenic to European honey bees with higher prevalence and replacing *N. apis* worldwide (Paxton et al., 2007; Higes et al. 2006; 2007; Klee et al 2007).

Nosema spores are transmitted via contaminated food and water or during cleaning up fecal material from infected bees or contaminated combs. These parasites exist outside the host cell only as metabolically inactive spores and disperse between hosts as spores that germinate within the midgut.

High number of spores can invade the midgut of a honeybee and multiply in a small time period after initial infection (Franzen, 2005; Bailey and Ball, 1991). Dysentery, reduction in colony size and life span and decrease in crop products and honey collection are the negative effects of *Nosema* on honey bees (Malone, Giacon and Newton, 1995).

Declines in population size, honey production, life-span and higher autumn/winter colony loss are signs of *N. ceranae* infection in honeybee colonies (Fries et al., 2006). The impacts of *N. ceranae* are different from *N. apis* related with pathology. *N. apis* was observed in midgut epithelium (Fries, 1988) and *N. ceranae* was detected in tissues such as malpighian tubules and hypopharyngeal glands but not seen in the muscles and fat tissues of infected bees (Chen et al., 2009a).

When honeybee health and survival of the colonies are considered, there are conflicting reports about the effects of *N. ceranae* infections. In Spain, *Nosema* causes colony collapse and Spanish *N. ceranae* isolates were higly virulent in laboratory infection experiments (Higes et al., 2008a).

The studies of Mayack and Naug (2009) and Paxton et al. (2007) did not support this extreme virulence. Many studies about the prevalence of *N.ceranae* did not result in collapse of the colonies while having the symptoms of nosemosis (Higes et al. 2006; Klee et al., 2007; Chauzat et al., 2007; Williams et al., 2008; Chen et al., 2008; Invernizzi et al., 2009; Cox-Foster et al., 2007; Paxton et al., 2007; Tapaszti et al. 2009, Fries et al., 2006).

1.3. Origin and current distribution of *Nosema ceranae*

The earlier detection of *N. ceranae* was reported in its original host, the Asian honeybee *Apis cerana* at China in 1994 by Fries et al. (1996). Despite its pathogenecity for *A. mellifera*, it was believed that geographic distribution of *N. ceranae* was limited to Asia where is the natural allocation of *A. cerana* (Fries, 1997). Before 1994, there wasn't much evidence for its existence out of Asia (Paxton et al, 2007, Klee et al., 2007). It has been taking place in USA since 1995 (Chen et al., 2008) and in Europe such as in Finland since 1998 (Paxton et al., 2007).

Huang et al. (2008) suggested that there aren't any transmission barriers for *N. ceranae* between *A. mellifera* and *A. cerana* by sequencing rRNA spacer regions in *N. ceranae* samples from both honey bee host species and couldn't find any differences between samples. Shortly after Higes et al. (2006) reported *Nosema ceranae* as the predominant cause of Nosemosis in Spain; the occurence of this microsporidium was also confirmed in many parts of Europe, America and Asia (Klee et al., 2007; Invernizzi et al., 2009; Chauzat et al., 2007; Williams et al., 2008; Chen et al., 2008; Huang et al., 2007; Fries et al., 2006; Cox-Foster et al., 2007; Paxton et al., 2007).

It seems that *N. ceranae* has become an arising pathogen that has broadened its distribution range via transfer from Asian honey bees, *Apis cerana*, to European honey bees by replacing *N. apis* (Klee et al., 2007). This gradual replacement process was showed by investigations of historic samples of bees infected with microsporidian spores (Paxton et al., 2007). No pure infections of *N. ceranae*, but only mixed infections (17%) and pure *N. apis* infections (83%) were observed with specific molecular detection techniques in a national survey study in Sweden (Fries and Forsgren, 2008).

Higher percentage of *N. apis* infection than *N. ceranae* infection was also observed among the bee samples from Australia (Giersch et al., 2009). This result might be a unique case for *Nosema* prevalence or result from a recent introduction of *N .ceranae* in Australia compared to the other regions in the world. Variety of climate conditions in different geographical regions might cause differences in distribution of *Nosema* (Giersch et al., 2009).

1.4. Natural and experimental infections by *Nosema*

In cage experiments, *N. ceranae* has increased virulence when injected into European honey bee individuals (Higes et al., 2007), and higher pathogenecity of *N. ceranae* causes reductions in honey yield and rising mortality in winter in field conditions as well (Higes et al., 2006). Starting from 1999 to 2005, a higher prevalence and losses during the season were found to be two characteristics of Nosemosis that were linked to *N. ceranae* in Spain (Martín- Hernández et al., 2007).

Paxton et al. (2007) also suggested a higher virulence for *N. ceranae* in comparison to *N. apis* in cage experiments.

Generally a positive link exists among parasite reproduction, high levels of virulence and increased transmission (Ebert, 1998). But fitness of the parasite may be reduced with high virulence in some circumstances if reduced transmission causes mortality. Experimental evidence using microsporidia infections demonstrated this complicated trade-off between transmisson and virulence in vivo (Berenos et al., 2009). Despite many studies from Spain claimed that *N. ceranae* lead to collapse of colonies (Martin- Hernandez et al., 2007; Higes et al., 2008a), cage experiments indicated that the parasite had no competing advantage inside the honeybee individuals. No correlation was observed between individual level virulence and colony level virulence in honey bee pathogens (Fries and Camazine, 2001).

Minor differences were seen in doses necessary for infections and duplication rates between *N. ceranae* and *N. apis*. Individual bee mortality caused by *N. ceranae* was not higher than the results of *N. apis*. When spores were given simultaneously, no competitive advantage for *N. ceranae* was observed within host in mixed infections in the study of Forsgren and Fries (2010). The high virulence of *N. ceranae* has not been defined in some other parts of the world due to some factors. One of them might be that *N. ceranae* has effects in association with different viruses and secondly different strains of *N. ceranae* might differ in virulence and show variation among regions. For example, two haplotypes with different levels of virulence were found in samples from North America, Europe and Asia (Williams et al., 2008). Bee race, the pathogen exposure time, climatic or regional discrepancies might be the other causes that influence the virulence of *N. ceranae* (Invernizzi et al., 2009).

Varying results and artificial conditions in cage experiments make them unsuitable to study the effects of infection on longevity. The finding about the higher individual level virulence of *N. ceranae* in comparison with *N. apis* (Paxton et al., 2007) needs to be proved in field colonies.

1.5. Colony collapse disorder (CCD) or honeybee colony depopulation syndrome (CDS)

Recently, there has been sudden and excessive loss of honey bee colonies (*Apis mellifera spp*.), firstly reported in Europe and then influencing other countries. In America, this situation has been named Colony Collapse Disorder (CCD) and Honey Bee Colony Depopulation Syndrome (CDS) in Europe. Depopulation syndrome or honeybee colony collapse has many economical and ecological impacts all over the world and many studies have tried to find out the reason for the incident in which strong colonies abruptly become powerless and seldomly die (Faucon et al., 2002; Stokstad, 2007). Reduced colony vigour, decreased honey production, severe mortality in winter without any observable signs, sudden loss of adult bees, poor brood attention are the outcomes of this syndrome. Colony breakdown is the final phase without any noticeable indication of a disase. *Nosema ceranae* have colonized *Apis mellifera* in both strong and unhealthy honey bee colonies at the same extent of time that this incidence has been reported worldwide and rapid dispersion of *N. ceranae* might be related with these problems (Martín-Hernández et al., 2007; Cox-Foster et al., 2007).

Higher virulence of *N.ceranae* was experimentally proved when injected into *A. mellifera* (Higes et al., 2007; Paxton et al., 2007), and natural infection showed six times higher risk of gradual depopulation syndrome with consequences of dying colonies in autumn or winter and reduced yield of honey (Martín-Hernández et al., 2007, Higes et al., 2008a). In the study of Paxton et al. (2007), *N. ceranae* and *N. apis* reached to the same equal numbers at individual level 14 days after infection and the decreasing lifespan of *N. ceranae* inoculated workers was not as striking as the results reported by Higes et al. (2006) but in the latter study no comparison was done with *N. apis* infected bees.

It has been reported that *N. ceranae* may have greater development within its host than *N. apis* at different temperatures in the environment (Martin-Hernandez et al., 2009) and additionaly *N. ceranae* places extra stress on bees related to nutrition (Mayack and Naug, 2009; Naug and Gibbs, 2009), which may cause dying foragers bees distant from the hive because of risky foraging activities. Shortage of resources (pollen and nectar) may worsen these effects of *N. ceranae* (Naug, 2009), offering a mechanistic model for CCD with habitat loss.

Researchers has not reached to an agreement about the main causes of CCD or CDS but it is believed that pathogens have a basic part in this situation (Higes et al., 2006; 2008a; Martín-Hernández et al., 2007). Many other reasons like altered in agricultural land practices (Williams, 2010; Naug, 2009), changes in the environment such as lack of food and application of pesticides) have been reported as potential effects of the honey bee colony lossess. The first report about this emergent invasive disease indicated *N. ceranae* in European *A. mellifera* as the main cause of heavy winter (2004-2005) colony collapse in Spain (Higes et al., 2006). *N. ceranae* was suggested as a colony level virulent parasite leading to colony collapse in professional apiaries in Spain in 18 months of time period (Martín-Hernandéz et al., 2007; Higes et al., 2008a).

But *N. ceranae* was recognized as a potential causative agent of CCD, not as the primary agent responsible for it in the metagenomic survey of CCD affected colonies of *A. mellifera* in the USA, although *N.ceranae* was overrepresented in the collapsing colonies (Cox-Foster et al., 2007). A similar opinion was also confirmed by vanEngelsdorp et al. (2009) and Johnson et al. (2009). According to Chen et al. (2008), *N. ceranae* have been present for more than a decade in the US. Furthermore, no clear causal link was found between the arrival of *N. ceranae* and increased colony losses in Uruguay and Germany (Invernizzi et al., 2009, Siede et al., 2008).

1.6. Biology of *Nosema*

Nosema disease affects the digestive tract in honey bees. Spores of *N. ceranae* were oval in shape and showing uniformity under the light microscope (Figure 2). By using electron microscopy, it was seen that *N. ceranae* consisted of structural characteristics of '*Nosema*' genus (Chen et al., 2009a). Spores of *Nosema* are transfered to the adult bee orally and as soon as they come to the mid-gut, they grasp the gut cells with their uncoiled polar-filament and the nucleus of the spore goes into the cell. While living as parasites in the epithelial cells that line the interior of the mid-gut, multiplication occurs rapidly resulting in more spores. The epithelial cells enable the bee to digest the pollen in its diet cell is by producing digestive enzymes. But the parasites exploit the contents of these cells and reduce the gut's efficiency in the food digestion and absorption and weaken the bee.

The cycle continues as the cell blows up and produces the new spores into the mid-gut and accumulate in rectum by passing through small intestine. Spores pass to other workers when they are shed in worker excreta. 8-20 eggs are laid by the females, and it takes 9-12 days for the parasite to complete its life cycle. A heavily infested worker bee's ventriculus can contain 30-50 million spores (Bailey, 1981) and spores pass to other bee's rectal contents during periods when workers are unable to take cleansing flights (e.g. winter) and are forced to defecate onto the comb. The spores can stay viable in honey for 3-4 months and in dried feces on combs for more than a year. Higes and colleagues (2008b) indicated an inceased viability of *N. ceranae* spores for at least 18 days. *N.ceranae* spores viability decreased significantly by freezing but were resistant to high temperature and desiccation (Fenoy et al., 2009)

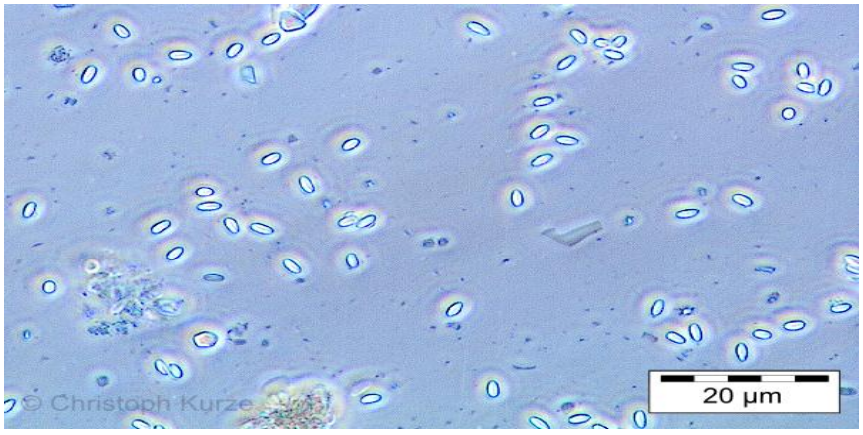

Figure 2 The oval-shaped *Nosema* spores with bright with dark smooth edges under phase-contrast light microscope

The same intracellular growth in the ventricular cells are observed for *N. ceranae* and *N. apis* (Higes et al., 2007; Fries et al., 1996;). But Chen et.al (2009a) detected the presence of *N. ceranae* in other tissues such as hypopharyngeal and salivary glands, fat bodies and malpighian tubules with PCR assay followed by sequencing. Royal jelly is secreted from hypopharyngeal glands and salivary glands of worker bees are the food sources of the queen and larvae. The detection of *N. ceranae* in these glands could indicate the horizontal transmission of the parasite via feeding and fecal contamination. The head, thorax, abdomen, and ovary tissues from naturally infected queens carried low levels of *N. ceranae* (Traver and Fell, 2012).

Although fat bodies are one of the main tissues for *Nosema* infection causing whitish and bloated gut and indicating impaired fat metabolism in some other insects, PCR signals of *N. ceranae* were weak in the fat bodies. *N. ceranae* was not detected in the muscle tissues which might result in the absence of crawling behavior in bees infected with *N. ceranae* (Sokolova et al. 2006).

Some metabolic changes are known to be triggered by *Nosema* in the host (Bailey, 1981) such as changed fatty acid structure in the hemolymph, decreased protein levels, causing a reduced hypopharengeal gland (Wang and Moeller, 1970, Malone and Gatehouse, 1998), and utilization of carbohydrates from the epithelial cells of the gut lining (Higes et al., 2007). It has reported that *N. apis* stimulates a high metabolic need and behavioral changes like increased age polyethism, deviations in task distribution among honeybees (Wang and Moeller, 1970b; Malone and Gatehouse, 1998). Behavior and physiology of honey bees may have shifts because of *N. ceranae* (Goblirsch et al. 2013; Dussaubat et al. 2013). *N. ceranae* infections lead to low levels of carbohydrate in the hemolymph of foragers. Additionally, *N. ceranae* causes energetic stress, which accelarates the hunger with an important impact on the feeding behavior of bees in the colony and alters their survival by being more prone to forage under unfavourable conditions (Mayack and Naug, 2009). Within-nest bees which were infected by *N .ceranae* were more reactive to sucrose and had less eagerness to share the food with other bees, suggesting that they had escalating levels of hunger (Naug and Gibbs, 2009). *N.ceranae* activates oxidative stress and changes hormone synthesis by raising ethyl oleate content (Dussaubat et al. 2010, 2012).

The similar effect of *Nosema* sp. and *V. destructor* on flight behavior such that infected foragers do not return to the colony, can be evaluated as a general response of honey bees to diseases to enhance the colony survival by decreasing pathogen load within the colonies (Kralj and Fuchs, 2010). Additionally, there is a relation between empty hives, acute paralysis virus (ABPV) and infection with both *Nosema* species (Cox- Foster et al., 2007).

1.7. Diagnosis of *Nosema*

Early spring or late winter is suitable for *Nosema* detection. Older bees are susceptible to infection, thus it is important to collect old bees (over 15 days old) for examination. Newly emerged ones or bees that are not older than 15 days are less prone to the disease. It is more proper to use alive bees for diagnosis, but dead ones can also be examined. Diagnosis of *Nosema* requires light microscopy, or molecular methods. Microscopic detection can be made by using the midgut ingridients or feces of the infected bees to diagnose the presence of spores (https://www.countryrubes.com).

Heavily infected bees may have whitish and bloated ventriculus (Figure 3) but there are no visible symptoms of *N. apis* infected bees (Fries, 1997) and *N. ceranae* as well. It is difficult to differentiate the two species under a light microscope although the spores of *N. apis* are a little bigger than in *N. ceranae*. The species can be seperated by using trasnmission electron microscopy based on the information that *N. ceranae* always have fewer polar filament coils compared to *N. apis* (Fries et al., 1996; Chen et al., 2009). There are many PCR based molecular protocols for the diagnosis and identification of *N. apis* and *N. ceranae.*

Figure 3 A healthy midgut which is brownish yellow in colour and *Nosema* infected midgut which is white in colour as a result of the accumulation of spores (Obtained from www.countryrubes.com).

The pathological consequences of *N. ceranae* in *A. mellifera* are different in comparison with *N. apis* and are not known well ,thus the virulence of *N. ceranae* causing colony depupulation should be described in European races of *A. mellifera* (Higes et al., 2006).

1.8. Transmission and infection of *Nosema*

There are several factors for the rapid and long distance occurence of *N. ceranae* . Transportation of the infected bees by beekeepers (Klee et al., 2007) and transfer of spores via hive structures are the main factors (Van der Zee, 2009). Within the hive, the infections are spread by defecation of diseased bees. Comb exchange between hives and apiary sites, feeding bees with contaminated honey, splitting and uniting contaminated hives enables the extent of *Nosema* from hive to hive.

Corbicular pollen and commercial royal jelly can also transmit *N. ceranae* spores (Cox-Foxter et al., 2007). The impact of time on the viability of *N. ceranae* spores in the hive needs to be investigated. Although *N. ceranae* viability was reduced significantly by freezing, viable spores were found in pellets which were thrown up by the bee eating bird *Merops apiaster* which showed the viability of spores of this parasite over long distances by flying behaviours of bee-eaters (Higes et al., 2008b; Valera et al., 2011).

The other factors contributing spore transmission are swarming, robbing, drifting, cleaning up the crushed bees with *Nosema* during housekeeping activities, infected package queen with worker companions. *Nosema* spores can exist in vectors like beekeeping equipment, honey, wax, etc. and spread quickly during normal hive / colony manipulations. (Edinburgh and Midlothian Beekeepers' Association, 2009).

The occurrence of *Nosema* disease varies throughout the year. The level of infection increases rapidly in early spring and decreases gradually in late spring. Unsuitable weather conditions restrict the flights of bees, thus bees clean feces from the combs at the beginning of the spring when brood rearing starts. Infections start to decrease in the late spring, because infected bees begin to fly and excrete outside. Newly emerged, nosema-free bees replace the old ones which die off. The combs become cleaner and the infection is not observed in summer but there is always a risk of reappearance of some infection within an untreated colony in upcoming spring (https://www.countryrubes.com).

Defecation as yellow or yellowish brown signs inside the hive is one of the symptoms of *Nosema* disease. Digestive tract injury of the bee leads to dysentery and makes the bee weaker, minimizes the fruitful life of the worker, and lessen its performance to produce brood food and digest food. As a result, colony growth and production of brood is influenced in earlier season (Figure 4). By using the epithelial cell and its enzymes to reproduce, the parasite deprives the bee of protein from digested pollen. The development of the hypopharyngeal glands and production of brood food is inhibited by the lack of the protein. Also reduction in the fat bodies of winter bees and reduction in the amino acid content of the haemolymph lead to a reduced life span of bees.

Infected bees may excrete in the hive unlike healthy workers because the can not fly or can only fly shortly outside the hive. Reduced egg production and life span of infected queens result in supersedure.

The rapid replacement of infected queens may be the result of *N. ceranae* disease that affects the physiological activities and modifies pheromone synthesis of queens (Alaux et al., 2011).

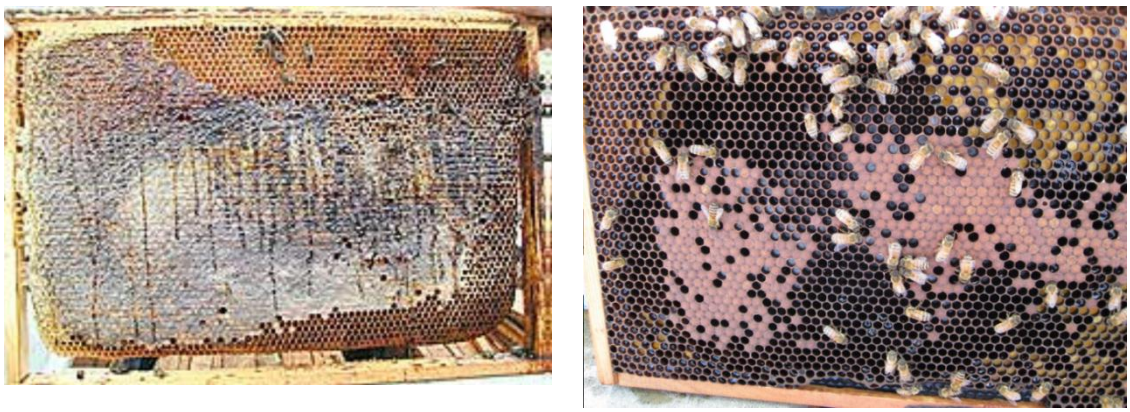

Figure 4 Dysentery symptoms on a comb caused by *Nosema* (left). A colony infected with *Nosema* including a few number of adult bees (right). (Photos obtained from www.countryrubes.com).

Diseased colonies usually have increased winter losses and can't increase the number of the population in spring with decreased honey production.

Sick bees crawling with their wings spread out or a mass of dead bees may be observed on the ground in front of the hive in spring. Some characteristic signs of *Nosema* such as crawling bees and dysentery may not be observed by *N .ceranae* infections (Higes et al., 2008a). More usually, reduced longevity, gradual depopulation (rather than fast-acting, short-duration syndrome) less honey yield and increasing winter-autumn losses are signs of *N. ceranae* (Fries et al., 2006).

1.9. Management of Nosema

Numerous control methods exist for *N. apis* in western honey bees, including heat treatment (Cantwell and Shimanuki, 1969), fumigation (Bailey, 1957), and replacement of aged equipment (Fries, 1988). Treatment and management of the disease *N. ceranae* is the same as that described for *N. apis*. Management includes the steps of hive examination, monitoring, controlling and apiary housekeeping. Examination of hive components and debris is important especially in spring to check for the abnormalities in the colony. Monitoring of the apiaries and colonies should be done regularly and examination of the dead colonies should be made to prevent robbing and spread of any disease present. Transferring the infected colony on to clean comb and disinfection of comb and hive parts is satisfactory. Comb exchange between colonies without disease check and introducing equipment or colonies without knowing their origin and disease status should be avoided. Acetic acid can be used for sterilization of contaminated equipment and the antibiotic Fumidil 'B' (dicyclohexylammonium fumagillin) can be used for preventing *Nosema* in early spring and autumn to inhibit reproduction of spore in bee gut but it is not effective to kill the spores. If overwintering bees receive antibiotic, the occurence of *Nosema* can become less in the following spring. (www. capabees.org/).

Fumagillin is effective in controlling *N. ceranae* infections (Williams et al., 2008). In some regions of the world, *N. ceranae* disease is treated by the major commercial medication fumagillin but in most parts of Europe except Spain, usage of antibiotics are banned (Fries, 2010). Treatment of fumagillin help the colonies to survive and all untreated colonies were dead within 15 months among ten colonies with endemic *N. ceranae* infections (Higes et al., 2008a).

Usage of the antibiotic fumagillin with sugar syrup during fall feeding and additional spring application in spring is the most common method for *N. apis* treatment in Canada and United States (Furgala and Gochnauer, 1969). Lower *N. apis* intensities were detected after medication with fumagillin during the following spring but there were no differences in survival profile with different concentrations of fumagillin (Szabo and Heikel, 1987a). In the bumble bee *Bombus occidentalis* fumagillin was not effective against the closely related species *N. bombi* (Whittington and Winston, 2003).

After giving 190 mg fumagillin (recommended dosage for *N. apis*) per colony for each *N .ceranae* treatment, no variation was observed among beekeeping operations (Williams et al., 2008, Williams et al., 2010).

Brood comb should be replaced on a regular and planned basis and 'frame change' techniques can be used in heavily infected colonies. As soon as the colony grows stronger and returns to the normal state after the infection, dead brood will be removed from the cells by the bees. The best method of controlling step is prevention by sustaining healthy and strong colonies with better hygienic tendencies. Placing hives at sunny sides instead of wet and damp areas with well-ventilation, preventing excess moisture within the hive during the winter, establishment of newly emerging bees that are *Nosema*-free, supplying adequate amount of honey and pollen during bad weather conditions and destroying the the bees and inner equipment with severe infection by burning are the precautions that should be held to prevent spread of *Nosema*. Fumigation with 60-80% acetic acid vapour can be used to disinfect the combs from *Nosema* spores which can survive more than one year. Heating the contaminated honey to 60ºC for 30 minutes provides disinfection. Queens can become infected with *Nosema* thus queen replacement should be considered from healthy and disease resistant colonies (https://www.countryrubes.com).

Thymol and resveratrol usage is an alternative way to control *Nosema* disease without being harmful to honey. Despite the fact that toxic effects can be observed in honeybees with high concentrations of thyme essential oil, bees which were fed by thymol and resveratrol candies had reduced *Nosema* infections and had prolonged life with resveratrol prepared candy application (Maistrello et al., 2008).

It was also reported that thymol supply was not toxic to adult honeybees (Ebert et al., 2007) and may restrain *Nosema* disease in honey bee colonies (Yucel and Dogaroglu, 2005, Rice, 2001).

1.10. Antimicrobial peptides in honeybee defense mechanism

Insects use behavioral, physiological, physical and immune defenses to get rid of the pathogens (Evans and Spivak, 2010). Cuticles and the membranes covering the digestive tract and changes in gut chemicals and pH (Crailsheim and Riessberger-Galle, 2001) are the first mechanical barriers to prevent infectious agents to enter and invade.

Cellular and humoral immunity occupy the second defence step (Boman, 2003; Lavine and Strand, 2002). Cellular immunity includes encapsulation, phagocytosis and melanization (Osta et al., 2004). Third way of innate immune response is the activation of prophenoloxidase (proPO) by a serine protease cascade resulting in the synthesis of melanin and quinones (Cerenius et al., 2008). Eater is the main receptor which recognizes and phagocytes bacteria in *Drosophila* (Kocks et al., 2005; Ertürk-Hasdemir and Silverman, 2005). Humoral immunity involves the production of antimicrobial peptides (AMPs) synthesis in response to pathogens (Evans et al., 2006). Four antimicrobial peptides; abaecin, apidaecin, defensin and hymenoptaecin are synthesized against a wide-spectrum of microorganisms (Casteels et al., 1989, 1990, 1993). Honey bee vitellogenin (Vg) is a protein that regulates immune functions and lifespan of honey bees and has a function in reproduction (Amdam et al., 2004). Division of labour, foraging specialization and combating with oxidative stress has been related to Vg that is produced in the fat tissue of the abdomen and transported to the ovaries and other tissues through haemolymph (Amdam and Omholt, 2003; Nelson et al., 2007, Corona et al., 2007).

Antimicrobial peptides are one of the evolutionary ancient protector mechanisms of invertabrates. Four antimicrobial peptide gene families with different functions in the innate immune system were described in *A. mellifera*. Apidaecin is the most conspicuous component against invading pathogens with strong antimicrobial activity to gram-negative bacteria by inhibiting enzymatic activities necessary for parasite replication.

Unlike defensin, abaecin and hymenoptaecin genes it doesn't need sizable experimental infections to be activated transcriptionally and it can be overproduced with its unique structure (Casteels-Josson et al., 1994). Abaecin is another main antimicrobial peptide which is active against gram-negative and gram-positive bacteria. Although it is weaker than apidaecin, abaecin can function as a backup and can affect some bacteria that has apidaecin resistance (Casteels et al., 1990). Hymenoptaecin prevents fungi, gram-negative and gram-positive bacteria and acts complementary to apidaecin by constraining the development of certain gram-negative bacteria that has apidaecin resistance (Casteels et al., 1993). The defensin peptide is slightly produced and have a significant function in the later stages of infections specifically targeting and lysing gram positive bacteria directly. Apidaecin and hymenoptaecin genes with the absence of abaecin end defensin were found in *A. mellifera* adults under slight exposure of pathogens in the wild. The important roles of apidaecin and hymenoptaecin peptides in suppressing or killing varying pathogens in the innate immune system of the honeybees was proved with this finding (Casteels-Josson et al., 1994).

There are many activation mechanisms of AMP synthesis in gut epithelial cells, hemocytes and adipocytes. Hemocyte mediators such as prostaglandins (Stanley-Samuelson, 1994) or endocrine system mediators (Glupov, 2001) can activate adipocytes and hemocytes. Phagocytes with absorbed microorganisms can trigger the AMPs synthesis by attaching to the adipocytes (Glupov, 2001). Four immune pathways – the Toll-pathway, the IMD-pathway, then JNK-pathway and the JAK/STAT-pathway have been described in the *A. mellifera* genome (Evans et al., 2006) and respond to *Nosema* infection (Antúnez et al., 2009). Protection against fungi and gram positive bacteria is mediated by Toll pathway and protection against gram negative bacteria is provided by IMD pathway (Hoffman, 2003; Evans and Spivak,2003)

1.11. Locomotor activity rhythms in honeybees

Circadian locomotor activity rhythms of individuals make contributions to the colony organization of honeybees (Fuchikawa and Shimizu, 2007a, Shimizu et al., 2001). In the first 2 to 3 weeks of adulthood, nurse bees give regular care for the brood and provide other tasks in the hive arhythmically and then switches to foraging for pollen and nectar out of the hive for the remaining four to seven week adult life with a well established internal circadian clock. Strong zeitgebers such as daily fluctuations in light and temperature are encountered by foragers than nurse bees because foragers consume more time out of the thermoregulated and dark hive (Bloch, 2001). Increased levels of 'clock' gene *per* mRNA in the brain of foragers were detected and the bahaviour of foragers were rhythmic. Additionally, low levels of *per* mRNA were recorded for young bees both with and without circadian rhythms of locomotor activity in the laboratory (Bloch, 2001). Arrhythmic behavior of foragers which shifted to brood care were observed under field conditions (Bloch and Robinson, 2001), but Bloch (2001) indicated that they were prone to have augmented *per* mRNA levels in the brain. There is simultaneous occurrence between daily floral rhythms and bee behavior, foraging behavior of honeybees occur whenever pollen and nectar levels are higher. They have preference to stay in the hive at remaining times to save energy levels and not to be weakened on non-fruitful foraging flights. They place themselves to rest in private locations of the hive away from the active bees on the floor (Moore, 2001). According to the examinations inside the hive and the locomotor activity, activities of young bees are based on the social structure like division of labor that influence their physiology, behavior and regulate their circadian rhythms in young bees. Activity 'around-the-clock' is associated to brood care and *per* mRNA expressions in the brain differ in foragers but not in nurse bees along the day. Rhytmic behaviors in isolated individual bees have been measured with different protocols both in natural and artificial conditions (Shemesh et al., 2007).

1.12. Honeybee pathogens and other associated microorganisms

The honey bee (*Apis mellifera* L.) has ecological importance as a natural pollinator of wild flora and crops. Moreover, managed honey bees have economical importance with hive products including honey, pollen, wax, propolis and royal jelly (Maheshwari, 2003). Recently, declines of managed colonies have been noted on many continents. Several causes of these large-scale losses have been reported, including honey bee parasites (*Varroa destructor*, *Acarapis woodi*); pathogens (*Nosema spp*. and bee viruses); pesticides, contaminated water, use of antibiotics, poor nutrition, and migratory beekeeping practices (Bacandritsos et al., 2010; Higes et al., 2008a; Naug, 2009; vanEngelsdorp and Meixner, 2010; vanEngelsdorp et al., 2009; Kevan et al., 2007). The high density of individuals and the exchange of food among *A. mellifera* colony members create a favourable environment for bacterial, viral, fungal, and protist pathogens and several studies have noted an increase in diversity and infection rates of pathogens in failing bee colonies. Here we will focus on honey bee pathogens and parasites and the use of modern sequencing techniques to identify these agents in healthy and declining colonies.

Among the honey bee pathogens, viruses are of special concern. Viruses are widespread in honey bees although most often without noticeable symptoms (Ball and Bailey, 1997). Multiple viral infections have been diagnosed in many bee colonies (Chen et al., 2004). At least 18 different viruses exist in honey bees (Bailey and Ball, 1991) with six of them; Sacbrood virus (SBV), Deformed wing virus (DWV), Acute bee paralysis virus (ABPV), Black queen cell virus (BQCV), Chronic bee paralysis virus (CBPV), and Kashmir bee virus (KBV) most commonly linked to bee disease. These viruses have strong impacts on managed bee populations, pollination services, and honey production on several continents (Allen and Ball, 1996; Nordstrom et al., 1999; Ellis and Munn, 2005, de Miranda et al., 2010). DWV and ABPV have been linked to parasitic mite loads, while Chronic bee paralysis virus (CBPV) and the related Lake Sinai viruses are also widespread and tied to significant losses in honey bee colonies (Runckel et al., 2011; Ravoet et al., 2013). Sacbrood virus is the only common virus of developing bees, and this virus is not generally implicated in adult bee mortality or morbidity (Anderson and Gibbs, l989).

Two species of Microsporidia, *Nosema apis* and *Nosema ceranae*, are widespread parasites of adult honey bees. *N. apis* is a long-standing infection agent of the European honey bee, *A. mellifera*, (Zander, 1909) that causes nosemosis, a disease with mild virulence. *N. ceranae* was first found as a parasite of the Asian honey bee, *Apis cerana* (Fries et al., 1996) although this species is now widespread throughout the range of *A. mellifera* (Fries et al., 2006, 1996; Paxton et al., 2007) arguably thanks to worldwide trade in bees (Klee et al., 2007) and perhaps pollen supplements. Honey bees can be co-infected with both *Nosema* species (Fries, 2010). Additionally, *N. ceranae* seems to be replacing *N. apis* worldwide (Klee et al., 2007). In association with *Nosema* infections, several viruses can significantly affect the apparent virulence of *Nosema* (Bailey and Ball, 1991).

Trypanosomes are a varied group of parasites that infect insects (Merzlyak et al., 2001). *Crithidia bombi*, a trypanosome that infects bumble bees, has effects on behaviour (Gegear et al., 2005) and longevity during stressful conditions (Brown et al., 2003). Trypanosomatid parasites parasitize the mid- and hindguts of their hosts (Lange and Lord, 2012) and can be widespread (Langridge and McGhee, 1967; Schmid-Hempel and Tognazzo, 2010). The current role of trypanosomes in honey bee health is not clear (Schwarz and Evans, 2013), although they have been recognized as possible correlates with bee declines in two field surveys (Runckel et al., 2011; Ravoet et al., 2013). The most common honey bee trypanosomatid currently is distinct from the species *Crithidia mellificae* described by Langridge and McGhee (1967), and has recently been named as *Lotmaria passim* (Schwarz *et al.*, 2015).

Spiroplasmas are particularly virulent pathogens that are found in various environments and implicated as pathogens of plants, vertebrates and insects. They have a seasonal occurrence associated with the nectarines and surfaces of flowers (Markham and Townsend, 1981; Williamson et al., 1989). Adult honey bees are parasitized by two species of bacteria, *Spiroplasma apis* (Mouches et al., 1983) and *Spiroplasma melliferum* (Clark et al., 1985). Upon invading the hemolymph, these bacteria can cause a fatal disease called spiroplasmosis or May disease. Gut microbiota of animals living in social communities may influence their health with their functions related to nutrition, immune responses and resistance against pathogens (Dillon et al., 2004, Round et al., 2009).

Surveys of 16S rDNA sequences from the honey bee indicate the presence of eight predominant species (defined as strains sharing >97% 16S rRNA identity) which account for 95% of the resident bacteria (Moran et al., 2012). These species include the beta-proteobacterium *Snodgrassella alvi* (family *Neisseriaceae*) and the gamma-proteobacterium *Gilliamella apicola* (family *Orbaceae*), the dominant Gram-negative members of the gut community, with each comprising up to 30–39% of the microbiota (Moran et al., 2012).

1.13. Metatranscriptomics analyses of Turkish honeybee subspecies

The surveillance and discovery of novel pathogens via high-throughput sequencing can provide a relatively unbiased view of pathogens and microbes associated with insects and other arthropods (Vayssier-Taussat et al., 2013; Bishop-Lilly et al., 2010; Ma et al., 2011). Recent efforts based on these technologies have uncovered novel and unexpected taxa associated with honey bees (e.g., Cox-Foster et al., 2007; Runckel et al., 2011; Cornman et al., 2013;) and have provided estimates of normal microbial levels versus those of diseased colonies. Turkey is one of the World's most important centers of apiculture, with managed and wild populations of 5 subspecies of *Apis mellifera* L. including *A.m caucasica*, *A.m syriaca*, *A.m anatoliaca*, *A.m meda* and an ecotype in Thrace belonging to the carnica subspecies group which is distinctly different from the subspecies found in Anatolia (Figure1) (Kandemir et al., 2000; Bodur et al., 2007; Tunca, 2009; Tunca and Kence, 2011). These subspecies cover approximately 20% of honey bee subspecies in the world. Our purpose here was to determine the regional prevalence of bacterial pathogens, viruses, fungi, parasites, protists, and symbionts and to compare their loads in areas where migratory and stationary beekeeping is practiced in Turkey. Monitoring viruses and other disease agents can help solve problems related to the health of stationary and migratory honey bee colonies and limit or avoid their spread. Although commercial migratory beekeeping practices are necessary for pollination and crop production, their effects on honey bee colony health and pathogen transmission should be addressed. We used transcriptome analyses to survey a wide range of pathogens and to detect unexpected or rare taxa.

RNA-seq technology allows the precise detection of rare transcripts by mapping reads against inclusive sequence databases, reducing the repetitive effort and possible biases of conventional molecular diagnostics (Minoche et al., 2011). Identification and confirmation of select virus and pathogen loads was confirmed by quantitative RT-PCR. Our survey was aligned with the European Honey bee colony loss network (www.COLOSS.org) survey, and was conducted to determine the colony losses in many regions representing honey bee diversity in Turkey. By using metagenomic analysis, our results provide new incidence records of the virome and microbiome in search of etiologically unexpected or previously unknown agents among 10 distinct provinces in Turkey and suggest a higher viral prevalence, and increased losses, in migratory beekeeping operations. *Apis mellifera* is an economically and ecologically important model organism and identification of pathogens and other microbes can have extensive implications for current practices in apiculture and agriculture.

CHAPTER 2

MATERIALS AND METHODS

2.1 Sampling for metatranscriptomics analyses

Colony loss surveys were carried out in 8 regions and 158 beekeeping operations in 2010. Among those, 98 were migratory beekeepers and 60 were stationary. In 2011, surveys of 221 beekeepers from 7 different regions were evaluated for this study. Samples analyzed for microbial loads comprised adult honey bees collected from field colonies in Turkey during summer and fall of 2010 and 2011. Efforts were made to collect from different regions of Turkey, and from a diverse set of beekeepers. Adult bees were collected from 134 colonies from different regions of Turkey from 38, 48, and 51 beekeepers in 2010- Fall, 2010-Spring, and 2011 respectfully and the samples were kept frozen until molecular diagnosis.

2.2. Sampling of worker bees for *N. ceranae* infection experiments

Frames of sealed brood were obtained from 10 healthy colonies of *A. m. carnica, A. m. syriaca, A. m. caucasica* subspecies, Mugla and Yigilca ecotypes of *A. m. anatoliaca* located in a common garden in the Middle East Technical University. Two colonies from each subspecies were used in this study. The brood (Figure 5) was incubated at 34 ± 1 °C 55 % humidity. Newly emerging bees from each colony were examined under light microscopy to show that they were *Nosema*-free

Figure 5 Healthy capped brood (with some uncapped cells) with honey bees (Obtained from cookevillebeekeepers.com)

2.3. *N. ceranae* inoculum preparation

The midguts of 20 heavily infected live bees were removed by using forceps. They were smashed in 1 ml of distilled water (or molecular grade water) in eppendorf tubes and centrifuged at 6000 or 10.000 rpm for 5-10 minutes. Then supernatant was removed and only the pellet was used. Pellet was dissolved in 1 ml water and the spores per microliter was counted by using light microscope.

2.4. *N. ceranae* spore count

The spore count was done with a Neubauer hemacytometer counting chamber (Figure 6). The spore suspension was added to the chamber and covered by a coverslip. Capillary action took place for the area under the coverslip to be filled. After waiting for the suspension to settle down, 40X objective was used to focus on the counting chamber.

Rulings covered 9 square millimeters. The central square composed of 25 middle sized square groups including 16 small squares. Triple lines separated each group and the middle line was the boundary. The volume of each 16 small squares is 0.00025 mm^3 because the ruled surface is 0.10 mm below the cover glass (https://www.mannlakeltd.com).

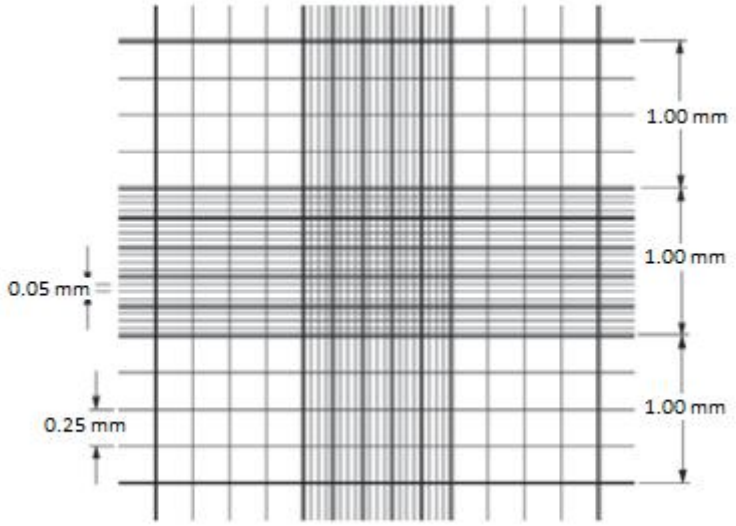

Figure 6 Neubauer hemacytometer ruling (Obtained from www.mannlakeltd.com)

All the spores were counted in the block bounded by the double lines. Also the spores were scored that cross the double lines on the upper or right side but the ones that were on the lower or left side weren't counted. 5 blocks of 16 squares were counted to maintain a good average.

2.5. *N. ceranae* infection experiments

Plastic hoarding cages with 25 cc volume, plastic pasteur pipettes to feed the bees with sugar solution, 10 and 20 µl pipettes to feed each bee with 5 µl sugar solution with/without *N. ceranae* spores, vortex for mixing the sugar solution with *N.cerenae* spores prior to infection trials and incubator that was adjusted to 34 ± °C were the materials used in the infection experiments.

One day old bees couldn't fly and sting, thus it was easy to handle them. One day after eclosion, each of the honey bees were inoculated with 5 µl of sucrose solution containing *N .ceranae* spores by touching a micropipette to their mouthparts until the entire droplet was consumed. 10.000 (10K) spores per bee which is a standard minimum dose (Fries et al., 2013) that has been shown to produce infection (Forsgren and Fries, 2010; Suwannapong et al., 2011, Goblirsch et al., 2013) were used for inoculations. The bees that did not consume the entire droplet were discarded.

Control bees consumed 5 µl of sucrose solution without *N. ceranae* spores. Workers from 10 colonies (n = 20 for each colony) were placed in plastic hoarding cages (Figure 7), which had a feeder (sterile pasteur pipette or 15 ml falcon tubes) containing 1:1 ratio of sucrose solution. All cages were kept in an incubator at 34 ± 1 °C with 55 % humidity.

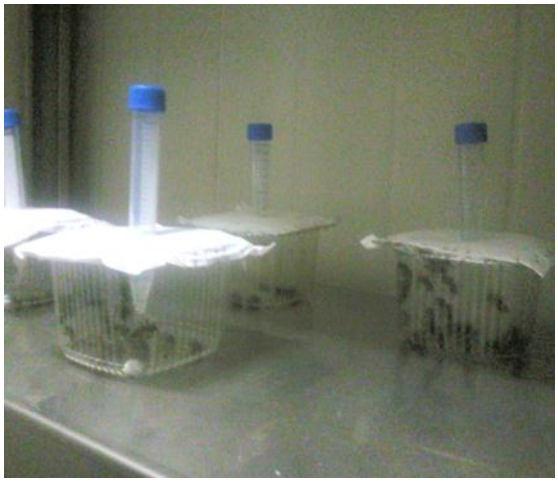

Figure 7 The plastic containers where the honey bees were kept for the experiments

2.6. Monitoring locomotor activity of *N .ceranae* infected honeybees

Drosophila activity monitoring (DAM) system was used to measure locomotor activity of 5 different subspecies of the honey bee, *Apis mellifera* L. for 12 days following the infection by *N .ceranae*. Activity monitoring system (Figure 8) is simple but experimentally operative and it has a high resolution. Each unit of the activity monitoring system has 32 independent activity channels which measure activity by using three infrared beams and sensors to guarantee that recordings are accurate. Every single bee was placed into the activity monitor in a 15 ml falcon tube. The movements of the honey bees were sensed via the infrared irradiation source around the middle portion of the tube. These movements were recorded by the computer. The activity monitor can take measurements with 1 sec., 2sec, 3 sec, 4sec, 5sec, 6sec, 10sec, 12sec, 15sec, 20sec, 30sec, 1 min, 2 min, 3 min, 4 min, 5 min, 6 min, 10 min, 12 min, 15 min, 20 min, 30 min and 60 minutes intervals. The measurements were made with 1 minute intervals in the experiments. The times that the honey bees had passed through the middle part of the tube in every minute was recorded.

The monitoring period outlasted for 24 hours (1440 minutes). Thus everyday 1440 movement data were recorded for every bee. The data were recorded by the TriKinetics Inc.'s DAM System v3.03 software of the computer that's connected to monitors (www.trikinetics.com).

Figure 8 Activity monitoring system

Other than the 4 activity monitors, also an environment monitor is connected to the computer to control light, temperature and humidity in the incubator. Throughout the monitoring period, the temperature, humidity and the light amount were recorded in every minute. The monitoring experiment was done in the incubator which provided identical conditions of the natural environment of the honey bees with 34 ± 1 °C with 55 % humidity in constant darkness. Any unusual change in experimental conditions could be determined by the environment monitor.

The activity of a total of 120 honey bees, 60 of which were controls, were recorded for 12 days. 12 bees from each of 5 subspecies (*A.m. caucasica*, *A.m. carnica*, *A.m. syriaca*, Mugla and Yigilca ecotypes of *A.m. anatoliaca*) were included in monitoring experiments. Controls and *N. ceranae* infected bees were distributed into monitors randomly. The bees were fed with a candy that was filled in the cap of the 15 ml falcon tube and the candy was covered with a cheese-cloth to ensure that only mouthparts of the bee would have access to the food, thus preventing the insect from getting stuck to the food.

Four holes were made on every tube to provide air circulation. After the monitoring period, the whole data was analyzed statistically with JMP™ (SAS Institute, Cary, NC, USA, v.9).

Trikinetics DAM system, originally generated for measuring locomotor activity in fruit flies and also facilitated high-throughput measurements of circadian rhythms in different honey bee groups. The commercial availability, cost-effectiveness, and integrated nature of this *Drosophila* activity monitoring (DAM) facilitates to measure the locomotor activity and circadian rhythms that regulates physiological and behavioral processes such as stages of sleep, spatiotemporal learning, sun-mediated navigation, time perception, reproduction, mating, and division of labor (Moore and Rankin, 1985; Goodwin and Lewis, 1987; Bloch et al., 2001). In *Drosophila*, genetics of thousands of mutant lines were screened by the existence of a simple, easily modified, low-cost and commercially available system for measurements of circadian locomotor activity and this easily modified system has been used in more than 600 publications (Kjærsgaard et al., 2010; Hardin, 2011; Peschel and Helfrich- Forster, 2011). A chamber with an infrared beam that recognizes activity as the number of beam interruptions, an interphase that will compile the data from the chamber and send it to a computer and data acquisition software to convert the interphase data into legible files, are the components that are necessary for infrared-based systems to measure locomotor activity (Guzman, 2014).

2.7. RNA isolation for metatranscriptomics analyses of honeybee subpecies

RNA was extracted from a pooled sample of 50 bees from each sampled colony, using an acid-phenol RNA extraction method. 50 intact bees were placed into mesh-divided Bioreba bag (or any 1-quart zip-loc bag) and 15 ml (or 25 ml for 50 bees) Lysis Buffer were added. Then the ingredients were smashed with rolling pin until bees fully broken. Liquid was massaged to mix fully. 620 ul of the mixture was drawn off and 380 ul acid phenol (pH 4, Sigma) was added, vortexed and incubated at 95^{o}C for ten mins. After cooling to room temperature, 200 ul chloroform was added. The mixturewas vortexed for 15 seconds and incubated at room temperature for 2 to 3 minutes. The samples were centrifuged at 10,000 X *g* for 15 minutes at 4°C and upper aqueous phase was collected into fresh tube containing 500 ul isopropyl alcohol and vortexed.

(At this stage, the samples can be shipped at room temperature one week). Samples were incubated at RT for 10 minutes and centrifuged at 10,000 X *g* for 10 minutes at 4°C. The supernatant was removed and 1 ml 75% ethanol was added to the pellet and vortexed briefly. Samples were centrifuged at 7,000 X *g* for 5 minutes at 2 to 8°C. The liquid was poured off and centrifuged 30 s and the remaining EtOH was pulled off with pipette. RNA pellet was air-dried for 5-10 minutes and dissolved in 50 ul RNase-free water by incubating for 10 minutes at 55 to 60°C (Evans et al., 2013).

2.8. RNA isolation for immune genes expression analyses

For immune genes expression analyses, RNAs of 12 control and 12 infected bees for each of the 5 subspecies were isolated. RNase-free bench, pipettes, tips, pestles and 1.5 ml microcentrifuge tubes are needed before the extraction. RNAse Zap (Ambion) was applied to treat bench tops and other glass and plastic surfaces to prevent RNAse contamination. RNase–free disposable tips, pestles, and microcentrifuge tubes and nuclease-free, cold 75-80% ethanol and 100% isopropanol were made ready to use prior to the exraction. TRIzol® (Invitrogen, Carlsbad, CA) and other reagents needed were at room temperature and the isolation process was done in a vented fume hood. It was important to work quickly with bee tissue to avoid RNA degradation (http://www.coloss.org/beebook).

500ml of TRIzol® was added to frozen bee abdomens in 1.5 ml tubes. The tissue was mashed with a sterile pestle until completely homogenized. Another 500ml TRIzol® was added and inverted several times to mix. The homogenized sample was incubated at room temperature for 3-5 minutes to allow complete dissociation of nucleoprotein complexes. 200 ul chloroform was added into homogenized sample. Sample tube was capped securely and shaked by hand for 50 times and incubated at room temperature for 3 minutes. The sample was centrifuged at 10, 000 rpm for 10 minutes at 4 °C. Following centrifugation, the mixture was separated into a lower red, phenol chloroform phase and a colorless upper aqueous phase. RNA remains in the aqueous phase. For the RNA precipitation and RNA wash steps, the aqueous phase was transferred to a fresh tube. 500 ul of isopropyl alcohol was added into the tube and the sample was incubated at room temperature for 10 minutes and centrifuged at 12, 000 rpm for 15 minutes at 4 °C. The RNA precipitate formed a pink pellet on the side and bottom of the tube.

The supernatant was poured off and the RNA pellet was washed with 1 ml of 75% ethanol. The sample was mixed by vortexing and centrifuged at 8000-10, 000 rpm for five minutes. The supernatant was poured off and the excess ethanol was removed. RNA was dissolved in 50 ul of RNAse-free water and stored at -80°C immediately (http://www.coloss.org/beebook).

RNAs were quantified by Nanodrop ND-1000 (Thermo Fisher Scientific,Inc.Wilmington, Delaware, USA) . By loading 2 ul of each sample, the absorbance ratios of A260/280 and A260/230 were measured and DNA concentrations were quantified in ng/ul (Appendix C and D).

2.9. cDNA synthesis and real time qPCR

RNA extracts were used to generate first and second-strand cDNA's using random hexamer primers and the reverse transcriptase Superscript II® (Invitrogen™), as described in vanEngelsdorp et al., (2009). Pathogen loads were estimated using real-time quantitative-PCR (qPCR) and a Bio-Rad CFX-96™ thermocycler. Complementary DNA (cDNA) was generated from 1 µg RNA template and was amplified in a separate 20 µl final reaction volume of Sso-Fast™ SYBR® Green reaction mix (Bio-Rad™) for each diagnostic primer pair. Published primers for *N. ceranae*, apidaecin, abaecin, defensin, hymenoptaecin, eater and vitellogenin genes were used (Table 1). We also used published primers to survey for SBV, KBV, IAPV, DWV, ABPV, BQCV, trypanosomes (vanEngelsdorp, et al., 2009), AFB (Evans, 2006), *Nosema ceranae* (Fries et al., 2013) and *Nosema apis* (Schwarz and Evans, 2013), *Spiroplasma apis* and *Spiroplasma melliferum* (Schwarz et al., 2014). Honeybee ribosomal protein S5 (RPS5) and ß-actin were used to normalize for cDNA content and to filter samples for degradation or experimental losses.

Table 1 Oligonucleotide primers and sequence identification for real-time quantitative RT-PCR

Locus	F-Primer (5'-3')	R- Primer (5'-3')
ß-actin	TTGTATGCCAACACTGTCCTTT	TGGCGCGATGATCTTAATTT
N. ceranae	GTCGCTATGATCGCTTGC	TATTGTAGAGAGGTGGGAGATT
Abaecin	CAGCATTCGCATACGTACCA	GACCAGGAAACGTTGGAAAC
Apidaecin	TAGTCGCGGTATTTGGGAAT	TTTCACGTGCTTCATATTCTTCA
Hymenoptaecin	CTCTTCTGTGCCGTTGCATA	GCGTCTCCTGTCATTCCATT
Defensin-1	TGCGCTGCTAACTGTCTCAG	AATGGCACTTAACCGAAACG
Eater	CATTTGCCAACCTGTTTGT	ATCCATTGGTGCAATTTGG
Vitellogenin	AGTTCCGACCGACGACGA	TTCCCTCCCACGGAGTCC

A thermal profile of 95° C for 30 s followed by 95° C for 5 s and 60° C for 30 s was used. Steps two and three were repeated for a total of 50 cycles and included plate reads for florescence during each 60^{o}C step. Following the cycle program, products were denatured for 10 s at 95° C., reannealed and then a dissociation profile was measured between 69° C and 95° C at an increment of 0.5° C to provide evidence for reaction fidelity (Evans, 2006). Positive and negative control reactions were run on each 96-well plate. Pathogen loads (ΔΔCT = CTcontrol- CTtarget) were determined as the difference between the CT of actin and the CT of each target (ΔCT), scaled up from the minimal ΔCT across all samples (Evans, 2004 and Evans et al., 2006). CT values for both studies were provided in Appendix C and D.

2.10. PCR Purification and sequencing

RT- PCR products were selected for sequencing to confirm the identities of products indicating the trypanosome, *L. passim*, and the bacteria *S. apis* and *S. melliferum*. These PCR products were purified using QIAquick PCR purification kit according to protocol recommended by manufacturer, and then were sequenced commercially by Macrogen (Rockville, MD, USA) DNA sequence similarity with trypanosomes, *S. apis* and *S. melliferum*, was confirmed using the BLAST search tool (Altschul et al., 1990) from the U.S. National Institutes of Health and searches against the National Center for Biotechnology Information (NCBI) *nr* database .

PCR purification was done by using QIAquick PCR Purification Kit. Spin-column technology with the selective binding properties of silica membrane and special buffers enables efficient recovery of DNA and removal of contaminants in each specific application. During purification process, in the presence of high concentrations of salt, DNA adsorbs to the silica membrane while contaminants pass through the column. Impurities are thoroughly washed away, and the pure DNA is eluted with Tris buffer or water. Buffer PB contains hydrochloride and isopropanol and allows the efficient binding of single or double-stranded PCR products as small as 100 bp and removes (99.5%)of primers up to 40 nucleotides. The buffer PB contain a pH indicator, for easy determination of the optimal pH for DNA binding. During the DNA adsorption step, unwanted primers and impurities do not bind to the silica membrane but flow through the column. Buffer PE which contains ethanol wash away the salts. Any residuals of Buffer PE that has the risk of interfering with subsequent enzymatic reactions, is removed by an additional centrifugation step.

Elution is most efficient under basic conditions and low salt concentrations and its efficiency dependent on the salt concentration and pH of the elution buffer with maximum efficiency between pH 7.0 and 8.5. Thus, DNA is eluted with 50 or 30 µl of the provided Buffer EB (10 mM Tris·Cl, pH 8.5) (www.quiagen.com).

5 volumes of Buffer PB was added to 1 volume of the PCR sample and mixed. Samples were applied to the QIAquick column (2 ml collection tube) and centrifuged for 30–60 s. Flow-through was discarded and the column was placed back into the same tube. To wash, 0.75 ml Buffer PE was added to the column and centrifuged for 30–60 s. Again, the flow-through was discarded and the column was placed back into the same tube and the column was centrifuged for an additional 1 min. The columns are transferred in clean 1.5 ml microcentrifuge tubes. 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water (pH 7.0–8.5) was added to the center of the QIAquick membrane to elute DNA and the column was centrifuged for 1 min (www.quiagen.com). Additionally, DNAs were stored at –20 °C.

2.11. High throughput sequencing and data analysis

High throughput sequencing was performed with the pooled samples from stationary colonies of each region and all RNA samples were pooled before sequencing (n= 6 ILLUMINA RNA libraries). Libraries were run on paired-end 100-cycle reactions and flow cells using Illumina Hi-Seq 2000 machines at the University of Maryland Institute of Genome Sciences. Sequences for all six libraries have been deposited at the US National Institutes of Health NCBI 'Honey Bee Disease Database" Bioproject (PRJNA52851).

After trimming and quality control of the generated sequences, transcriptomic analysis was done with the support of CLC Genomics Workbench 7.0.3 (CLC Bio, Aarhus, Denmark) by mapping sequencing reads and counting and distributing the reads across genes and transcripts based on annotated reference genes (Figure 9). During the alignment, 2 mismatches were allowed with deletion cost of 3, insertion cost of 3, length fraction of 0.8, similarity fraction of 0.8 and maximum 10 hits for a read. For statistical analyses, proportions-based tests were used for the comparison of the counts by considering the proportions that they comprise of the total sum of counts in each sample.

Multi-group comparison was done by weighted t-type test statistic Baggerly's test (Baggerly et al., 2003). FDR (False Discovery Rate) corrected p- values were calculated according to the methods of Benjamini and Hochberg, (1995) to determine the statistical significance of the pathogen load.

Real time q-PCR data statistics were performed by using JMP™ (SAS Institute, Cary, NC, USA, v.9). A one-way analysis of variance was used to test differences between group means. The total variability in the response was divided into two parts; within-group variability and between-group variability. The differences between the group means were considered to be significant if the between-group variability was broader relative to the within-group variability. Multiple comparisons of group means were done by using pooled variance estimates for these means. Student's t-tests were computed for each pair of group levels and individual pairwise comparisons.

Matrix of correlation coefficients that summarized the strength of the linear relationships between each pair of response variables was calculated and Pearson product-moment correlations for each pair of variables were listed. Correlations and the significance probabilities were calculated by the pairwise deletion method and the count values differed if any pair had a missing value for either variable.

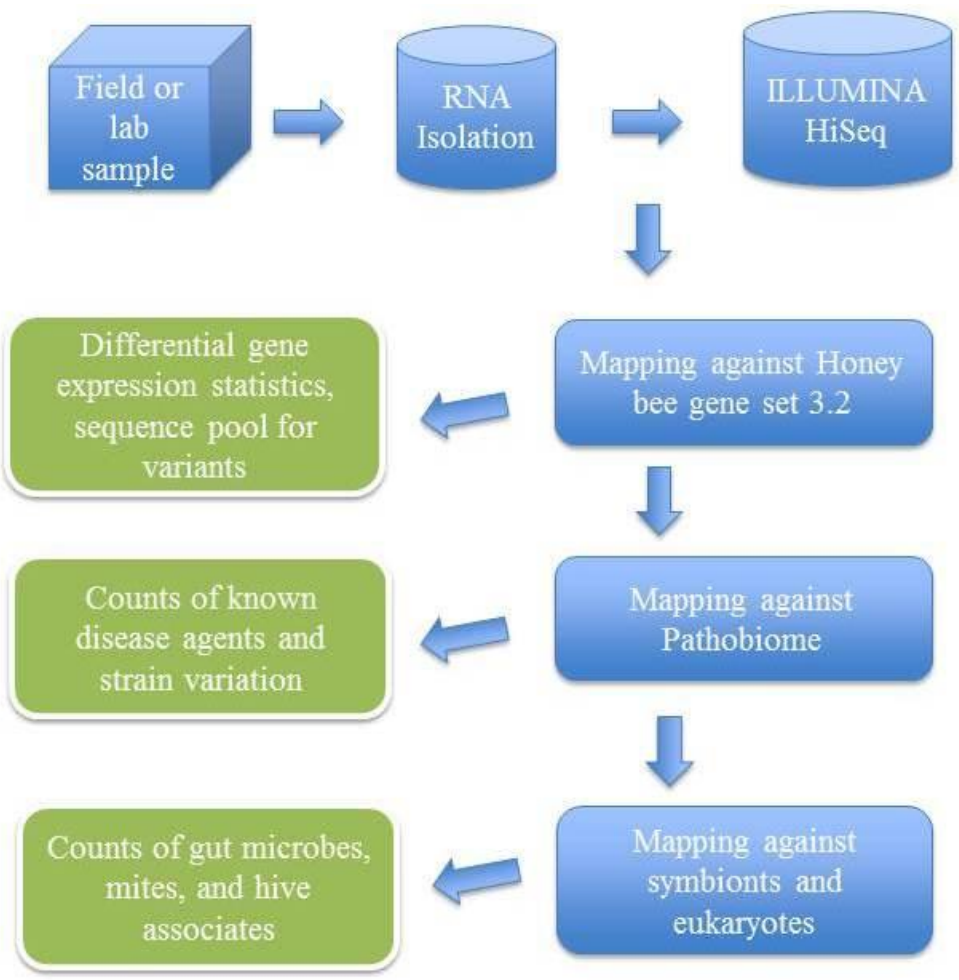

Figure 9 Flow chart showing analytical steps for mapping ILLUMINA RNA-seq reads against an annotated bee microbial dataset.

2.12. Statistical details of JMP™ analysis

Statistical analysis of immune gene expression results were done by JMP™ (SAS Institute, Cary, NC, USA, v.9). Scatterplots and some graphs were used to visualize relationships between variables.

Those relationships between variables were analyzed and described numerically and predictions about the average value of one variable (*Y*) from the value of another variable (*X*) can be made by using a *model*. The RSq value is the measure of model accuracy and the percentage of variability and a value closer to 1 means a model is making a good prediction.

2.12.1. Leverage plot details

Effect leverage plots were generalized to apply to any linear hypothesis by Sall (1990). According to a hypothesis that accepts an effect as zero, effect leverage plots shows observations when the other effects are in the model. The whole model leverage plot indicate the observations regarding all effects are hypothesized to be zero.

2.12.2. Construction details

The hypothesis of interest is supposed as,

$$L\beta = 0$$

The parameter assessment restricted by the hypothesis can be written

$$b_0 = b - (X'X)^{-1}L'\lambda$$

Here b is the least squares measure

$$b = (X'X)^{-1}X'y$$

and lambda which is 'Lagrangian multiplier' for the hypothesis constraint, calculated by

$$\lambda = (L(X'X)^{-1}L')^{-1}Lb)$$

The restricted and hypothesis-restricted residuals are, respectively,

$$r = y - Xb$$

$$r_0 = r + X(X'X)^{-1}L'\lambda$$

x-axis value can be considered as v_x which is the restricted residual minus the unrestricted residual, r_0 - r, and *y*-axis value v_y which is the unrestricted residual plus x-axis value. The points includes coordinates of x and y from the basis of the leverage plot with the response mean is 0 and slope of the solid line is 1,

$$v_x = \mathrm{X}(\mathrm{X}'\mathrm{X})^{-1}\mathbf{L}'\lambda \quad \text{and} \quad v_y = \mathbf{r} + v_x$$

A dotted horizontal is present line at the mean of the response $\bar{y}$ in leverage plots. The plotted points are given by ($v_x + \bar{y}$, v_y) (http://www.jmp.com/support/help).

2.12.3. Superimposing a test on the leverage plot

The confidence limits for the expected value of the response can be plotted as a function of the predictor variable **x**

$$\text{Upper (x)} = \mathbf{xb} + t_{\alpha/2} s\sqrt{\mathrm{x}(\mathrm{X}'\mathrm{X})^{-1}\mathrm{x}'}$$

$$\text{Lower (x)} = \mathbf{xb} - t_{\alpha/2} s\sqrt{\mathrm{x}(\mathrm{X}'\mathrm{X})^{-1}\mathrm{x}'}$$

where **x** = [1 *x*] is the 2-vector of predictors.

The significance of the hypothesis test can be assessed visually with these confidence curves. The confidence curves cross the horizontal line at the response mean when the slope parameter is significantly different from zero. But if there is no significance, they do not cross the horizontal line at the mean of response. The confidence curve does not reach the horizontal line at the mean response if the *t* test for the slope parameter is situated right on the margin of significance.

When a point on the *x*-axis is marked by *z*, and functions are defined as,

$$\text{Upper}(z) = \mathbf{z} + \sqrt{s^2 t_{\alpha/2}^2 \bar{h} + (F_\alpha / F) z^2}$$

and

$$\text{Lower}(z) = \mathbf{z} - \sqrt{s^2 t_{\alpha/2}^2 \bar{h} + (F_\alpha / F) z^2}$$

F refers to *F* statistic for the hypothesis and F_{α} is the reference value for significance α. $\bar{h} = \bar{x}(X'X)^{-1}\bar{x}'$, where $\bar{x}$ represents a row vector including suitable middle values for the predictors, such as their means. Upper(*z*) - Lower(*z*) is an accurate confidence interval for the predicted value at z. The confidence functions cross the *x*-axis when the *F* statistic is greater than the reference value. However they do not cross, if *F* statistic is less. The confidence functions have the *x*-axis as an asymptote in some cases as *F* statistic is equal to the reference value (http://www.jmp.com/support/help).

The illustration of a Generic Leverage Plot indicates how residuals are interpreted in the leverage plot (Figure 10). The residual for a model including the effect is represented by the distance from a point to the line of fit and the residual error without the effect in the model is the distance from the point to the horizontal line. This can be stated as the hypothesized value of the effect is restricted to zero and the model is represented by the mean line in the leverage plot.

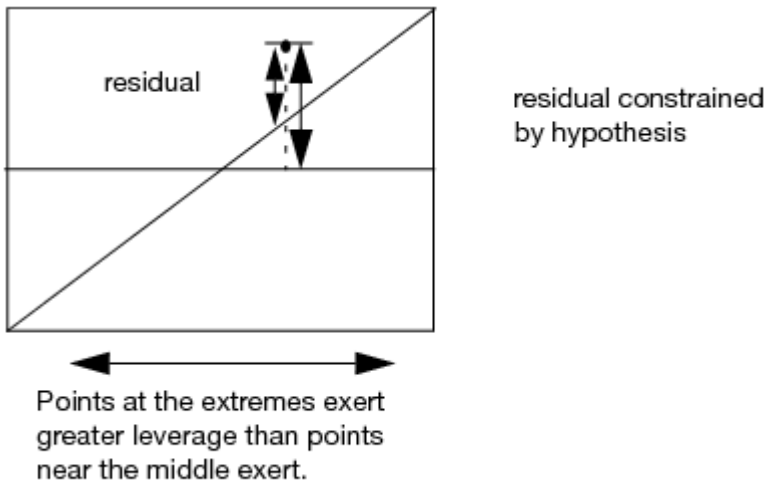

Figure 10 Illustration of a Generic Leverage Plot (Obtained from http://www.jmp.com)

2.12.4. Correlation of estimates

The correlation matrix is computed to estimate the presence of collinearity. For insight on the construction of this matrix, consider the typical least squares regression formulation. For the construction of the matrix, in the formula, the response (*Y*) is a linear function of predictors (*x's*) plus error (ε):

$$Y = \beta_0 + \beta_1 x_1 + \ldots + \beta_p x_p + \varepsilon$$

The predictor values are considered to be fixed and the response value is considered to be a comprehension of a random variable for each observation.

Thus, the coefficients $\beta_0, \beta_1, \ldots, \beta_p$, can be estimated for any set of Y values. The correlations of the parameter estimates based on the predictor values and a term symbolizing the intercept. The response values has no impact on the correlation between two parameter estimates (Belsley et al., 1980).

In a data set with *n* observations and *p - 1* predictors, **X** is the *n* by *p* matrix and its first column consists of *1s* and the remaining *p - 1* columns consist of the *p - 1* predictor values. The measure of the vector of the coefficient of regression is,

$$\hat{\beta} = (X'X)^{-1}X'Y$$

where response values are represented by *Y*. Under the standart assumptions, the covariance matrix of $\hat{\beta}$ is

$$Cov(\hat{\beta}) = \sigma^2(X'X)^{-1}$$

where σ^2 refers to the variance of the response (http://www.jmp.com/support/help).

When each entry in the covariance matrix is divided by the product of the square roots of the diagonal entries, the correlation matrix can be estimated. *V* is described to be the diagonal matrix having the entries as the square roots of the diagonal entries of the covariance matrix:

$$V = Sqrt(Diag(Cov(\hat{\beta})))$$

Then the correlation matrix is :

$$Corr(\hat{\beta}) = \sigma^2 V^{-1}(X'X)^{-1}V^{-1}$$

2.12.5. Degrees of freedom

The degrees of freedom are calculated using the Kenward and Roger correction for tests including only linear combinations of fixed effect parameters (Kenward and Roger (1997).

2.12.6. Multiple comparisons

The aim of making multiple comparisons is to compare group means with an overall average mean (Analysis of Means) and with a mean of a control group. The Student's *t* Tests are conducted to make pairwise comparisons of the effect least squares means with confidence intervals by just controlling the error rate for an individual comparison. Least Squares Means estimates can be computed to make comparisons when the model consists of nominal and ordinal effects. The Least Square Means Student's *t* Test depends on the standard independent samples with specified significance level (http://www.jmp.com/support/help).

2.12.7. LSMeans table

By using the model for the levels of a categorical effect, least squares means are predicted while the other model factors are set to *neutral* values. Standard error of the least squares mean for each level are also computed with upper and lower 95% confidence limits. The neutral value is the sample mean for a continuous effect. The average of the coefficients for an effect is described as the neutral value for a nominal effect which is not included in the effect of interest. The first level (baseline or control level) of the effect in the value ordering can be defined as the neutral value for an uninvolved ordinal effect. Comparisons can be made among Least squares means which are also called *adjusted means* or *population marginal means.* They can differ from simple means (response sample mean) when the values for other effects in the model do not balance out across the effect. Least Square Means Plots are constructed to show least square means for nominal and ordinal main effects and their interactions (http://www.jmp.com/support).

2.12.8. One way analysis of variance

Using the One way or Fit Y by X platform, you can explore how the distribution of a continuous Y variable differs across groups defined by a single categorical X variable. A one-way analysis of variance tests for differences between group means. The total variability in the response is composed of within-group variability and between-group variability. The differences between the group means are accepted to be significant, if the between-group variability is large in comparison to the within-group variability. The response points for each X factor value and the comparison of the distribution of the response across the levels of the X factor are displayed by One-way plot (http://www.jmp.com/support/help).

2.12.9. Rsquare and Adj Rsquare

R^2 is calculated according to this equation,

$$\frac{\text{Sum of Squares (Model)}}{\text{Sum of Squares (C Total)}}$$

Adj is calculated with this formula,

$$1 - \frac{\text{Mean Square (Error)}}{\text{Mean Square (C Total)}}$$

(http://www.jmp.com/support/help).

CHAPTER 3

RESULTS

The RNA expression levels of seven genes were determined 6 days after *Nosema ceranae* infection.

3.1. *N.ceranae* expression

The infectivity of *N. ceranae* was successful according to One-way ANOVA analysis ($p \leq .0001$) (Figure 11 and Table 2). *N. ceranae* mRNA expression augmented significantly after infection with *N. ceranae* when compared with the controls for all of the subspecies included in the study (Figure 12 and Table 3). Relationship between the effects of races and treatment on the expression of *N. ceranae* was also proven by two-way analysis of variance with interaction ($p < .0001$) (SS = 2012.09, F ratio = 28.9, $p < .0001$). *N. ceranae* transcript levels showed a strong relation with either of race and treatment factors as a result of two-way analysis of variance ($p < .0001$) (Table 4)

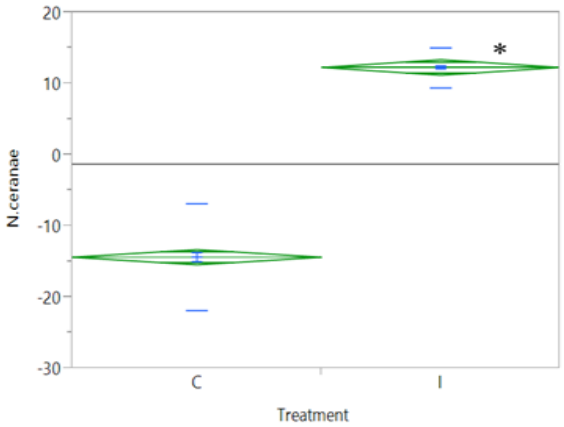

Figure 11 One way analysis of *N.ceranae* expression by treatment (C = Control, I = Infected) ('*' significant).

Table 2 Variance analysis of *N.ceranae* expression by treatment (‘*’ significant).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	38513.232	38513.2	1186.159	<.0001*
Error	215	6980.804	32.5		
C. Total	216	45494.036			

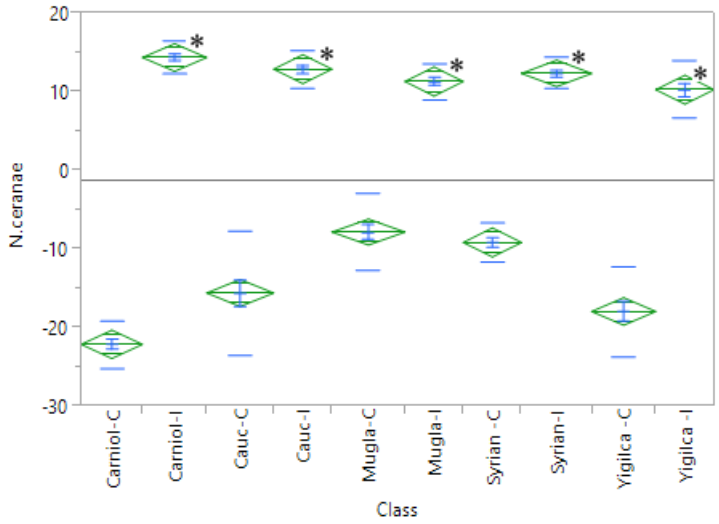

Figure 12 One way analysis of *N.ceranae* expression by class (C = Control, I = Infected) (‘*’ significant)

Table 3 Variance analysis of *N.ceranae* expression by class (‘*’ significant).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Class	9	41898.486	4655.39	268.0161	<.0001*
Error	207	3595.550	17.37		
C. Total	216	45494.036			

Table 4 Effect tests on *N. ceranae* expression ('*' significant).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Race	4	4	1320.979	19.0126	<.0001*
Treatment	1	1	38581.776	2221.198	<.0001*
Race*Treatment	4	4	2012.092	28.9596	<.0001*

3.2. Vitellogenin expression

Vitellogenin expression significantly increased as a result of exposure to *N .ceranae* for Carniolan race when comparisons were done by using Student's *t*- test (p = 0.0005). It also increased for infected Syrian race when compared with control bees (non-significant). There was variation among subspecies for Vg expression (p < .0001) (Figure 13 and Table 5). The expression of Vg did not differ between the control and infected bees of Mugla ecotype and decreased a little for Caucasian and Yigilca honeybees. But these results were not statistically significant. Pairwise differences between the subspecies were analyzed by *t*- test. Two-way analysis of variance indicated the interaction between the race and treatment effects on vitellogenin response (SS = 86.1, F ratio = 3.2, p = 0.0132). Two-way ANOVA also showed a strong relation between the vitellogenin expression and race effect (SS = 279.8, F ratio = 10.5, p < .0001) (Table 6). Vitellogenin transcripts of Carniolan subspecies (r =0.4296, p = 0.0029) and Syrian subspecies (r = 0.3408, p = 0.0205) showed correlations with *N. ceranae.*

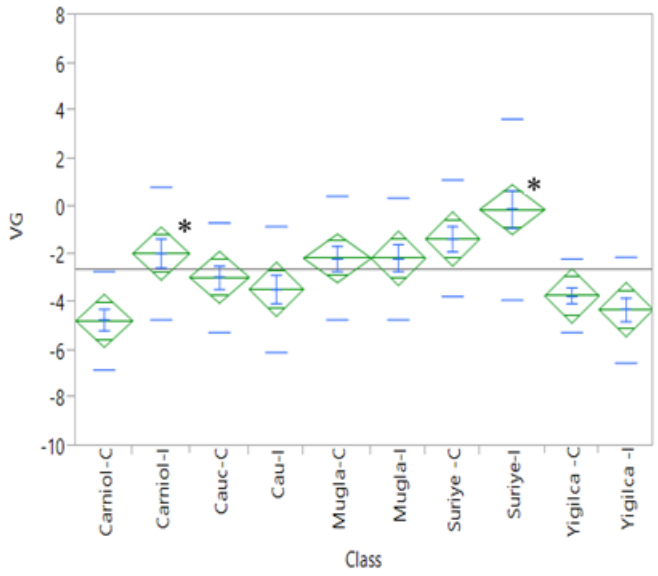

Figure 13 One way analysis of vitellogenin expression by class (C = Control, I = Infected) ('*' significant).

Table 5 Variance analysis of vitellogenin expression by class ('*' significant).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Class	9	395.6631	43.9626	6.6259	<.0001*
Error	203	1346.9002	6.6350		
C. Total	212	1742.5633			

Table 6 Effect tests on vitellogenin expression ('*' significant).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Race	4	4	279.75542	10.5409	<.0001*
Treatment	1	1	18.63311	2.8083	0.0953
Race*Treatment	4	4	86.11923	3.2449	0.0132*

3.3. Defensin expression

Defensin expression was upregulated significantly following the treatment with *N.ceranae* (ANOVA, $p < .0001$) (Figure 14 and Table 7). A rapid response as an increase in the transcript levels of defensin was observed for the Caucasian (*t*-test, $p = 0.0042$), Carniolan (*t*-test, $p = 0.0053$), Syrian (*t*-test, $p = 0.0048$) and Yigilca (*t*-test, $p = 0.0102$) races 6-days after infection with *N. ceranae* (Figure 15 and Table 8). Defensin expression did not differ significantly between control and *N. ceranae* infected bees of Mugla ecotype. Among the subspecies, the highest increase in mRNA levels of defensin was observed in Caucasian race ($p = 0.0004$). Student's t-test was used to detect the pairwise differences between the races. Transcript levels of defensin showed strong relationship with either race (SS = 211.2, *F* ratio = 6.5, $p < .0001$) and treatment (SS = 223.9, *F* ratio = 27.4, $p < .0001$) by two-way analysis of variance (Table 9). Correlations between defensin and *N .ceranae* was found in Carniolan ($r = 0.3939$, $p = 0.0068$), Suriye ($r = 0.4451$, $p = 0.0019$) and Yigilca($r = 0.3895$, $p = 0.0082$) subspecies. Defensin levels also correlated with apidaecin in Carniolan ($r = 0.7411$, $p < .0001$) Caucasian ($r = 0.7299$, $p < .0001$), Suriye ($r = 0.6798$, $p < .0001$), Mugla ($r = 0.3970$, $p = 0.0052$) and Yigilca ($r = 0.7411$, $p < .0001$).

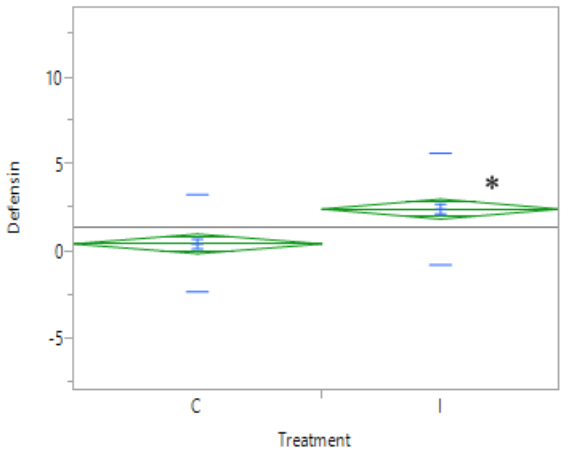

Figure 14 One way analysis of defensin by treatment (C = Control, I = Infected) ('*' significant)

Table 7 Variance analysis of defensin by treatment ('*' significant).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	216.2335	216.233	24.1035	<.0001*
Error	215	1928.7698	8.971		
C. Total	216	2145.0033			

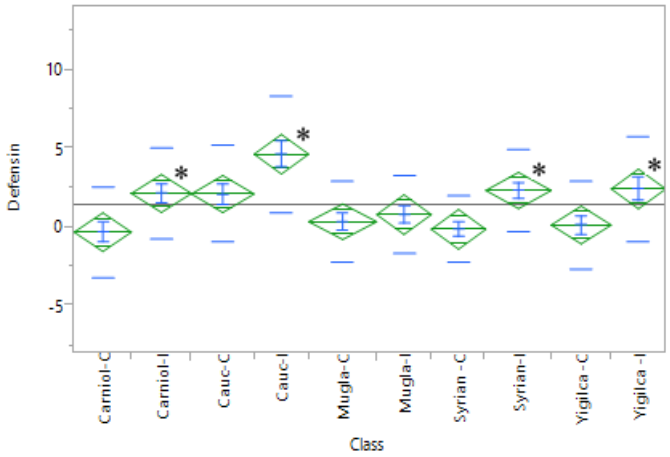

Figure 15 One way analysis of defensin by class (C = Control, I = Infected) ('*' significant).

Table 8 Variance analysis of defensin by class ('*' significant).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Class	9	453.6651	50.4072	6.1693	<.0001*
Error	207	1691.3382	8.1707		
C. Total	216	2145.0033			

Table 9 Effect tests on defensin expression ('*' significant).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Race	4	4	211.21992	6.4627	<.0001*
Treatment	1	1	223.95010	27.4089	<.0001*
Race*Treatment	4	4	35.20035	1.0770	0.3689

3.4. Hymenoptaecin expression

N. ceranae exposure generated a significant increase in expression between control and treated bees of the subspecies (ANOVA, $p < .0001$) (Figure 16 and Table 10). A significant response towards increased expression of hymenoptaecin was detected for infected Yigilca (*t*- test, $p < .0001$) and Carniolan (*t*- test, $p = 0.0013$) subspecies (Figure 17 and Table 11). A two-way analysis of variance indicated a strong relationship between race and treatment effects on hymenoptaecin expression (SS = 96.1, *F* ratio = 2.7, $p = 0.0338$) (Table 12). Hymenoptaecin transcripts were strongly correlated with *N. ceranae* treatment rather than race (SS = 168.3, *F* ratio = 18.6, $p < .0001$). Correlations were found between hymenoptaecin and abaecin for Carniolan ($r = 0.3222$, $p = 0.0290$), Caucasian ($r = 0.3786$, $p = 0.0095$), Syrian ($r = 0.3210$, $p = 0.0296$), Yigilca ($r = 0.3848$, $p = 0.0091$) and between hymenoptaecin and defensin for Carniolan ($r = 0.8429$, $p <.0001$), Caucasian ($r = 0.3562$, $p = 0.0151$), Syrian ($r = 0.8310$, $p <.0001$), Yigilca ($r = 0.7411$, $p < .0001$) and Mugla ($r = 0.5234$, $p = 0.0001$).

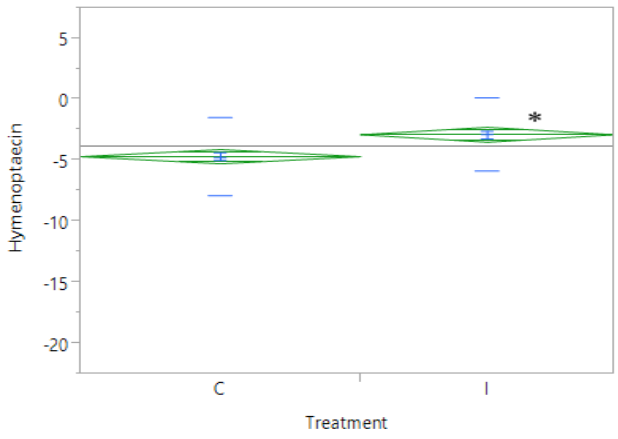

Figure 16 One way analysis of hymenoptaecin by treatment (C = Control, I = Infected) ('*' significant).

Table 10 Variance analysis of hymenoptaecin by treatment ('*' significant).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	164.6783	164.678	17.3964	<.0001*
Error	204	1931.1132	9.466		
C. Total	205	2095.7915			

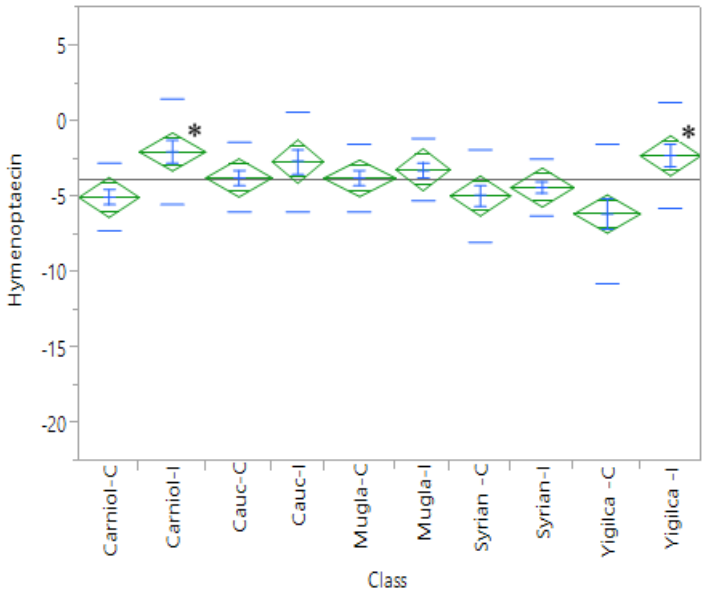

Figure 17 One way analysis of hymenoptaecin by class (C = Control, I = Infected) ('*' significant).

Table 11 Variance analysis of Hymenoptaecin by class ('*' significant).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Class	9	327.7065	36.4118	4.0364	<.0001*
Error	196	1768.0850	9.0208		
C. Total	205	2095.7915			

Table 12 Effect tests on hymenoptaecin expression ('*' significant).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Race	4	4	57.93983	1.6057	0.1744
Treatment	1	1	168.29337	18.6561	<.0001*
Race*Treatment	4	4	96.11265	2.6636	0.0338*

3.5. Apidaecin expression

In the case of Apidaecin mRNA levels, significant differences were found between *N. ceranae* infected and control bees (One –way ANOVA, $p < .0001$) (Figure 18 and Table 13). The results were significant with a rapid increase in the apidaecin expression for infected Carniolan ($p <. 0001$), Caucasian ($p = 0.0002$) Syrian (0. 0299), Yigilca ($p = 0.0104$) and Mugla (non-significant) races (Figure 19 and Table 14). Apidaecin expression showed variation among the races (ANOVA, $p = 0. 0445$). A significant effect of race (SS = 57.0, F ratio = 2.7, $p < 0.0302$) and treatment(SS = 279.0, F ratio = 53.4, $p < .0001$) and a substantial relationship between race and treatment (SS = 76.5, F ratio = 3.6, $p = 0.0066$) was indicated by two-way ANOVA with interaction (Table 15). Apidaecin levels were correlated with *N .cerenae* transcripts for Carniolan ($r = 0.5674$, $p <.0001$), Caucasian ($r = 0.3732$, $p =0.0106$), Suriye ($r = 0.3790$, $p = 0.0094$) and Yigilca ($r = 0.3158$, $p = 0. 0346$) and they substantially correlated with hymenoptaecin transcripts for Carniolan ($r = 0.7897$, $p <.0001$), Caucasian ($r =.3412$, $p =0.0203$), Suriye ($r = 0.7118$, $p <.0001$), Yigilca ($r = 0.6507$, $p <.0001$) and Mugla ($r = 0.5636$, $p <.0001$).

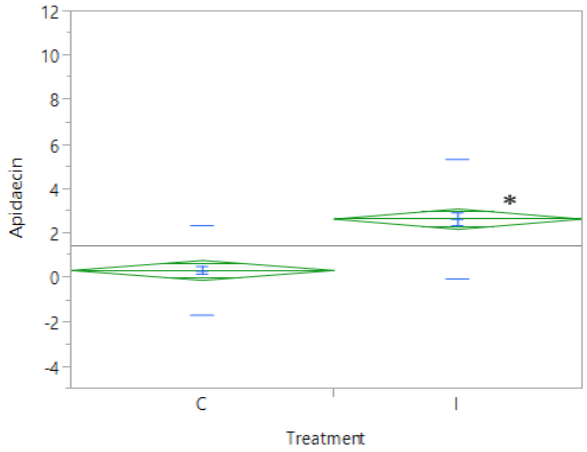

Figure 18 One way analysis of apidaecin by treatment (C= Control, I = Infected) ('*' significant).

Table 13 Variance analysis of apidaecin by treatment ('*' significant).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	284.8478	284.848	50.6118	<.0001*
Error	212	1193.1550	5.628		
C. Total	213	1478.0027			

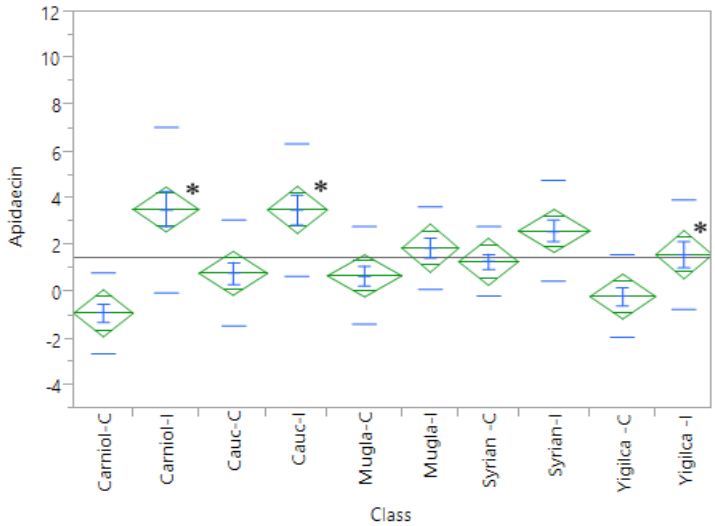

Figure 19 One way analysis of apidaecin by class (C= Control, I = Infected) ('*' significant).

Table 14 Variance analysis of apidaecin by class ('*' significant).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Class	9	413.2368	45.9152	8.7970	<.0001*
Error	204	1064.7659	5.2194		
C. Total	213	1478.0027			

Table 15 Effect tests on apidaecin expression ('*' significant).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Race	4	4	57.03525	2.7319	0.0302*
Treatment	1	1	279.01216	53.4563	<.0001*
Race*Treatment	4	4	76.59140	3.6686	0.0066*

3.6. Abaecin expression

Abaecin expression augmented in response to *N.ceranae* infection for each of the subspecies. Although this increase was apparent for Carniolan, Caucasian and Mugla, ANOVA analysis indicated a significant up-regulation in abaecin for only Caucasian race (*t*- test, $p = 0.0185$). (Figure 20 and Table 16). Significant differences were observed when comparisons were made among races (ANOVA, $p < .0001$). Pairwise comparisons between the races were analyzed by student's *t*-test. Race (SS = 909.6, *F* ratio = 12.5, $p < .0001$) and treatment (SS = 92.1, *F* ratio = 5.0, $p = 0.0255$) factors had independent impacts on abaecin expression according to two-way analysis of variance (Table 17). Significant correlations were observed between abaecin and defensin for Carniolan ($r = 0.3658$, $p = 0.0124$), Caucasian ($r = 0.4984$, $p = 0.0004$), Mugla ($r = 0.2923$, $p = 0.0438$) and Yigilca ($r = 0.5014$, $p = 0.0005$) and between abaecin and apidaecin for Carniolan ($r = 0.4042$, $p = 0.0053$), Caucasian ($r = 0.6396$, $p < .0001$), Yigilca ($r = 0.4368$, $p =0 .0027$) and Mugla ($r = 0.3255$, $p = 0.0240$).

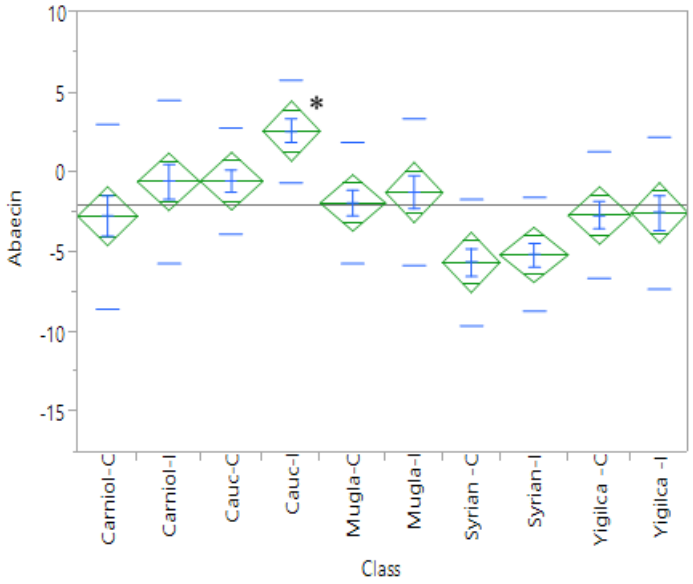

Figure 20 One way analysis of abaecin by class (C = Class, I = Infected) ('*' significant).

Table 16 Variance analysis of abaecin by class ('*' significant).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Class	9	1048.9458	116.550	6.4076	<.0001*
Error	204	3710.5918	18.189		
C. Total	213	4759.5376			

Table 17 Effect tests on abaecin expression ('*' significant).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Race	4	4	909.61899	12.5022	<.0001*
Treatment	1	1	92.15448	5.0664	0.0255*
Race*Treatment	4	4	68.15106	0.9367	0.4436

3.7. Eater expression

Significant reduction in the levels of eater transcripts after *N. ceranae* treatment was observed (ANOVA, p <.0001) (Figure 21 and Table 18). Transcript levels of eater in *N. ceranae* inoculated-bees were lower than controls for Caucasian ($p \leq .0001$), Mugla (p = 0. 0021) Yigilca (0.0117) and Syrian (not significant) subspecies. The expression of eater gene was not influenced by *N. ceranae* inoculation for Carniolan race (Figure 22 and Table 19). A significant difference in declining levels of eater transcripts after *N.ceranae* exposure was observed among races (p = 0.0404). Pairwise mean differences between control and infected bees of Caucasian bees showed the highest difference in the expression of eater gene. Impacts of race (SS = 50.1, F ratio = 2.9, p = 0.0212) and treatment (SS = 114.7, F ratio = 27.0, p < .0001) on eater expression and the relationship of these two factors (SS = 67.6, F ratio = 3.9, p = 0.0040) were analyzed by two-way ANOVA (Table 20). The expression of eater encoding gene indicated correlations with vitellogenin transcripts for Caucasian (r = 0.3861, p = 0.0080), Suriye (r = 0.4856 p = 0.0006) and Yigilca (r = 0.3093, p = 0.0387). Additionally, eater transcripts had significant correlations with apidaecin (r = 0.3904, p = 0.0080), defensin (r = 0.3525, p = 0.0176) and hymenoptaecin (r = 0.3318, p = 0.0260) for Yigilca ecotype and it correlated with *N. ceranae* (r = 0.4970, p = 0.0004) for Caucasian subspecies.

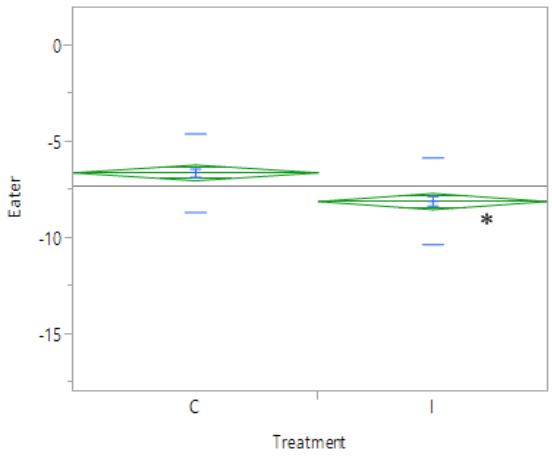

Figure 21 One way analysis of eater by treatment (C = Control, I = Infected) ('*' significant).

Table 18 Variance analysis of eater by treatment (‘*’ significant).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	1	116.7037	116.704	25.0422	<.0001*
Error	207	964.6771	4.660		
C. Total	208	1081.3809			

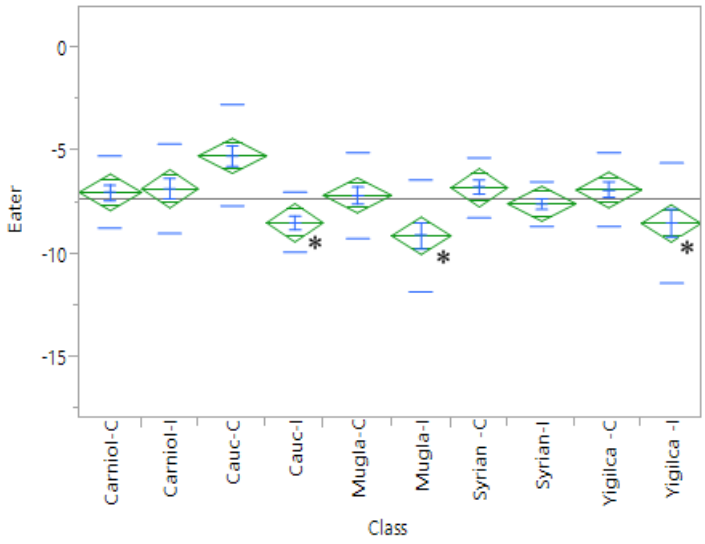

Figure 22 One way analysis of eater by class (C = Control, I =Infected) (‘*’ significant).

Table 19 Variance analysis of eater by class (‘*’ significant).

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	9	236.4855	26.2762	6.1889	<.0001*
Error	199	844.8954	4.2457		
C. Total	208	1081.3809			

Table 20 Effect tests on eater expression ('*' significant).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Race	4	4	50.12263	2.9514	0.0212*
Treatment	1	1	114.76091	27.0299	<.0001*
Race*Treatment	4	4	67.60076	3.9805	0.0040*

3.8. Two-factor nested effects

The plot effect leverage option was used to produce a leverage plot for treatment effect on the expression of each immune genes in the model. These plots helped to view the impacts of points on the test while the effect is in the model. A point which was horizontally located away from the center of the plot had more impact on the effect test than a point which is closer to the center. The actual by predicted plot was used to view the test when all the parameters in the model were accepted as zero. When analyzing the effects of race and treatment on the expression of immune genes, treatment was nested within race category. Significance of these effect tests are shown in tables for each target gene. The Whole Model plot tested for all effects, race and treatment.

3.8.1. Response abaecin whole model

The whole model plot, tested for race and treatment was significant. Abaecin actual levels ($p < .0001$, $RSq = 0.22$, $RMSE = 4.2649$) (Figure 23). Two factor nested random effects of treatment ($p = 0.0255$) and Race(treatment) ($p < .0001$) on the expression of Abaecin were significant (Table 21) and they were shown by leverage plots (Figure 24). Least square plot for nested race-treatment effect (Figure 25) and only treatment effect (Figure 26) confirmed the previous One-way ANOVA results for Abaecin.

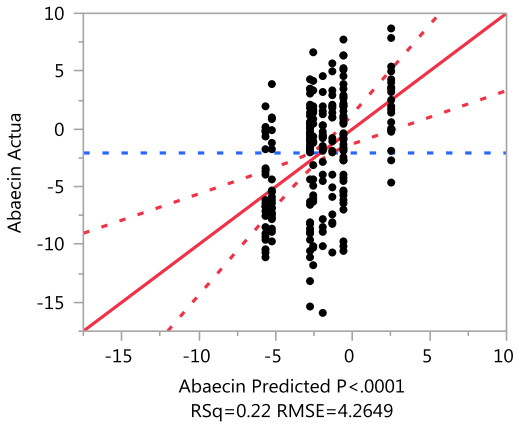

Figure 23 Whole model actual by predicted plot for abaecin response

Table 21 Two-factor nested effects of race and treatment for abaecin expression

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Race[Treatment]	8	8	981.09353	6.7423	<.0001*
Treatment	1	1	92.15448	5.0664	0.0255*

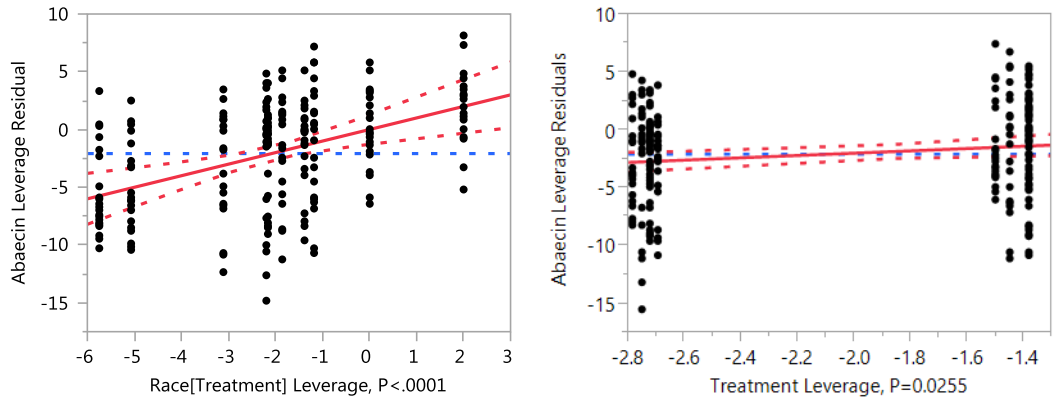

Figure 24 Race(Treatment) leverage plot (left) and Treatment leverage plot (right) for Abaecin Expression

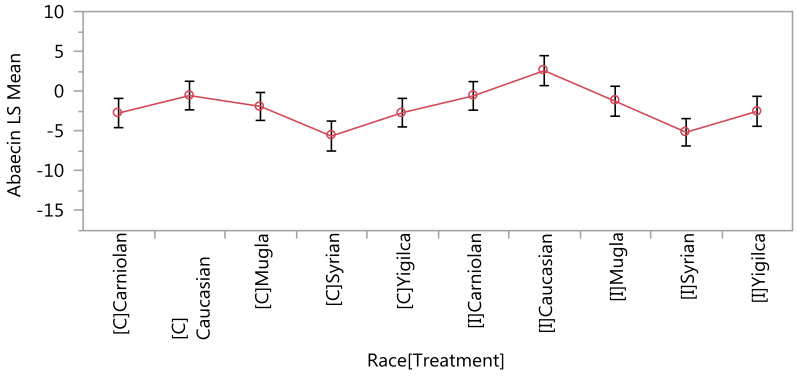

Figure 25 LS means plot of abaecin expression for Race(Treatment) nested effect on control and *N .ceranae* infected subspecies

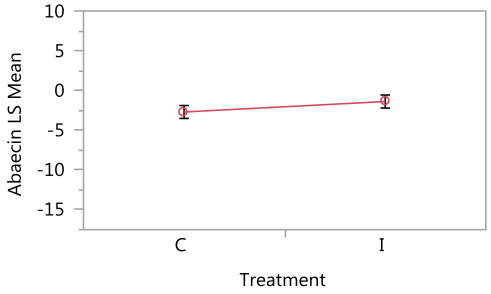

Figure 26 LS means plot of abaecin Expression for treatment effect (C= Control, I =Infected)

3.8.2. Response apidaecin whole model

According to the whole model plot tested for race and treatment, Apidaecin actual levels were significant (p < .0001, *RSq* = 0.28, *RMSE* = 2.2846) (Figure 27). Two factor nested random effects of Race(treatment) (p = 0. 0027) and treatment (p <.0001) on the expression of Apidaecin were also significant (Table 22) and they were shown by leverage plots (Figure 28). Least square plot for nested race-treatment effect (Figure 29) and only treatment effect (Figure 30) confirmed the previous One-way ANOVA results for Apidaecin.

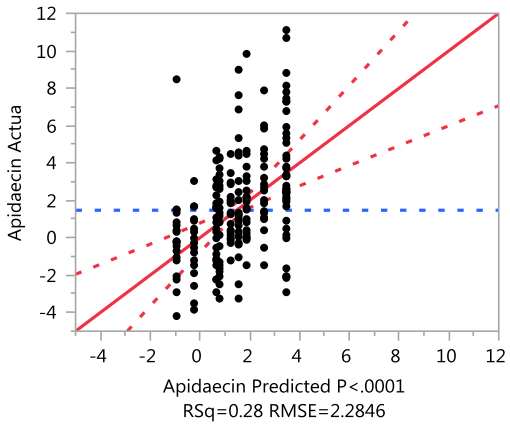

Figure 27 Whole model actual by predicted plot for apidaecin response

Table 22 Two-factor nested effects of Race and Treatment for apidaecin expression

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Race[Treatment]	8	8	128.38905	3.0748	0.0027*
Treatment	1	1	279.01216	53.4563	<.0001*

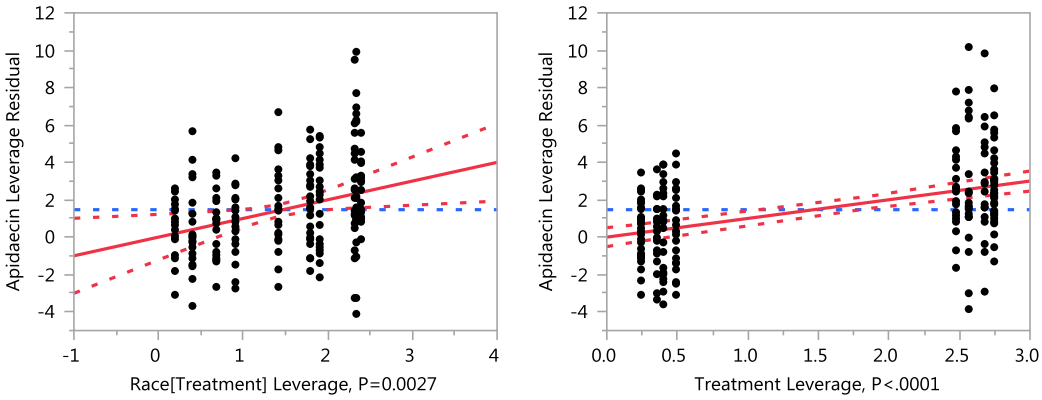

Figure 28 Race(Treatment) leverage plot (left) and Treatment leverage plot (right) for apidaecin expression

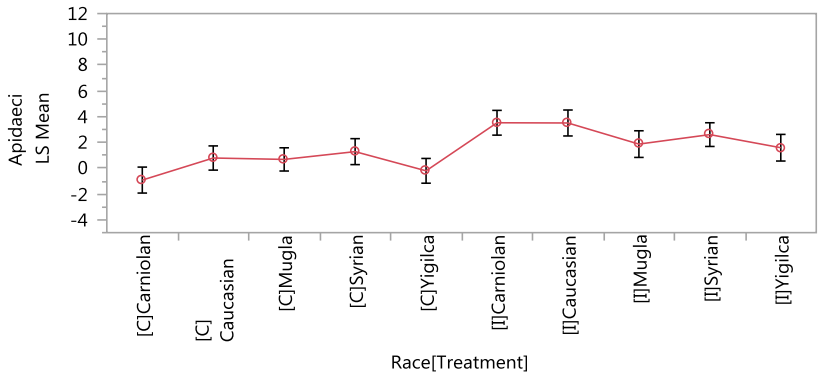

Figure 29 LS means plot of apidaecin expression for Race(Treatment) nested effect on control and *N .ceranae* infected subspecies

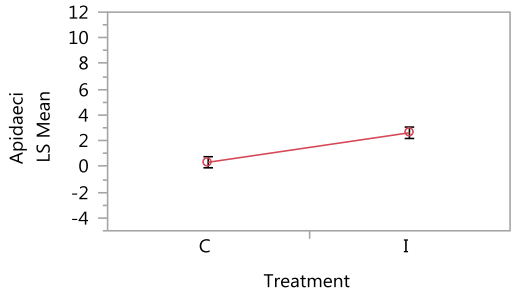

Figure 30 LS means plot of apidaecin expression for treatment effect (C= Control, I =Infected)

3.8.3. Response hymenoptaecin whole model

Based on the whole model plot which was conducted for race and treatment, Hymenoptaecin actual levels were significant (p < .0001, *RSq* = 0.16, *RMSE* = 3.0035) (Figure 31). Two factor nested random effects of treatment (p <.0001) and Race(treatment) (p = 0. 00249) on the expression of Hymenoptaecin were also significant (Table 23) and they were shown by leverage plots (Figure 32). Least square plot for nested race-treatment effect (Figure 33) and only treatment effect (Figure 34) confirmed the previous One-way ANOVA results for Hymenoptaecin.

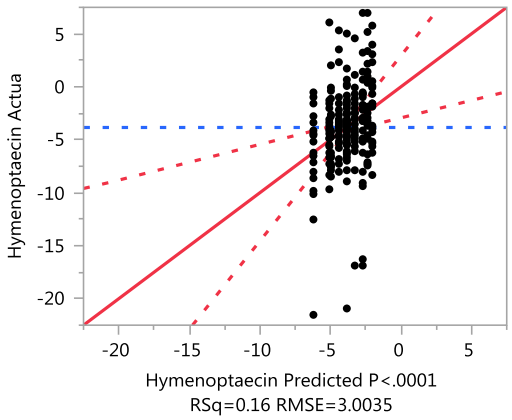

Figure 31 Whole model actual by predicted plot for hymenoptaecin response

Table 23 Two-factor nested effects of Race and Treatment for hymenoptaecin expression

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Race[Treatment]	8	8	163.02826	2.2590	0.0249*
Treatment	1	1	168.29337	18.6561	<.0001*

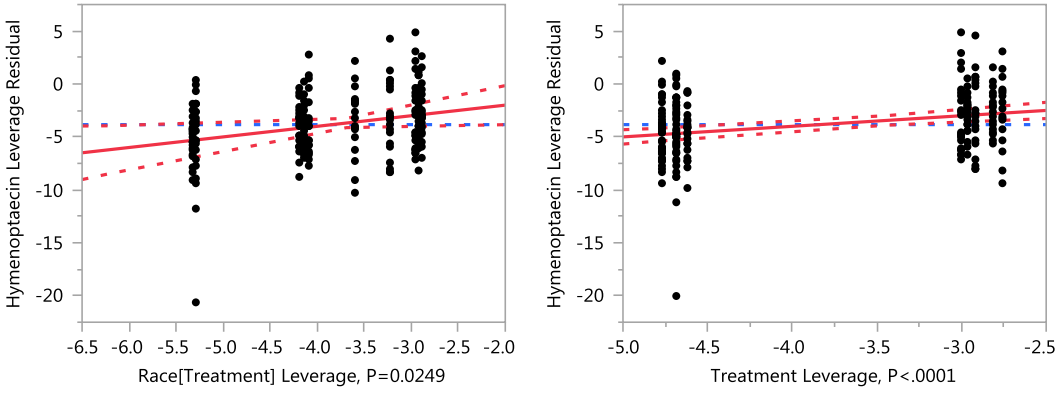

Figure 32 LS means plot of hymenoptaecin expression for Race(Treatment) nested effect on control and *N .ceranae* infected subspecies

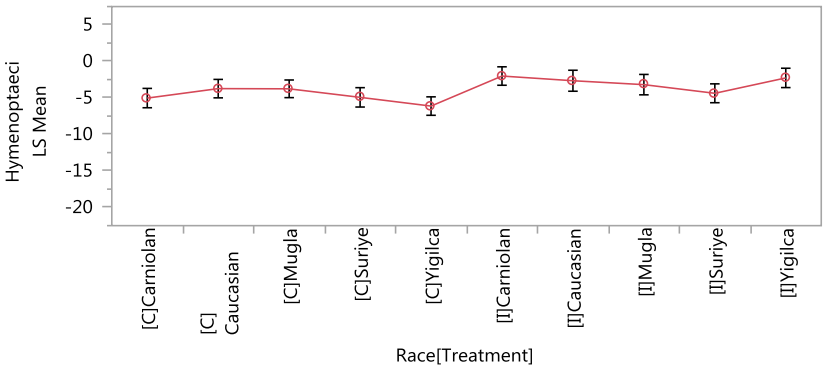

Figure 33 LS means plot of hymenoptaecin expression for Race(Treatment) nested effect on control and *N .ceranae* infected subspecies

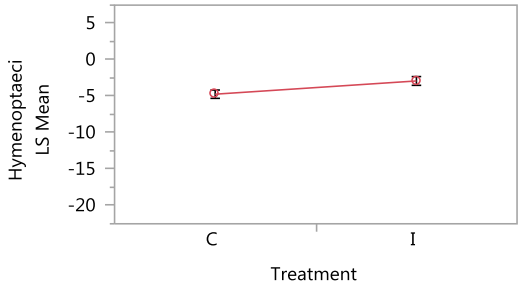

Figure 34 LS means plot of hymenoptaecin expression for treatment effect (C= Control, I =Infected)

3.8.4. Response defensin whole model

The whole model plot which was computed for race and treatment showed that actual defensin levels were significant ($p < .0001$, $RSq = 0.21$, $RMSE = 2{,}8584$) (Figure 35). Two factor nested random effects of treatment ($p < .0001$) and Race(treatment) ($p = 0.0006$) on the expression of Defensin were also significant (Table 24) and they were shown by leverage plots (Figure 36). Least square plot for nested race-treatment effect (Figure 37) and only treatment effect (Figure 38) confirmed the previous One-way ANOVA results for Defensin.

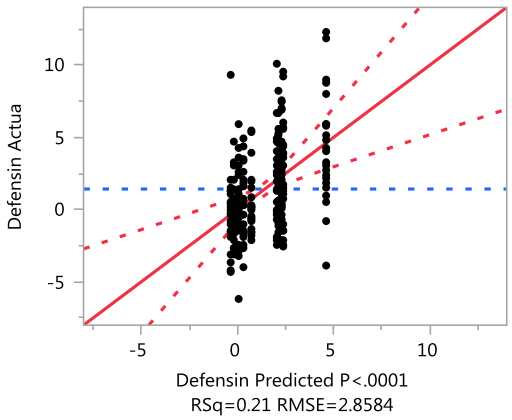

Figure 35 Whole model actual by predicted plot for defensin response

Table 24 Two-factor nested effects of Race and Treatment for defensin expression

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Race[Treatment]	8	8	237.43162	3.6324	0.0006*
Treatment	1	1	223.95010	27.4089	<.0001*

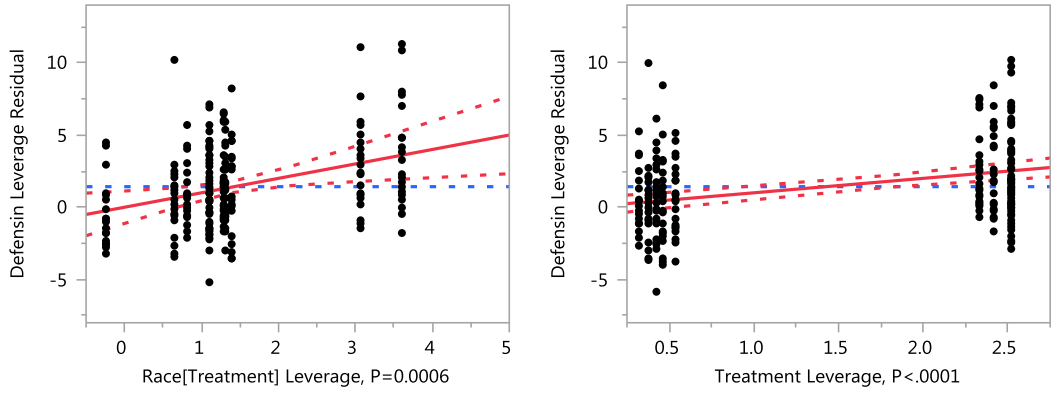

Figure 36 Race(Treatment) leverage plot (left) and treatment leverage plot (right) for defensin expression

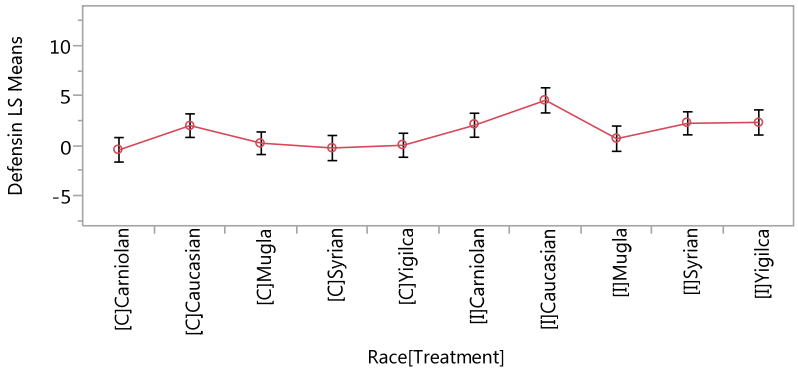

Figure 37 LS means plot of defensin expression for Race(Treatment) nested effect on control and *N .ceranae* infected subspecies

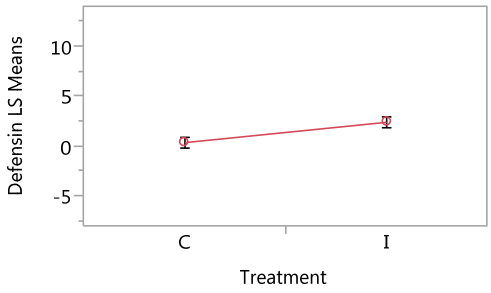

Figure 38 LS means plot of defensin expression for treatment effect (C= Control, I =Infected)

3.8.5. Response eater whole model

Based on the whole model plot which was tested for race and treatment; actual Eater levels were significant (p < .0001, *RSq* = 0.22, *RMSE* = 2.0605) (Figure 39). Two factor nested random effects of treatment (p <.0001) and Race(treatment) (p = 0. 0008) on the expression of Eater were also significant (Table 25) and they were shown by leverage plots (Figure 40). Least square plot for nested race-treatment effect (Figure 41) and only treatment effect (Figure 42) confirmed the previous One-way ANOVA results for Hymenoptaecin.

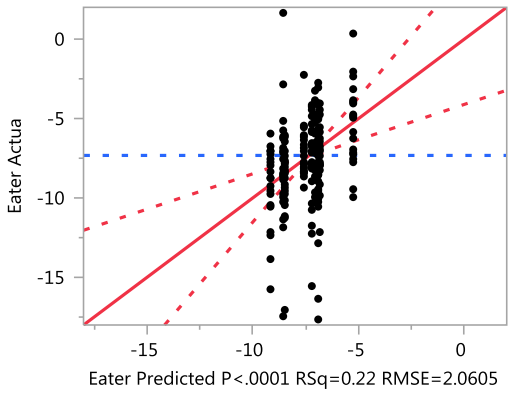

Figure 39 Whole model actual by predicted plot for eater response

Table 25 Two-factor nested effects of Race and Treatment for eater expression

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Race[Treatment]	8	8	119.78176	3.5266	0.0008*
Treatment	1	1	114.76091	27.0299	<.0001*

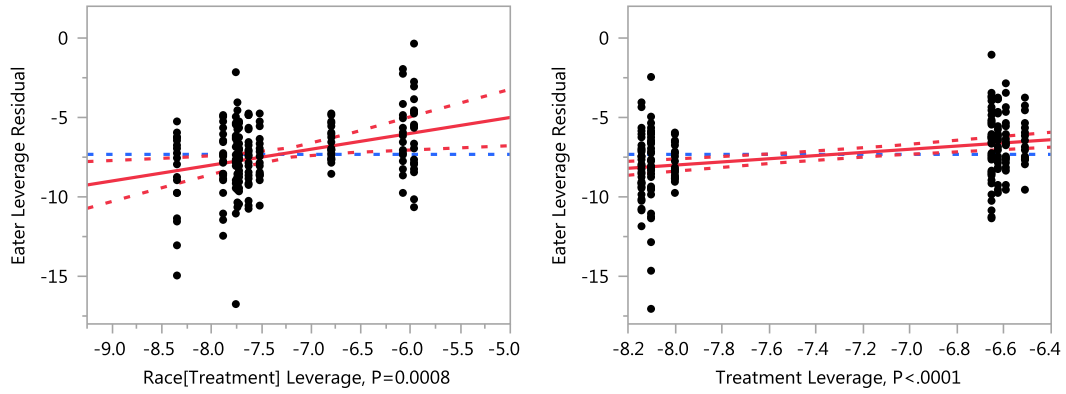

Figure 40 Race(Treatment) leverage plot (left) and Treatment leverage Plot (right) for eater expression

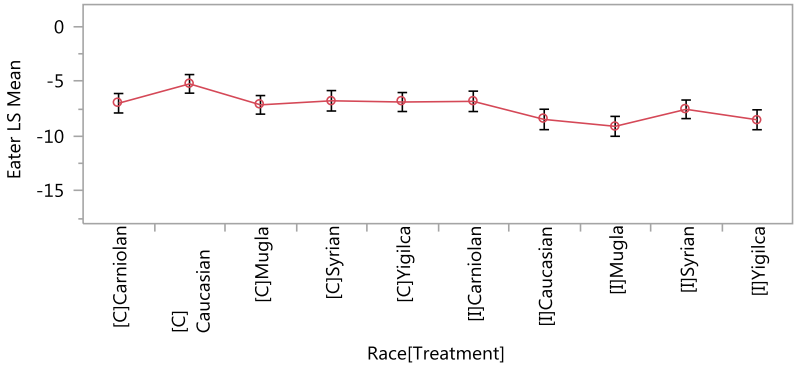

Figure 41 LS means plot of eater expression for Race(Treatment) nested effect on control and *N .ceranae* infected subspecies

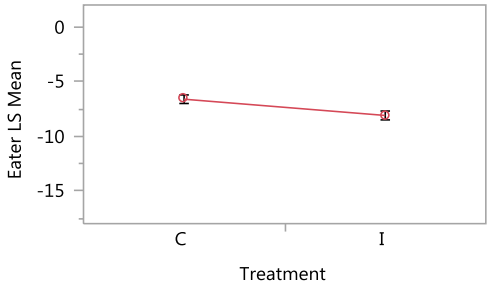

Figure 42 LS means plot of eater expression for treatment effect (C= Control, I =Infected)

3.8.6. Response vitellogenin whole model

According to the whole model plot that was computed for race and treatment, vitellogenin actual levels were significant ($p < .0001$, $RSq = 0.23$, $RMSE = 2.5758$) (Figure 43). Two factor nested random effects of treatment ($p = 0.0953$) and Race(treatment) ($p < .0001$) on the expression of Vitellogenin were also significant (Table 26) and they were shown by leverage plots (Figure 44). Least square plot for nested race-treatment effect (Figure 45) and only treatment effect (Figure 46) confirmed the previous One-way ANOVA results for Vitellogenin.

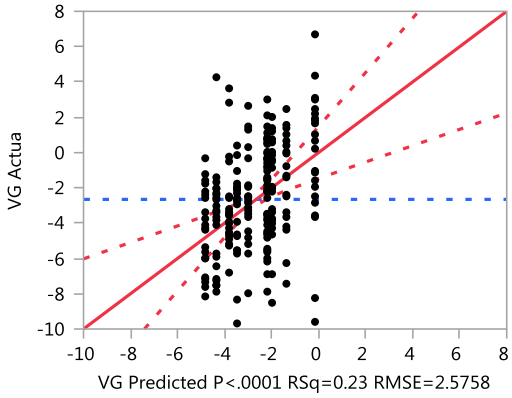

Figure 43 Whole model actual by predicted plot for vitellogenin response

Table 26 Two-factor nested effects of Race and Treatment for vitellogenin expression

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Race[Treatment]	8	8	372.36813	7.0153	<.0001*
Treatment	1	1	18.63311	2.8083	0.0953

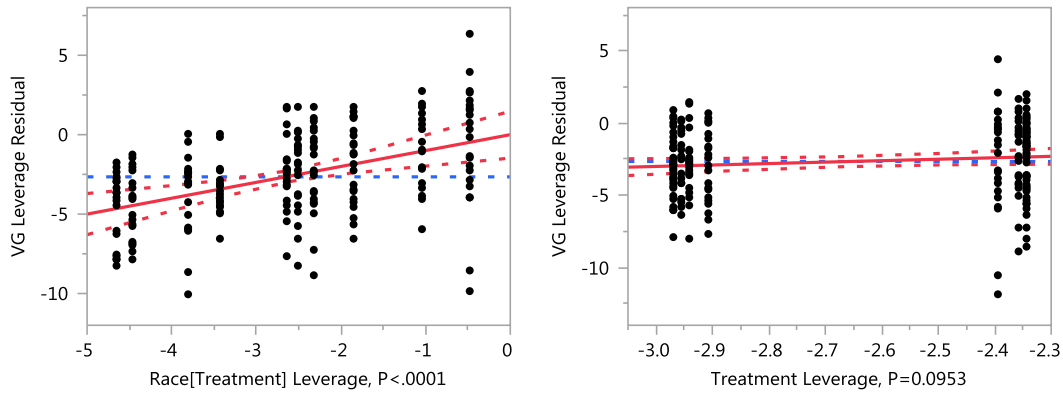

Figure 44 Race(Treatment) leverage plot (left) and Treatment leverage plot (right) for Vitellogenin Expression

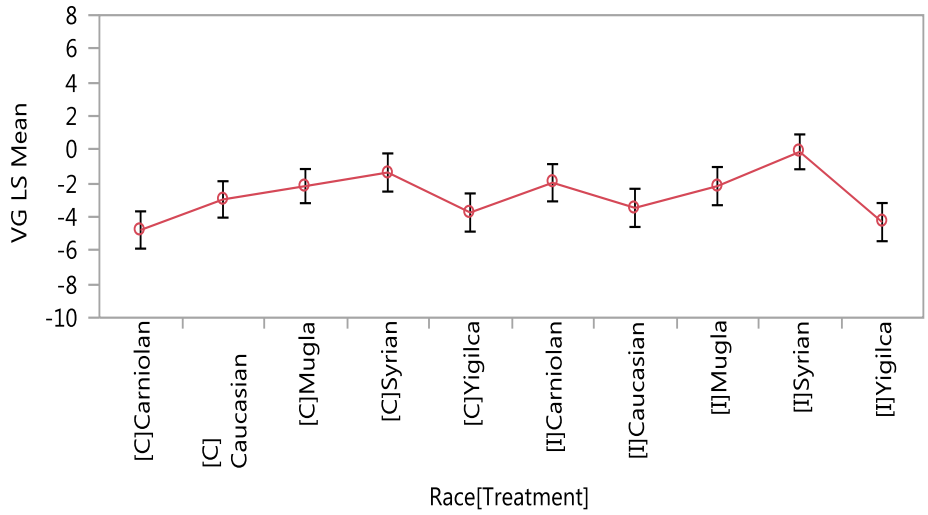

Figure 45 LS means plot of vitellogenin expression for Race(Treatment) nested effect on control and *N .ceranae* infected subspecies

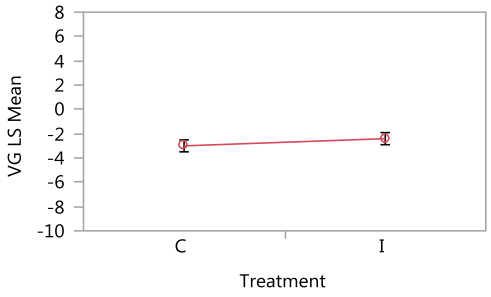

Figure 46 LS means plot of vitellogenin expression for treatment effect (C= Control, I =Infected)

3.9. Locomotor activity monitoring results

Mean activity of *N. ceranae* infected bees of Mugla and Caucasian honeybees were always lower than the control ones along 12 days of monitoring period. The mean activity of infected Caucasian and Mugla bees showed a decreasing trend starting from day 3 as well as control bees (Figure 47 and 48). In contrast, the mean activity of *N .ceranae* infected and control bees of Yigilca ecotype did not indicate a steady decrease and they showed variation in their activity throughout the experiment. But the mean activity of *N .ceranae* infected bees of Yigilca ecotype was lower than the controls (Figure 49).

At the beginning, the mean activity of infected bees of Carniolan subspecies were higher than the controls but their activity started to decrase at day 4 and their mean activity become lower than the controls starting from day 6 (Figure 50). Similarly, the mean activity of infected bees of Syrian subspecies also exceeded the activity levels of controls but after day 6, the activity levels of *N. ceranae* infected ones indicated apparent decreasing response in comparison with the controls (Figure 51).

Comparisons among subspecies were done by analysing cumulative activity data. One-way analysis of variance indicated significant differences when comparisons were done among the control and *N .ceranae* infected subspecies throughout 12 days of monitoring period (Figure 52). During the 6^{th} day of monitoring, the highest mean activity level was observed in Mugla, Caucasian, Syrian, Yigilca, Carniolan controls, respectively ($F = 2.8015$, $p = 0.0369$) and they varied in their mean activity levels on 8^{th} and 12^{th} days of monitoring period.

When comparison was done for *N .ceranae* infected subspecies, Syrian race had the highest activity and it was followed by Mugla, Caucasian, Carniolan and Yigilca on day 6 of *N .ceranae* infection ($F= 3.2785$, $p = 0.0180$) and the subspecies showed similar order with declining activity levels on 8^{th} day of *N .ceranae* infection ($F= 2.7964$, $p = 0.0354$). All of the races followed a decreasing trend in their activity levels along the monitoring period except for Yigilca ecotype. Although the mean activity levels of Yigilca ecotype seems to be increasing according to the cumulative activity data, actually it showed insignificant variation in the mean activity levels throughout the experiment. The mean activity levels of control bees of Caucasian race and Mugla, Yigilca ecotypes were higher than the *N .ceranae* infected ones. Despite Carniolan and Syrian subspecies seems to have increased mean activity of *N .ceranae* infected bees than controls according to the cumulative data, when the data was evaluated day by day, it is clear that the mean activity values of *N .ceranae* infected Syrian and Carniolan bees start to fall behind the controls after 6^{th} day of monitoring period. Activity level differences among the subspecies were also confirmed by effect tests. There was a significant effect of race on cumulative activity data till 8^{th} day of infection (Day 1 ($p = 0.0164$), day 2 ($p = 0.0082$), day 3 ($p = 0.0088$), day 4 ($p = 0.0037$), day 5 ($p = 0.0030$, day 6 ($p = 0.0061$), day 7 ($p = 0.0393$).

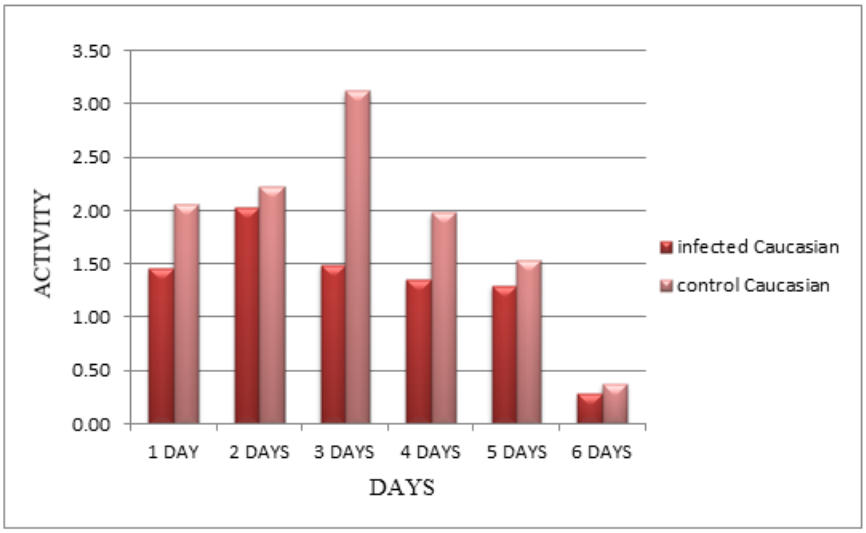

Figure 47 Locomotor activity levels of Caucasian subspecies throughout 6-days of *N. ceranae* infection period

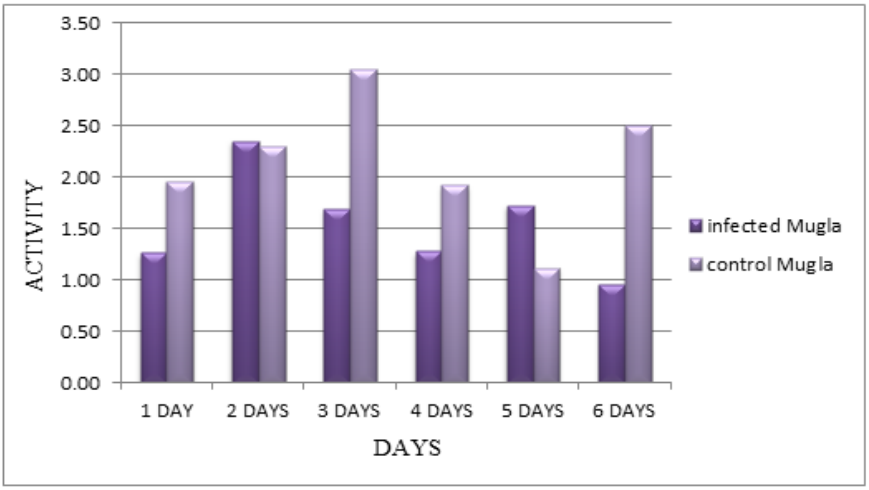

Figure 48 Locomotor activity levels of Mugla ecotype of A. m. anatoliaca throughout 6-days of *N. ceranae* infection period

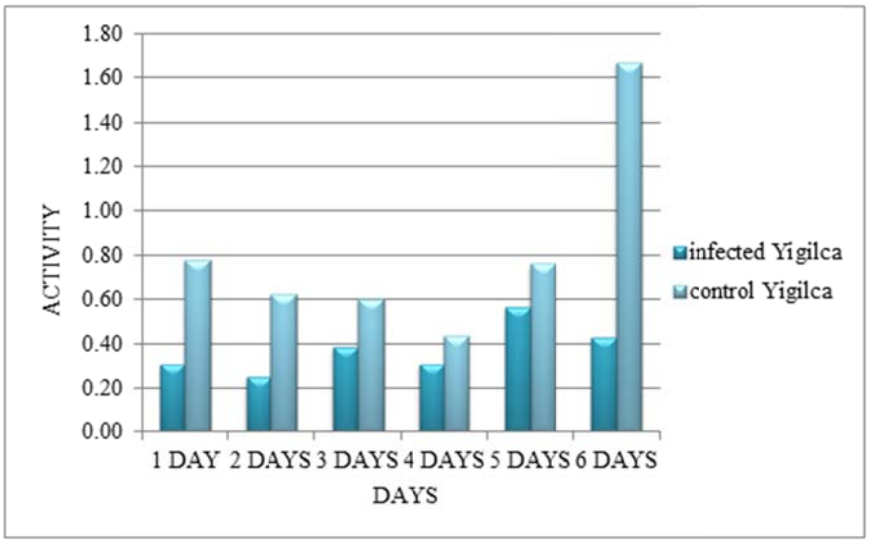

Figure 49 Locomotor activity levels of Yigilca ecotype of *A. m. anatoliaca* throughout 6-days of *N. ceranae* infection period

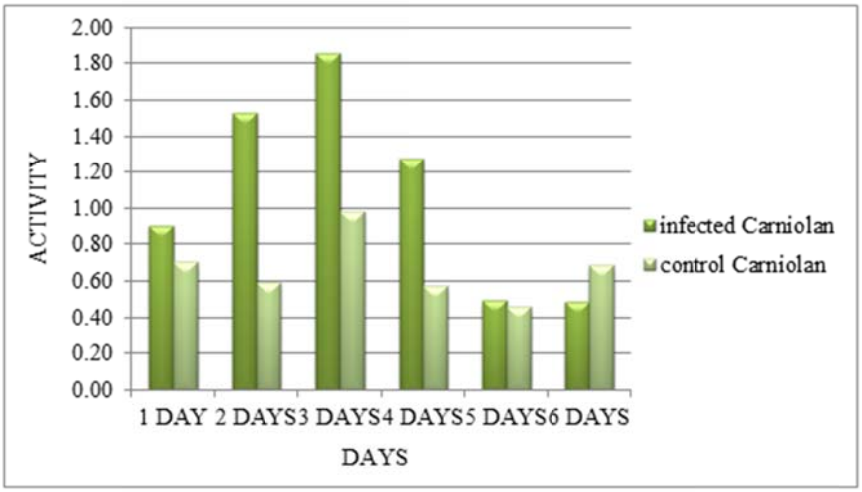

Figure 50 Locomotor activity levels of Carniolan subspecies throughout 6-days of *N. ceranae* infection period

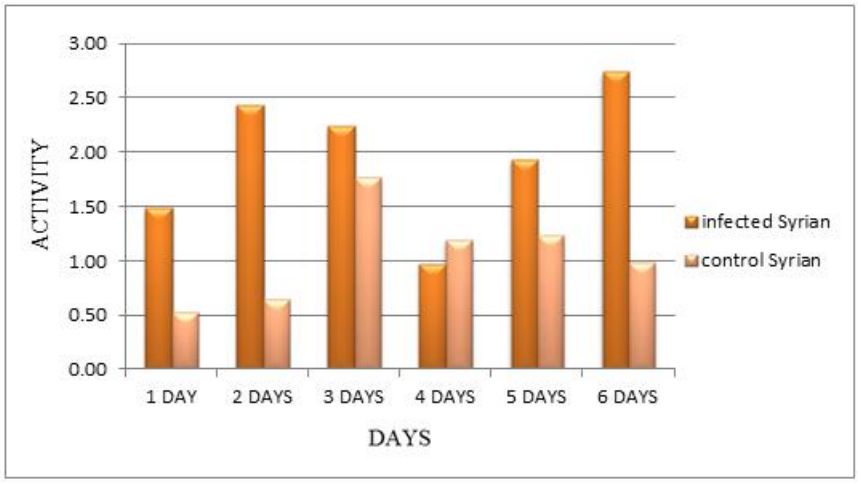

Figure 51 Locomotor activity levels of Syrian subspecies throughout 6-days of *N. ceranae* infection period

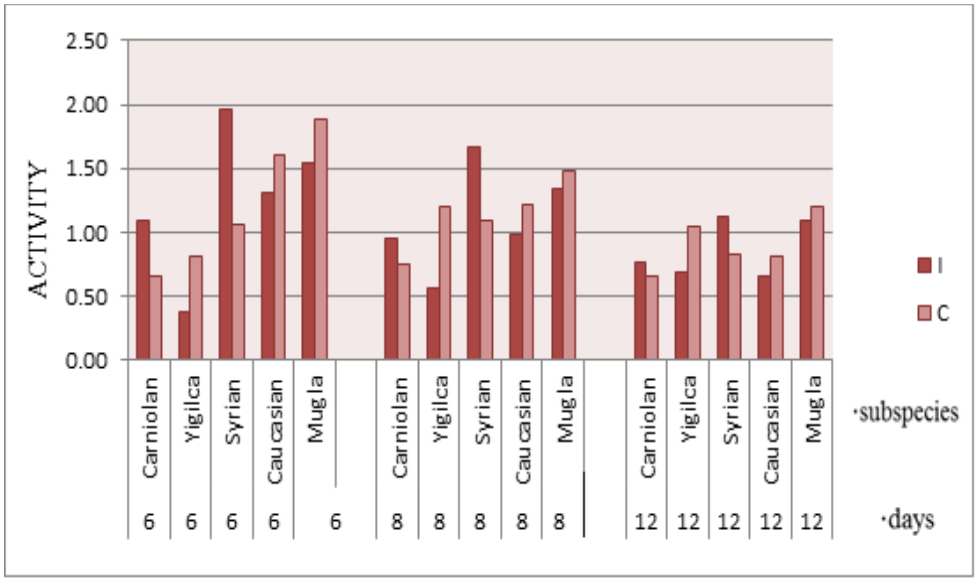

Figure 52 Locomotor activity levels of the honey bee subspecies during 6th, 8th and 12th day of *N. ceranae* infection.

3.10. RT-qPCR results of pathogen prevelance of honeybee samples collected from different regions of Turkey

Kashmir bee virus (KBV), Israeli acute paralysis virus (IAPV), American foulbrood (AFB) and Sacbrood virus (SBV) were not detected by PCR in any of the samples, nor was the microsporidian pathogen *N. apis* (except for only 3 colonies in 2011) by RT-PCR.

Present in these samples were Acute bee paralysis virus (ABPV), Deformed wing virus (DWV), Black queen cell virus (BQCV), and *N. ceranae*. Mixed infections of ABPV, DWV, BQCV, and *N. ceranae* were detected (Table 27 and 28).

Table 27 The results of pathogen analysis of honey bee samples collected from different regions of Turkey. '*' Detected among samples of 2011only

Sampling Date	Locations	Pathogens
2010-2011	Muğla	ABPV, BQCV, DWV, *Nosema ceranae*, Trypanosomes
2010-2011	Hatay	ABPV, BQCV, DWV, *Nosema ceranae*, Trypanosomes
2010	Ankara	ABPV, BQCV, DWV, *Nosema ceranae*, Trypanosomes
2011	Yığılca	ABPV, BQCV, DWV, *Nosema ceranae*, Trypanosomes
2010-2011	Kırklareli	ABPV*, BQCV, DWV, *Nosema ceranae*, Trypanosomes
2010-2011	Ardahan	ABPV, BQCV, DWV, *Nosema ceranae**, Trypanosomes
2010-2011	Artvin	ABPV, BQCV, DWV, *Nosema ceranae**, Trypanosomes
2010	Bitlis	ABPV, BQCV, DWV, *Nosema ceranae*, Trypanosomes
2010	Edirne	BQCV, DWV, Trypanosomes
2010	Elazığ	BQCV, DWV, Trypanosomes

Table 28 Normalized pathogen loads across sites and between stationary and migratory beekeepers for 2010 and 2011. Abundances are log-based-two scale so every increase by one number reflects a doubling of pathogen RNA. 'ND' Not detected, '/' Sampling was not done.

	Stationary beekeepers									
	DWV		**ABPV**		**BQCV**		***N.ceranae***		**TRYP**	
	2010	***2011***	***2010***	***2011***	***2010***	***2011***	***2010***	***2011***	***2010***	***2011***
All sites	4.99	11.59	1.34	13.87	5.36	6.31	0.19	10.97	8.82	14.36
Muğla	7.02	12.57	0.37	11.6	5.83	3.94	0.42	3.81	ND	11.74
Hatay	10.99	15.16	3.42	8.69	8.86	5.99	ND	ND	12.36	1.91
Yığılca	/	17.55	/	19.40	/	ND	/	9.48	/	20.83
Kırklareli	3.88	9.15	ND	12.37	4.26	5.02	0.91	15.20	0.94	7.04
Ardahan	7.56	9.00	2.57	17.80	ND	13.00	0	13.67	ND	16.28
Artvin	ND	6.13	2.26	13.37	5.78	9.92	0	23.67	24.90	28.37
Ankara	4.31	/	0.78	/	11.64	/	0	/	21.59	/
Edirne	1.14	/	ND	/	1.15	/	0	/	1.92	/
	Migratory beekeepers									
	DWV		**ABPV**		**BQCV**		***N.ceranae***		**TRYP**	
	2010	***2011***	***2010***	***2011***	***2010***	***2011***	***2010***	***2011***	***2010***	***2011***
All sites	4.81	17.08	6.32	18.09	8.13	5.99	1.54	4.69	13.92	15.89
Muğla	6.62	17.77	3.60	15.42	8.64	6.52	2.25	5.34	23.04	18.54
Hatay	8.21	16.38	5.05	20.75	10.32	5.45	2.69	4.04	14.77	13.24
Ardahan	ND	/	4.63	/	13.16	/	ND	/	26.24	/
Ankara	ND	/	4.91	/	7.28	/	0.71	/	2.15	/
Bitlis	11.56	/	19.72	/	6.33	/	3.57	/	12.92	/
Elazig	2.44	/	ND	/	3.05	/	ND	/	4.38	/

The distribution of bee pathogens was significantly different among provinces and with beekeeping practices in 2010 (Appendix A). Generally, DWV loads were higher in Bitlis, Hatay, Muğla and Ardahan than in other regions. ABPV was the most common virus in Bitlis and was especially high in the samples of migratory beekeepers. Among provinces that were sampled, BQCV loads were lowest in Edirne and highest in colonies of migratory beekeepers sampled in Ardahan, Hatay and Muğla (Table 28).

In 2011, DWV, BQCV and *N. ceranae* loads were significantly different among the regions (Appendix A). DWV loads were higher in Muğla, Hatay and Yığılca. BQCV levels were high in Ardahan and Artvin and was not detected at all in Yığılca. *N. ceranae* was more frequent among Artvin, Ardahan and Kırklareli but not detected among stationary colonies of Hatay (Table 28). ABPV occurrence was the highest in samples from stationary apiaries of Yığılca and Ardahan and samples from migratory ones of Hatay and Muğla. The lowest level was in the stationary colonies of Hatay. ABPV loads showed difference between Yığılca-Kırklareli and Yığılca-Muğla ($p = 0.0398$ and $p = 0.0478$).

ABPV loads in Muğla and Ankara, BQCV loads in Ardahan, Muğla, and *N.ceranae* loads in Hatay were significantly higher in the samples of migratory beekeepers than the samples of stationary beekeepers in 2010. Among 2011 samples, the pathogen loads of migratory colonies were also higher in Muğla and Hatay but the results were not significant (Appendix A).

In 2010, *N. ceranae* was widespread in migratory colonies of Muğla, Hatay and Bitlis and less frequent in samples from Ankara and Kırklareli. *N. ceranae* was not found in samples of beekeepers from Edirne, Artvin, Ardahan, and Elazığ in 2010 (Table 28). In contrast, *N. ceranae* showed high incidence in all sites except for the stationary colonies of Hatay in 2011 (Table 28). *N. ceranae* loads were higher among migratory colonies than stationary ones in both years. *N. apis* was not observed among the samples of 2010 and most of the samples of 2011 although the analysis was repeated twice. In 2011, *N. apis* was detected in three stationary colonies in total, from three beekeepers in Muğla-Bodrum, Düzce-Nas and Ardahan-Posof.

Trypanosome loads were significantly different among regions in both years. Trypanosome abundance was higher within the samples of migratory beekeepers than those of stationary ones (Appendix A). 10/10 positive samples were confirmed by DNA sequencing as reflecting the 28S rRNA locus of trypanosomes. In general trypanosome levels were higher among samples from Muğla, Ankara, Artvin and Ardahan compared to the samples from Kırklareli, Edirne and Elazığ, for 2010. In 2011, levels were especially high in samples from Artvin, Yığılca and Muğla, and low in samples of stationary colonies in Hatay and Kırklareli (Table 3.27). Seasonal

variation was observed among samples collected in 2010 (ANOVA $p = 0.0023$). Specifically, trypanosome loads were highest in spring as opposed to fall. In Ardahan and Hatay, trypanosomes were not detected among any of the fall samples. Among the samples with trypanosome infection, thirteen 2010 samples for which products were assayed by DNA sequencing were all confirmed as being part of the 'SF' strain of *L. passim* (identified as *C. mellificae*) (Runckel et al., 2011) and ten were positive among 51 samples in 2011, based on qPCR and melt-curve analyses (7 products were confirmed by DNA sequencing). 4 of the 'SF' positive samples were from Muğla, 6 from Hatay, 8 from Artvin-Ardahan, 4 from Yığılca, and one from Elazığ. GAPDH sequence analyses from Artvin and Yığılca all matched *L.* passim, haplotype A (Morimoto et al. 2013), indicating that this haplotype was more common among the samples when compared with haplotype B.

S. melliferum was detected for 2 samples in Artvin province in 2010 while 13 samples were positive for Artvin, Ardahan, Muğla, Yığılca and Kırklareli during 2011. Six of the positive samples were confirmed by DNA sequencing. *S. apis* was detected in only 2 samples from Muğla and Elazığ in 2010 and in one sample from Yığılca in 2011.

Dual infections of DWV and BQCV were detected in both migratory and stationary beekeepers in 2010 (50-100 % of honey bees in 6 provinces sampled). Dual infections with these two viruses were less commonly observed in 2011 (18-40 % of honey bees in 4 of the provinces sampled). Triple infections of ABPV, BQCV and DWV were seen in 3 regions (50-100 % of honeybee samples) in 2010, whereas occurred less (18-40 % of honey bee samples) at 4 regions in 2011. Among 2010 samples, *N. ceranae* loads were correlated with DWV loads ($r = 0.25$, $p = 0.0186$). ABPV loads were correlated with both DWV ($r = 0.40$, $p = 0.0001$) and *N. ceranae* ($r = 0.35$, $p = 0.0009$). BQCV correlated with DWV within samples from Ankara, Bitlis, Edirne and Muğla. In Bitlis, ABPV levels were positively correlated with DWV ($r = 0.89$, $p = 0.0061$). In Hatay, BQCV correlated with *N. ceranae* ($r = 0.47$, $p = 0.0443$). Among 2011 samples ABPV showed correlations with DWV ($r = 0.29$, $p = 0.0381$) and in some regions with *N. ceranae*. A positive correlation was observed between trypanosome load and *N. ceranae* ($r = 0.28$, $p = 0.0083$) and between trypanosomes and BQCV ($r = 0.59$, $p < 0.0001$).

According to the survey results, there was an increase in colony losses in 2011 when compared with 2010 losses (Figure 53). Kırklareli, Muğla , Hatay, and Ankara had relatively higher colony losses in both years while Ardahan and Artvin tended to have lower losses than the other provinces. High colony losses were also observed in Edirne and Bitlis in 2010 and especially in Yığılca in 2011. Average colony losses of migratory beekeepers were significantly higher than those of the stationary beekeepers.

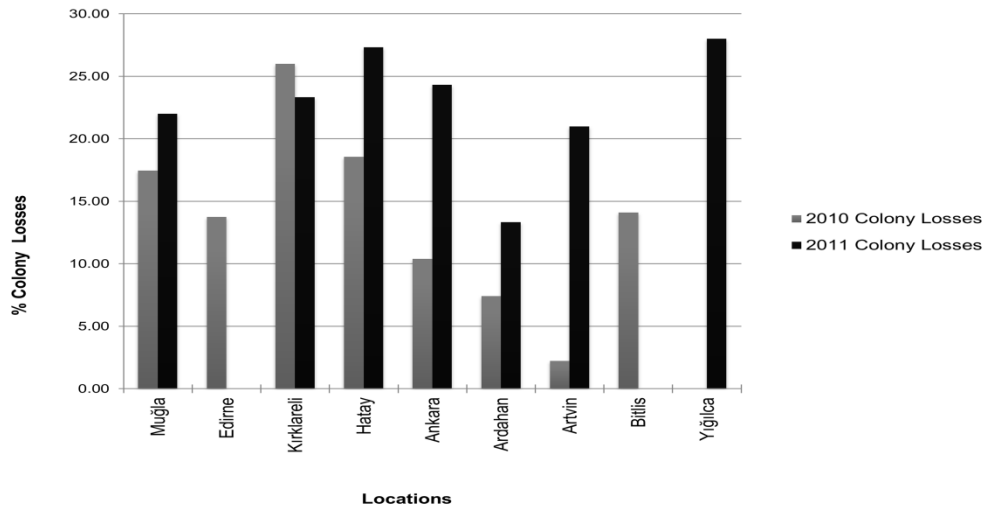

Figure 53 Percent colony losses of surveyed beekeepers by location in 2010 and 2011.

3.11. Read Mapping results of pathogen prevelance of honeybee samples collected from different regions of Turkey

DWV reads were detected in sequenced RNA libraries from all of the regions (Figure 54 and Appendix B), although they were most highly represented in Hatay, Yığılca, and Muğla, matching the results from real time q-PCR. We found strong evidence for *Varroa destructor* virus-1 as well, a first for these regions. Overall, the DWV/VDV group of iflaviruses was the predominant viral taxon (Figure 3.44). RNA sequences of BQCV were highest in the Ardahan region, although BQCV reads were also abundant in other provinces.

Consistent with the fact that BQCV was not detected in Yığılca by RT-qPCR very few reads were found in this province during RNA sequencing According to the sequence analysis, ABPV contigs were common in most regions. Consistent with RT-PCR results, they were most frequently observed in Yığılca and Ardahan.

Very few CBPV reads were found in Kirklareli province and this virus was not detected or was very few in other regions. In contrast, the related Lake Sinai viruses were highly represented both in diversity across this large clade, and in abundance. IAPV and KBV were notably rare or were not present across all regions. Sacbrood virus was similarly rare, although 1885 SBV reads were detected in Ardahan region. SBPV reads (Slow Bee Paralysis Virus) were not observed in any of the regions (Appendix B).

Nosema ceranae was highly prevalent in our RNA-seq analysis. *N.ceranae* was especially common in Artvin province, again matching our RT-qPCR analyses. *N. ceranae* was not detected in stationary colonies of Hatay province but it was evident there by RNA-seq analysis. We found minimal, if any reads matching *N. apis* in the RNASeq data, those present were largely the result of regions with high sequence similarity to *N. ceranae*. Reads for the betaproteobacterium, *Snodgrassella alvi* were common among all of the provinces with highest frequency in Kırklareli, Muğla, Yığılca and Ardahan. The gamma-proteobacterium, *Gilliamella apicola* was prevalent in all regions, while the related *Frischella perrara* was rare (Appendix B). Consistent with both RT-qPCR and DNA sequencing, the presence of *Spiroplasma melliferum* was confirmed in most of the regions. The abundance of congener *S. apis* was confirmed with the RNA-seq analysis despite being missed by PCR, yet *S. apis* remained the minor of the two species.

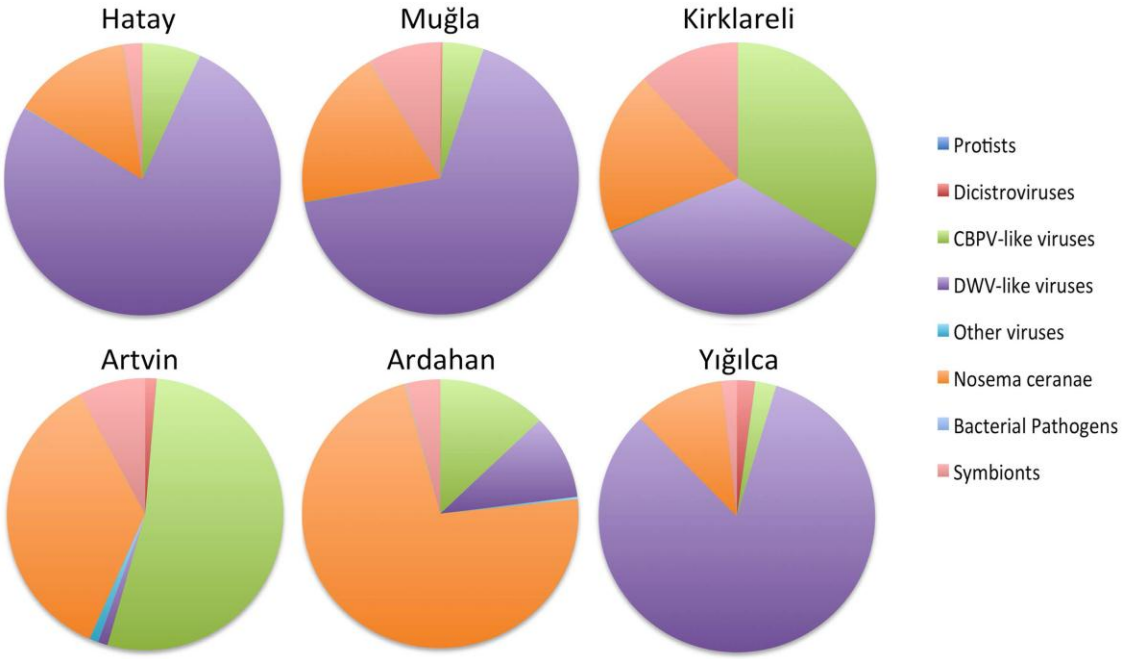

Figure 54 Relative microbial loads across the six regions, as inferred by RNA-Seq matches. Taxa are binned by taxonomic group.

CHAPTER 4

DISCUSSION

4.1. Immune gene responses

Immune responses of honeybees were examined 6 days after *N. ceranae* infection. It was observed that honeybee defence machinery were activated by honeybee immune components for all of five the subspecies after infection with *N .ceranae*. This activation involved the expression of antimicrobial peptide encoding genes with rapid increments. Six days old bees were analyzed in this study because investment in immune function becomes utterly variable as they age and shift physiological state, behavioral task or initiate immunosenesence (Amdam et al., 2005b).

Pairwise differences between infected and control bees indicated higher increase in mRNA expression in response to *N. ceranae* for Caucasian, Carniolan subspecies and Yigilca ecotype. Accordingly, a rapid increase was observed in hymenoptaecin, abaecin, defensin and apidaecin transcripts of these subspecies. Overall, mRNA means of *N. ceranae* in both control and infected bee groups of Suriye race and Mugla ecotype were higher than the others. These subspecies also showed a trend towards an increase in the expression of antimicrobial peptides but this increase was not stronger than the other races. More susceptibility to *N. ceranae* might be the result of weaker humoral immunity of these subspecies than the other races. Especially Caucasian bees demonstrated highest immune response following the inoculation by *N. ceranae.* Varying immune responses may indicate the discrepancies in the ability of the honey bee subspecies to get rid of *N. ceranae* infection. In the study of Werner et al. (2000), dissimilarities among bees in their capability to perceive pathogens were also attributed to genetic variation in immune responsiveness. Variation in AMPs response intensity is based on honey bee genetic background (Decanini et al., 2007).

Immune defences of honeybees were activated rapidly six days after inoculation with *N .ceranae* with increasing expression levels of four antimicrobial peptide encoding genes defensin, hymenoptaecin, apidaecin and abaecin that are associated with bee humoral immunity. Production of antimicrobial peptides is a sign of an active humoral immune mechanism. This is supported by the data of Schwarz et al. (2013) that showed honey bees showed escalating immune responses to *N .ceranae* experimental infections identical to those found in honey bees having natural *N. ceranae* infections (Jefferson et al., 2013) and to artifical infections in drones (Huang et al., 2012). The results of Chaimanee et al. (2012) showed the downregulation of these four genes 3 and 6 days after infections but the genes did not express any differences between control and infected *A. mellifera ligustica* bees after 12 days of infection. *N. apis* inoculated *A. mellifera iberiensis* showed increased expression levels of abaecin, defensin and hymenoptaecin during 4th day of the injection in the study of Antunez et al. (2009). Although the pathogen invasion had already started (Higes et al., 2007), abaecin and hymenoptaecin expression was not influenced 4 days after *N .ceranae* infection but their expression diminished 7 days after infection proposing that *N. ceranae* suppressed humoral immunity. But this reduction might be related to spore dosage 10-fold greater than that needed for a successful infection (Forsgren and Fries, 2010). No significant differences were observed in expression of abaecin, hymenoptaecin, defensin mRNA levels between *N. ceranae* infected bees from colonies with higher levels of infection and lower infection levels or between control bees from both type of colonies by using hybrids of *A. mellifera mellifera* and *A. mellifera scutellata* in another study of Antunez et al. (2013). Downregulation of hymenoptaecin was found when honey bees were infected with *N .ceranae* alone or in application with an insecticide (Aufauvre et al., 2014). Also the Asian honeybee *Apis ceranae* indicated variation in antimicrobial peptides (especially hymenoptaecin peptides) (Xu et al., 2009). Induction of three immune peptides (hymenoptaecin, defensin 1 and abaecin) was also revealed after infection by bacteria in a similar study. Hymenoptaecin was the most outstanding antimicrobial peptide detected in young bees and larvae (Randolt et al., 2008) and in drone larvae and adult drones after bacterial infection (Gatschenberger et al., 2012). Toll and Imd pathways, two of four signaling cascades contribute to innate immunity, have significant roles in the control of transcription of antimicrobial peptide encoding genes (Hultmark, 2003).

Imd signaling pathway mediates the expression of abaecin and hymenoptaecin (Schluns and Crozier, 2007) and defensin expression is carried out by the interaction of Toll and Imd pathways (Arronstein et al., 2010). Thus, production of hymenoptaecin, abaecin and defensin is an apparent sign for activation signaling pathways of the innate humoral system. It was suggested that honey bee immune response can be fairly non-specific according to the results of increased abaecin mRNA levels when honey bee larvae was treated with either *P. larvae* or nonpathogenic bacteria (Evans and Lopez, 2004).

The expression of abaecin, defensin, apidaecin and hymenoptaecin transcripts increased in correlation with each other for all of the subspecies included in this study. Augmented immune gene expression might be one of the ways of successfully preventing the pathogen development. Apidaecin which is known to have a role against the pathogen attacks in the first step (Casteels et al., 1989), significantly elevated especially in relation with hymenoptaecin for all the five subspecies. Hymenoptaecin is also known to be complementary to apidaecin (Casteels et al., 1993). Abaecin functions as a backup and defensin takes part in the later stages of infections (Randolt et al., 2008). When comparisons were made for the expression levels of AMPs in a study, it was evident that abaecin expression levels were not influenced from *N .ceranae* exposure in four different honey bee species. *A. ceranae* and *A. florea* indicated upregulation of mRNA levels of abaecin and an increase in mRNA levels of hymenoptaecin in *A. ceranae* after inoculation with Canadian isolate of *N .ceranae* in the same study (Chaimanee et al., 2013). The up-regulation of apidaecin gene was also detected in the gut of *N. ceranae* infected queen bees with different ages following the 6^{th} day of the infection and the results showed that *N. ceranae* triggers apidaecin expression in early periods of infection (Chaimanee et al., 2014). In the study which explores the impacts of *N. apis* and *N .ceranae* infections on immune responses of honeybees, an increase in abaecin expression was observed 4 days after infection with *N. apis*. Although defensin expression increased following *N .apis* infection at day 4 and 7, there weren't any changes observed following the infection with *N. ceranae*. Hymenoptaecin expression levels were augmented 4 days after infection by *N. apis*, while *N .ceranae* reduced its expression level (Antunez et al., 2009).

Significant reduction in the levels of eater transcripts six days after *N. ceranae* treatment was observed in this study. No changes were observed in the expression of eater gene in *N. ceranae* infected bees in a similar study (Chaimanee et al., 2012). Also gut samples of 1, 6 and 12 days old honey bee queens revealed a decrease in expression of eater gene 6 days after infection (Chaimanee et al., 2013). Eater is an EGF-like repeat transmembrane receptor that is expressed in *Drosophila* hemocytes and also described in the honey bee genome for its role in phagocytosis of broad range of bacterial pathogens (Kocks et al., 2005; Evans et al., 2006). In this study, the transcript abundances of eater gene decreased in correlation with the other antimicrobial peptides while AMPs were increasing in response to *N .ceranae.* Six days after *N. ceranae* infection, it seems that humoral immune response is activated before cellular immune response. Humoral immunity involves antimicrobial peptide (AMPs) synthesis into the hemolymph by fat body and it was reported that host resistance was increased by the synthesis of AMPs that manage bacteria for the Eater-based phagocytosis during their attempts to pass through the gut (Chung et al., 2011). *Toll* pathway was reported to be the basic immune pathway in defence against *N. ceranae* (Huang et al., 2012). After bacterial infection, normal responses were found in NF-_B-like Toll and IMD signaling pathways with ineffective phagocytosis and lower ability to survive in *Drosophila* flies which did not have the *eater* gene (Kocks et al., 2005). In the same study, no protective role for eater after injury with *E. coli* or after infection with a fungal pathogen was also consistent with the finding that eater is not required for IMD or Toll pathway signaling. These findings might be one of the explanations for the reduction in eater gene expression levels after *N .ceranae* infection. Dainat et al (2012) observed reduction in hemocyte numbers of winter bees accompanied by a decrease *eater* expression levels from summer to fall, although humoral immunity was activated.

Vitellogenin expression levels increased in Carniolan and Suriye subspecies with a strong correlation with *N .ceranae* transcripts and weakly expressed in other subspecies after *N .ceranae* infection in this study. Carniolan and Suriye subspecies might be more capable of coping with the *N .ceranae* and initiating their defense mechanisms especially cellular defence against this pathogen.

Although other subspecies had decreased levels of eater transcripts, only Carniolan subspecies increased its expression and Syrian race had the lowest reduction in eater expression showing that these two subspecies might have better cellular response against the pathogen.

Vitellogenin (Vg) is is thought to act in reproduction, protect the bees from oxidative cellular damage, regulate immune function and lifespan of honey bee. Increasing levels of vitellogenin point out the life-span and immunocompetence of worker and queen bees (Amdam et al., 2004, 2005b; Corona et al., 2007). According to Nelson et al (2007), honey bees had low levels of Vg expression in *N .ceranae* infected bees in field conditions and they were expected to have fewer resistance to oxidative stress with shortened lifespan. For example, slower senescence lead to extended longevity of queen bees (Remolina et al., 2007). In the study of Chaimanee et al. (2012), Vg expression levels did not yield in significant differences between control and *N. ceranae* infected bees. *N .ceranae* infection suppressed the antimicrobial peptides in the study Antunez et al. (2009), and consistent with that finding; they found suppressed Vg expression with reduced lifespan of bees. Vitellogenin transcripts elevated in one and twelve days old queen bees inoculated with *N .ceranae* but during the age of six, they indicated lower expression (Chaimanee et al., 2014). Vitellogenin levels rised about 58 % in *N .ceranae* inoculated honey bee queens (Alaux et al., 2011). In the study about antibacterial immune competence of honey bees; they detected high levels of vitellogenin with hymenoptaecin and defensin 1 activity in winter bees infected with *E. coli*. Reactions of humoral and cellular defence were also activated by newly emerging adult workers and drones (Gatschenberger et al., 2012). Some authors claimed that components of innate immune system do not rely on vitellogenin expression. Because larvae and adult drones reacted with a strong immunity following the bacterial challenge when vitellogenin was not present (Gatschenberger et al., 2012). In contrast, no significant changes in vitellogenin transcripts were detected after *N .ceranae* infection indicating that higher vitellogenin expression levels in 9 and 20 days old bees from low level of infection colony could be ordinary, not induced by infection in those bees.

Also they recorded similar expression levels of AMPs, thus they concluded that different degrees of *N .ceranae* infection could be associated to a differential expression of vitellogenin (Antunez et al., 2013). Data analysis of the study related with predictive markers and colony losses, no variation was detected in vitellogenin for the collapsed colonies that did not survive winter. Oppositely, a very high increase was observed in vitellogenin transcripts for the colonies that survived winter, thus vitellogenin seemed to be a viable marker (Dainat et al., 2012).

Discrepancies in immune responses of honey bees among studies may arise from the differences in host genotypes, *Nosema* strains, spore doses used for infections, methodologies used for feeding the bees with spores, tissues used for isolation or physiological factors.

4.2. Activity Monitoring

Drosophila activity monitoring (DAM) system was used to measure locomotor activity of 5 different subspecies of *Apis mellifera* L. for 12 days following *N .ceranae* challenge. It was reported that honey bees (*Apis mellifera*) displayed distinct free-running rhythms in activity at the level of individual and also at the colony level (Moore and Rankin, 1985). Disease-free newly emerged bees were used in this experiment. Measuring circadian rhythms of honey bees was not the purpose of this study, and it is known that newly emerging honeybees which were isolated individually in a stable environment did not have rhythmic circadian pattern of locomotor activity, consumption of oxygen or temperature adjustment in their first days of adult life but they reveal circadian rhythms in later days (Moore, 2001, Nijland and Hepburn 1985). Thus keeping the environmental conditions constant during monitoring period was important. One of the restrictions of automatic data acquisition systems is acquiring high-quality data is efficient only for individual animals that are isolated and it may not be sociobiologically appropriate. The circadian system and locomotor activities are influenced by numerous social components in natural colonies. Meshi and Bloch (2007) found that young bees that stayed together with older forager bees had powerful rhythms in locomotor activity for 24-hours in comparison with the bees kept with the same number of younger bees, offering that the impact of social regulation on locomotor activities was not alike with the the age-depended division of labor.

For example, circadian rhythm in honey bees may be affected by socially-adjusted hive temperature conditions. It was reported that circadian rhythms were displayed by small experimental colonies of honeybees in cluster temperature (Nijland and Hepburn, 1985). Honey bees are endothermic insects which can internally arrange their body temperaturean and decrease was observed in the body temperature of a forager honeybee decreased at night (Kaiser, 1988). Although the influence of social factors were excluded, locomotor activity rhythms of isolated bees in the labarotory conditions, has provided compherensive knowledge on the locomotor system of individual honey bees (Fuchikawa and Shimizu, 2007a; Shimizu et al., 2001; Moore and Rankin, 1993)

In this study, infected Syrian subspecies had the highest activity levels among the races throughout six days period of *N. ceranae* infection despite this subspecies indicated higher *N .ceranae* expression and did not show stronger increase in the expression levels of antimicrobial peptides at day six when compared with the other subspecies. Increased activity levels of this subspecies after infection was also supported by high vitellogenin mRNA levels of Syrian honey bees during day six which might be the sign of its ability to resist earlier periods of *N .ceranae* infection. But after day six, the activity levels of this subspecies started to drop. The activity levels of infected honeybees started to decrease after six days and fall behind the activity levels of control Syrian honeybees.

mRNA means of *N.ceranae* in both the control and infected Mugla ecotype were also one of the highest. Unlike Syrian race, the activity levels of Mugla honey bees decreased following the infection by *N .ceranae.* Carniolan subspecies was one of the races that showed rapid increase in humoral immunity with increasing activity levels throughout 6-days of infection. Augmented vitellogenin levels at day six which means that this race is better to defend itself first 6-days after the *N.ceranae* infection but the activity levels started to diminish after day-6. Caucasian bees demonstrated highest immune response following the inoculation by *N. ceranae* with decreasing mean activity values. Yigilca ecotype demonstrated a rapid increase in antimicrobial peptide expression but a certain comment about the relation of their immunity and activity can’t be made since this ecotypes showed variation in the activity levels along the monitoring period.

Decreasing activity levels in spite of increasing humoral immunity can be explained in the context of cost-benefit. Producing higher levels of antimicrobial peptides might be leading to energetic demand and decreasing their activity. In the hive environment during their adulthood, costs of high immune responsiveness was documented as reduced longevity or colony productivity (Evans and Pettis, 2005) of impaired learning ability (Mallon et al., 2003). A more complete experiment involving evaluation of food consumption of experimental bees in the hoarding cages or activity monitoring system is needed to make precise comments.

These results can be supported by similar experiments with the bees exposed to *N .ceranae* under field conditions. The immune defenses of individual honey bees might be compensated by behavioral interactions in terms of social immunity mechanisms at the colony level (Cremer et al., 2007).

4.3. Evaluation of Real Time q-PCR results of pathogen prevelance of honeybee samples collected from different regions of Turkey

The surveyed regions comprise important centers of beekeeping in Turkey. Kashmir Bee Virus (KBV) and Israel Acute Bee Paralysis Virus (IAPV) were not detected in sampled colonies by RT-qPCR, while the related virus Acute Bee Paralysis Virus (ABPV) was abundant in most of the samples and was present in the regions where colony declines were observed. Historically, KBV has been found most frequently in the United States and Australia (Allen and Ball, 1996) and this virus is thought to be an exotic bee virus in central Europe (Berenyi et al., 2006). Our discovery of just one of these three species could reflect climatic and environmental conditions (Anderson, 1991) or differential abilities of these viruses to infect different honey bee subspecies.

DWV was detected in both migratory and stationary operations in all of the regions, consistent with the high prevalence of this virus worldwide (Tentcheva et al., 2004b; Berenyi et al., 2006; Kukielka et al, 2008, Nielsen et al., 2008; Welch et al., 2009). The presence of DWV has been reported in colonies of *Apis mellifera* L. in Ordu province of Turkey (Gülmez et al., 2009). BQCV was the second most widespread bee virus in our study.

BQCV is also the second-most prevalent virus in honey bee colonies in Asia and Europe (Tentcheva et al., 2004b) and its presence was confirmed in many studies (Berenyi et al., 2006; Choe et al., 2012; Kukielka et al., 2008, Welch et al., 2009). BQCV was previously reported in 21.42% of 28 bee samples from 6 provinces of the Black Sea Region in Turkey (Gümüşova et al., 2010). BQCV was more prevalent in migratory bee colonies, consistent with the results found for migratory bees sampled in the U.S. (Welch et al., 2009).

In most of the regions we studied, BQCV and DWV were found together more often than expected by chance. This result is consistent with the findings in a previous study of virus infections (Chen et al., 2004) for which these distantly related viruses were found coinfecting honey bees at a high frequency. Triple infections of ABPV, BQCV and DWV were detected in some colonies. Nielsen et al. (2008) also found a high incidence of dual and triple infections. According to Chen et al. (2004), 50% of colonies in the USA had dual infections while 7% had triple infections.

Simultaneous multiple infections were also common in Austria (Berenyi et al., 2006), Southwest England (Baker and Schroeder, 2008), Brazil (Teixeira et al., 2008) Jordan (Haddad et al., 2008), France (Tentcheva et al., 2004b) and Hungary (Forgach et al., 2008).

In Turkey, the presence of *N. apis* was confirmed earlier (Aydın and Cakmak, 2005; Whitaker et al, 2010; Muz et al, 2010). Using qPCR, we detected *N. apis* in only three colonies, indicating the replacement of *N. apis* by *N. ceranae* in many regions included in this study. Worldwide, *N. ceranae* is now far more common than *N. apis* (Chen et al., 2009; Valera et al., 2011; Yoshiyama and Kimura, 2011).

In 2006, *N. ceranae* was found in the provinces of Artvin, Hatay and Muğla (Whitaker et al., 2010). Collapsed colonies from the Hatay overwintering region and Southeastern Marmara were found to show infections of *N. ceranae* (Muz et al., 2010). In our 2010 samples, *N. ceranae* was observed in Bitlis, Hatay and Muğla and not in Edirne, Artvin, Ardahan and Elazığ in 2010. Interestingly, *N. ceranae* was widely common for all of the regions involved in the study in 2011. Detection of *N. ceranae* among 2011 samples of Ardahan-Artvin might be the reason for the increase in colony losses in these provinces in 2011.

The incidence of *N. ceranae* in Kırklareli province in 2011 was much more than the previous year. *N. ceranae* loads of samples from migratory beekeepers were significantly higher than the stationary ones and *N. ceranae* loads were correlated the viruses ABPV, BQCV and DWV. We propose that the infectivity of *N. ceranae* expands with migratory beekeeping activities and in association with different viruses and trypanosomatids. In July, the Thrace region is a highly frequented location by migratory beekeepers seeking to harvest sunflower honey. It is thought that these beekeepers in this region had colony losses because of the application of pesticides rather than honey bee disease factors. Hatay and Ankara are also important locations for migratory beekeepers. Caucasian bees which are used by most of the migratory beekeepers, are raised in Ardahan and Artvin. Bitlis also suffered high colony losses in 2010, combined with high disease loads among samples of migratory beekeepers. In contrast, while honeybee samples in Yığılca were derived from stationary colonies, high colony losses and high pathogen loads were observed. Probably some other factors were contributing to colony losses in this province. All five viruses and pathogens were also detected in Muğla, the center of migratory beekeeping.

These results could reflect the differences in pathogen exposure of local and migratory colonies, varying resistance levels, or perhaps a differential ability to handle stress. Genetic impacts of migratory beekeeping has become an important concern in Turkey and elsewhere. Population structure can be disturbed with hybridization, leading to a loss of regionally adaptive traits and perhaps decreasing colony fitness.

Trypanosomes were highly prevalent in our samples. Trypanosomes have also been reported in Australia (Langridge and Mcghee, 1967), China (Yang et al., 2013), France (Dainat et al., 2012), Japan (Morimoto et al., 2013), Switzerland (Schmid-Hempel and Tognazzo, 2010), USA (van Engelsdorp et al., 2009; Runckel et al., 2011) and Spain (Orantes-Bermejo, 1999). Trypanosome levels were higher in summer samples than the fall samples in our samples. In southern Spain, this parasite appears in July and August (Orantes-Bermejo, 1999) and *L. passim* (recently named as the primary trypanosomatid in lieu of *C. mellificae*, Schwarz et al., 2015) was common in summer colonies in Belgium (Ravoet et al., 2013). These findings are in

contrast with the results of Runckel et al. (2011) which show a peak in *L. passim* levels in January in U.S. colonies.

Synergistic effects can make colonies more vulnerable to other pathogens (Cornman et al., 2012) and we noted a positive correlation between *L. passim* and *N. ceranae* prevalence. Similarly, this relationship was documented in field surveys from the U.S. (Runckel et al, 2011) and the co-occurence of *L. passim* and *N. ceranae* in summer was tied to higher colony mortality in Belgium (Ravoet et al., 2013). Complex dynamic immune responses of honey bees to both *Nosema* and trypanosomatids were recently reported (Schwarz and Evans, 2013) and it will be interesting to test further for mechanistic explanations for any synergisms between these parasites.

4.4. Metagenomic sequencing of pathogen prevelance of honeybee samples collected from different regions of Turkey

As in prior studies, iflaviruses related to Deformed wing virus (DWV) were especially prevalent. While DWV amounts were higher, reads for the closely related *Varroa destructor*-1 virus (VDV-1) were fairly abundant in Hatay, Yığılca and Muğla. CBPV reads were relatively rare in most of the regions and were not detected in Yığılca and Ardahan. CBPV was reported in 7 of 28 (25%) samples from 6 provinces of Black Sea region in Turkey (Gümüşova et al., 2010). CBPV was detected in 4 of 96 apiaries in survey study of Denmark, in 73% of the samples from Greek and 9% of apiaries in China (Nielsen et al., 2008, Bacandritsos et al., 2010, Ai et al., 2012). A recently described relative of CBPV, Lake Sinai virus, was prevalent in all regions, second only to the DWV and VDV group. In fact, this group was the most prevalent virus in Kirklareli and Ardahan provinces. The pathogenic or epidemiological significance of Lake Sinai viruses are not well known. The LSV species complex is diverse, with members sharing between 70% and 99% sequence identity, hence this group is often missed in screenings based on PCR. LSV4 appears to be especially abundant, along with LSV1 and LSV2. There was significant variation across the regions in the specific lineages seen for this group. LSV2 was the most abundant single component of the honey bee microbiome in the study of Runckel et al., 2011. The presence of LSV was also confirmed in honey bees from Spain by high-throughput sequencing (Granberg et al., 2013). A new fourth strain of Lake Sinai Virus (LSV) was identified in the study of Ravoet et al. (2013).

In this study, despite tens of millions of microbial gene reads, few reads matching IAPV, KBV and SBV were seen. IAPV, KBV and SBV were not detected using RT-qPCR, indicating that RNA-seq sensitivity was higher than that of qPCR, or that current qPCR primers for this group must be redesigned to capture all strains. Given the RNA-Seq data, we can consider that these pathogens are quite rare in our samples. KBV has been absent in some other European surveys (e.g., Berenyi et al., 2006 Forgach et al., 2008) and rare in surveys in France, Denmark and United Kingdom (Tentcheva et al., 2004b, Nielsen et al., 2008, Ward et al., 2007). IAPV is prevalent in the Middle East and Australia (Maori et al., 2007a; Palacios et al., 2008) and this species was reported in 71 samples containing 10 bees; each from 20 provinces in Turkey (Ozkırım and Schiesser, 2013). In this study IAPV wasn't confirmed by RT-qPCR in our samples and RNA sequence analysis revealed that this virus was not present in Muğla and Hatay and was incredibly rare among samples from Artvin, Yığılca and Ardahan. SBV was similarly scarce in this study. This conflicts with results of Tentcheva et al. (2004b) but overlaps with the results of Baker and Schroeder (2008) and Forgách et al., (2008). As with most surveys, we examined only adult honey bees. SBV causes a fatal disease in honey bee larvae (Bailey, 1975), thus brood samples could provide more evidence for its prevalence.

Apis mellifera filamentous virus (AmFV) was not detected within our samples, nor was VdMLV (Varroa Macula-like virus). A plant-pathogenic RNA virus, tobacco ringspot virus (TRSV) was not detected in any of the RNA samples in our study.

Recent culture-independent studies reported eight bacterial phylotypes inhabiting the gut of the honey bee, *Apis mellifera* from several continents (Jeyaprakash et al. 2003; Mohr & Tebbe 2006; Babendreier et al. 2007; Cox-Foster et al. 2007; Olofsson & Vasquez 2008). Colonies founded by swarms, interactions within the colony, intercolony interactions like robbing food in neighboring hives and mixing of colonies by beekeepers all might affect the gut microbiota (Engel et al., 2012). In our study, unique gene reads of *Snodgrassella alvi* and *Gilliamella apicola* were highly represented and showed differences among provinces because of geographic, environmental and subspecies differences of hosts. The differences in social behaviors of the subspecies, the dietary sources and exposure to varying pathogens and pesticides might influence the abundance of these bacteria among regions.

One gamma-proteobacterial member of the gut microbiota *Candidatus Schmidhempelia bombi*, was present in 90% of bumble bee individuals in the study of Martinson et al., (2014). This symbiont was prevalent in all of our surveyed locations and widely represented in Hatay, Yığılca and Muğla. The recently described gamma-proteobacterium *Frischella perrara* (Engel et al., 2013) was present in our study albeit at low levels.

Lactobacillales symbionts have been proposed as actors in both nutrition and parasite defenses of honey bees. *Lactobacillales* stimulate the innate immune system, arguably increasing honey bee defenses against disease agents (Evans and Lopez, 2004). Along with their impacts on immunity, the microbial symbionts have been proposed to nutritionally compete with pathogens by occupying the available niches (Crotti et al., 2012). In this study, very low number of 16S *Lactobacillus* reads were observed among regions. Overall bacterial loads were especially high in Hatay, Yığılca and Artvin. The prevalence of the bacterial pathogens *S. apis* and *S. melliferum* is low among Belgian honey bee colonies (Ravoet et al., 2013), and these bacteria are present only seasonally in North and South America (Runckel et al., 2011, Schwarz et al., 2014). We found low *Spiroplasma* levels, with highest incidence in Artvin province. *S. melliferum* was the more common species, matching results from the Americas (Schwarz et al., 2014). The bacterial brood diseases European Foulbrood (EFB) caused by the bacterium *Melissococcus plutonius* (Bailey, 1983) and American Foulbrood (AFB) caused by the bacterium *Paenibacillus larvae* (Genersch et al., 2006) are globally important diseases of honey bees. *P. larvae* reads were not common among our samples. Similarly, *M. plutonius* was not prevalent among the regions but was more ubiquitous in Hatay, Muğla and Yığılca. Environmental conditions at these sites can be conductive for the expression of the disease. Like AFB, EFB transmission is also linked to larval immune responses (Evans, 2004), hygienic behavior (Spivak and Reuter, 2001) as well as interaction between *M. plutonius* and the intestinal microbiota of the honey bee larvae (Gilliam, 1997; Olofsson and Vasquez, 2008), nutritional and stress conditions, weather and geography (Bailey, 1961).

Sequences for *Ascosphaera apis,* the causative agent for Chalkbrood disease, were generally rare, with the highest incidence from samples of in Hatay province. Similarly, neogregarines (nominally *Apicystis bombi*) persisted at extremely low levels among regions in this study. Among arthropod parasites of honey bees, we found no genetic evidence for the presence of the tarsonemid tracheal mite, *Acarapis woodi* within our samples. The Asian parasitic mite *Tropilaelaps* is considered more dangerous to *A. mellifera* than the parasitic mite *Varroa destructor* (Rath et al., 1995), and this mite is worthy of screening. Our deep sequencing analysis showed no sign of *Tropilaelaps*. The presence of phorid flies (*Apocephalus borealis*) in the study of Ravoet et al. (2013) proves their existence in Europe, but our deep sequencing did not reveal signs of this parasite.

4.5. Implications of the metagenomic analyses

Based on the survey results about the colony losses, differences were observed between the years 2010 and 2011 among regions. Generally, pathogens, pests and management problems due to intensive production are the main forces acting on colony losses. Multiple causes may act together affecting the resistance ability of honey bees to declines by suppressing their immune system. These causes involves natural habitat loss, weather events, stress, insufficient nutrition, poor genetic diversity (Potts et al. 2010) and starvation, queen failure, mites, long distance transportation of the colonies (van Engelsdorp, 2010). Also use of pesticides contribute to the colony decline. Low levels of pesticide exposure have negative impacts like impairment of immune system functioning, odor discrimination, learning ability, foraging behavior, memory in honeybees (Desneux et al. 2007; Alaux et al. 2010; Yang et al. 2008; Williamson and Wright, 2013). Chronic pesticide exposure lead the colonies that are vulnerable to diseases to harmful consequences (Thompson, 2003). It is thought that the application of pesticides rather than honey bee disease factors lead to colony losses in Thrace region because of the activities of migratory beekeepers who visits this location to harvest sunflower honey. Inappropriate usage of the pesticides can cause residual problems in honey and effect the quality of some hive products.

One of the main results of this study was the correlation between higher disease loads and migratory beekeeping and the dispersion of parasites and pathogens among the regions across the country. Especially *N. ceranae* expanded with migratory beekeeping activities and in association with different viruses and trypanosomatids in this study. Trade of honey bees, bee products and equipments has a strong impact on spreading honey bee pathogens worldwide; for example the transfer of *N. ceranae* and *V. destructor* from the Asian honey bee (*A. ceranae*) to European honeybee *A mellifera*) and introduction of *V. destructor* and tracheal mite (*Acarapis woodi*) to the Americas (Mutinelli, 2011). In Turkey migratory beekeeping is very common and honey bee management is scheduled around natural nectar flows which differs among the regions in Turkey. Maximum strength is expected from the colonies before the nectar flows begin (Delaplane, 1999) and generally the honey bee colonies are transferred to the citrus groves and thyme areas in spring, to the fir forests in June, to the cotton, clover and sunflowers plants in summer and to the pine forests in August, September and October (Santas, 1990). Because of intense management practices, transfer of honey bees into new locations permit them to meet with wild native pollinators that causes struggling for resources, transmission of pathogens and receiving the same environmental risks. Homogenization of the genetic structure of the honey bee populations by lowering the genetic variation is another threat of migratory beekeeping .

ABPV, DWV and BQCV were the most common viruses and detected as dual and triple infections in this study. VDV-1 which is closely related with DWV was also abundant. There is no registered chemical treatment against adult viruses and the best time to take some precautions is during spring and fall. Methods of treatment are establishing strong, healthy colonies by controlling mites and *Nosema*, replacement of heavily infected combs with the new ones, using disinfected materials for queen rearing, disinfecting combs of dead outs and requeening with new or hygienic queens. The treatments against Varroa without disrupting bee behavior or life-span includes chemical and mechanical control. Thymol, coumaphos, sucrose octanoate esters, fluvalinate, Amitraz, formic acid and oxalic acid are the chemical control strategies which can be used against Varroa mites. The effectiveness of these chemicals may decrease as the mites develop resistance to some of them.

Disrupting the lifecycle of mites and establishing mite invasion within the tolerance levels of the colonies are typical mechanical controls. Other control mechanisms include drone brood sacrifice, powdered sugar dusting to encourage cleaning behaviour, screened bottom boards, interrupting the brood and decreasing the brood cell (http://www.moraybeekeepers.co.uk/).

N. ceranae was also prevalent in association with the viruses and trypanosomatids in this study. Proper ventilation through the hive or antibiotic usage such as fumagillin are alternative ways to combat *Nosema*. Removing much of the honey from the beehive in the late fall and feeding the bees on sugar water are other options to prevent or minimize the disease. Although refined sugar includes less nutrients than naturally produced honey, it reduces the risk of dysentery, keeps the colonies healthy when the resources are scarce (or in the late winter when brood production starts and colonies need supplemental feeding) and reduces the risk of pathogen transmission by preventing robbing behavior (Nakamura, 1999).

As well as pathogen presence or absence, clinical data over time is important to be given in specific colony loss situation. Longitudinal epidemiological datasets about the land use, weather conditions, distribution and prevalence of pathogens and *Varroa destructor* for managed and native bees, pesticide exposure are needed to provide better solutions for the beekeeping problems. Effective comparisons can be made among different geographic areas via determining the disease incidence and pathogen prevalence in single populations, with standardized sampling and data collection protocols and identification of the roles of human manipulation of wildlife and intense use of managed species in agriculture (Smith et al., 2014). Management practices can be achieved successfully by retraining beekeepers to improve product quality and develop better beekeeping technology (Tolon, 1999). This epidemiological survey which also reflects the interactions between pathogens can help to understand the spatial and temporal dynamics in pathogen distribution. Future studies about honey bee immunology and strain variation may provide supportive information about individual or colony level responses of the honey bees.

CHAPTER 5

CONCLUSION

In this study we addressed the immune defence reactions, locomotor activity differences of five honey bee subspecies upon *N. ceranae* infection and confirmed the phylogenetic relationship between these subspecies. As a parameter for immune strength, the increase in the production of antimicrobial peptides was investigated for each subspecies as an indication for active humoral immune system. Subspecies included in the study showed variation in the ability to generate antimicrobial peptides as well as eater and vitellogenin. The role of genetic diversity in decreasing the pathology and failure of the colonies against the diseases in honey bees were suggested in many studies (Tarpy, 2003; Evans, 2004). Thus determination of immune responses across diverse honey bee genotypes may provide preliminary data and useful genetic information in this research field for future preservation and breeding studies of certain subspecies.

We screened bee-derived RNA against the most complete sequence set for honey bee associates used to date. We assessed levels of known and novel parasites, pathogens, and symbionts. We present quantitative data for bacterial pathogens (*Melissococcus plutonius*, *Paenibacillus larvae*, *S. apis*, *S. melliferum*), protists (*Apicytis*, trypanosomatids), viruses (Lake Sinai virus, Chronic bee paralysis virus, Deformed wing virus, *Varroa destructor* virus, Sacbrood, and Dicistroviruses), symbionts (*Candidatus Schmidhempelia bombi*, *Frischella perrara*, *Snodgrassella alvi*, *Gilliamella apicola*, *Lactobacillus spp*., *Acetobacteracea*), microsporidia and fungi in *A. mellifera* colonies in distinct regions of Turkey. The presence of KBV, SBPV, Tobacco ringspot virus, VdMLV (Varroa Macula like), *Acarapis spp*., *Tropilaelaps claerae* and *Apocephalus* (phorid fly) were also examined. As in other countries, bee viruses were correlated with colony losses in Turkey.

In comparison with 2010, the increase in pathogen loads in 2011 might be a factor for increased colony losses observed in this study. It seems likely that migratory beekeeping practices enable the spread of disease factors among honey bees in places where they visit and causing an important threat to the honey bee colonies. In addition, the impacts of parasites and pathogens varies between regions, perhaps reflecting different honey bee genetic traits. Migratory beekeeping was correlated with both higher disease loads and a potential risk of dispersing regional parasites and pathogens across the country. This practice also allows for greater gene flow between migratory honey bee populations and local populations. Current diversity and local genetic structure can be preserved with selection strategies and establishing broad areas of isolation to reduce the risks of migratory beekeeping practices. While experimental work and longitudinal analyses will be needed to confirm causes of bee declines, our analyses, reference sequences, and strategy will help reduce the set of likely causes.

Natural subspecies are under threat of urbanization, migratory beekeeping habits of honey producers, commercial breeding and quuen importation which blends the original gene pool with commercial strains of diverse characteristics and leads to the loss of genetic variability. Genetically distinct populations with high amount of genetic diversity should be identified and conserved as genetic sources. Also it is necessary to improve the local strains that can be easily managed because of their adaptation ability to their local environment. Genetically specific populations can be used in breeding efforts to strengthen the resistance of imported strains against harsh local conditions. Some precautions against the distribution of local honey bee queens across the country and importing honey bee colonies from the other countries should be immediately taken into account.

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APPENDIX A

ONE WAY ANOVA RESULTS OF PATHOGEN PREVALENCE

Table A.1 One-way Anova results among provinces, in all regions with beekeeping type and in each province according to the beekeeping type in 2010 and 2011. 'ND' not detected, '/' sampling was not done, '*' significant

Year	2010					2011				
Pathogens	**DWV**	**ABPV**	**BQCV**	***N.ceranae***	**TRYP**	**DWV**	**ABPV**	**BQCV**	***N.ceranae***	**TRYP**
Among provinces	<0.0001*	<0.0001*	<0.0001*	0.0153*	0.0013*	0.0349*	0.1954	0.0329*	0.0004*	<0.0001*
With Beekeeping	0.6805	<0.0001*	<0.0001*	0.0005*	0.0001*	0.4168	0.0512	0.7546	0.4632	0.0271*
Mugla	0.8054	0.0004*	0.0252*	0.0799	<0.0001*	0.3874	0.4789	0.6602	0.7764	0.1436
Hatay	0.0013*	0.2757	0.2805	0.0065*	0.6752	0.8698	0.0652	0.9351	0.2199	0.0024*
Ardahan	0.0073*	0.115	<0.0001*	ND	<0.0001*	/	/	/	/	/
Ankara	0.0009*	0.0273*	0.0009*	0.2162	<0.0001*	/	/	/	/	/

APPENDIX B

NORMALIZED READ COUNTS FOR HITS TO PARASITES, PATHOGENS AND SYMBIONTS IN RNA SEQUENCING

Table B.1 Normalized read counts for hits to parasites, pathogens and symbionts in RNA Sequencing efforts for six sites in Turkey

Target	Hatay	Mugla	Kirklareli	Artvin	Ardahan	Yigilca
Bacterial Pathogens						
Melissococcus_plutonius	2131	1324	153	490	83	1172
Paenibacillus larvae	67	40	6	16	15	33
Spiroplasma apis	56	20	5	107	2	22
Spiroplasma melliferum	59	20	4	1482	20	24
Protists						
Apicystis (Neogregarine)	5	6	4	2	4	3
Crithidia mellificae 30254	0	0	0	2	0	1
Crithidia sp. Haplotype A	0	1	0	4	0	3
Crithidia sp. Haplotype B	0	0	0	0	0	0
Crithidia sp. GAPDH	0	0	0	17	0	30

Table B.1 (Cont'd

Virus						
ABPV	63	5587	4	76	22506	144296
BQCV	2164	1614	2655	4954	15445	350
CBPV	21	3	1967	3	0	1
DWV	2317199	306436	36061	5520	7410	854713
Apis filamentous virus	0	0	0	2	1	0
IAPV	0	0	0	3	45	4
KBV	0	0	0	0	0	0
LSV1Genome	129807	47613	247873	124359	195420	53052
LSV3	4880	115	4405	107	21367	3980
LSV4	62466	20842	133211	85805	88629	15873

Table B.1 (Cont'd)

Virus						
LSV5	3	110	435	0	734	90
LSV5	3	391	64	0	141	614
LSV5	0	0	0	0	2	1
LSV5	11	281	699	200	438	11
LSV5	0	0	1	0	0	3
LSV5	184	0	2211	0	15702	230
Sacbrood	45	8	13	10	1885	32
SBPV	0	0	0	0	0	0
Tobacco_ringspot_virus	0	0	0	0	0	0
Varroa_Macula_like	0	0	0	0	0	3
VDV	1112041	1213796	550109	177304	11614	4703331

Table B.1 (Cont'd)

Symbionts						
Overall bacterial load	600967	70382	20793	116926	14449	598188
Candidatus Schmidhempelia bombi	8871	3716	2962	1706	265	5041
Frischella	37	10	4	5	2	20
Gilliamella apicola	45087	127562	147515	47120	71743	46403
Lactobacillus spp.	314	82	16	29	8	104
Snodgrasella alvi	41237	60276	49704	22323	58094	65751
Fungi						
Ascosphaera apis	2031	728	400	618	58	862
Nosema ceranae	624056	437735	327516	1302005	597540	704281
Parasites						
Acarapis spp. (*Acari*)	0	0	0	0	0	0
Tropilaelaps claerae (*Acari*)	0	0	0	2	0	1
Apocephalus (*Phoridae*)	0	0	0	0	0	0

APPENDIX C

RT-QPCR CT VALUES OF THE SAMPLES FOR EACH GENE TARGET

Table C.1 RNA quality, the ratio of 260/280 absorbance and RT-qPCR Ct values of the samples for each gene target with province and management information in 2010 and 2011. Positive and negative control reactions were run on each plate.

Sample ID	Year	Management	Region	ng/ul	260/280	RPS5 Ct	ABPV Ct	BQCV Ct	DWV Ct	IAPV Ct
ARDH1	2011	Stationary	Ardahan	1170.1	2.03	35.14	27.9	23.16	35.72	N/A
ARDH2	2011	Stationary	Ardahan	761.4	1.95	31.56	32.81	27.56	44.65	N/A
ARDH3	2011	Stationary	Ardahan	1155.1	2	32.66	30.79	32.69	36.01	N/A
ARDH4	2011	Stationary	Ardahan	1282.1	2.05	47.03	N/A	N/A	36.33	N/A
ARDH5	2011	Stationary	Ardahan	767.4	1.96	38.27	33.67	38.62	37.92	N/A
ARDH6	2011	Stationary	Ardahan	1013.7	2.04	37.5	18.04	N/A	44.1	N/A
ART1	2011	Stationary	Artvin	1637.7	1.92	38.62	41.34	N/A	N/A	N/A
ART2	2011	Stationary	Artvin	1250	2.07	34.8	35.86	47.28	38.43	N/A
ART3	2011	Stationary	Artvin	917.2	1.95	31.44	32.02	32.58	41.19	N/A
ART4	2011	Stationary	Artvin	1754	2.01	43.83	28.65	27.74	37.05	N/A
H1	2011	Migratory	Hatay	1613.3	2.03	44.38	32.44	40.15	27.3	N/A
H2	2011	Migratory	Hatay	1544	2.1	34.88	22.97	33.65	35.47	N/A
H3	2011	Migratory	Hatay	2176.3	2	N/A	32.34	40.73	38.09	N/A
H4	2011	Stationary	Hatay	1240.2	2.06	40.01	32.74	N/A	39.7	N/A
H5	2011	Stationary	Hatay	2100.1	2	N/A	36.96	34.21	37.02	N/A
H6	2011	Stationary	Hatay	1496	2.04	43.06	36.85	N/A	N/A	N/A
H7	2011	Stationary	Hatay	1747.3	2.01	34.45	46.35	35.86	25.47	N/A
H8	2011	Stationary	Hatay	1246.8	2.02	29.28	N/A	43.18	22.02	N/A

Table C.1 (Cont'd)

Sample ID	Year	Management	Region	ng/ul	260/280	RPS5 Ct	ABPV Ct	BQCV Ct	DWV Ct	IAPV Ct
K1	2011	Stationary	Kirklareli	1529.2	2.02	47.26	38.21	N/A	N/A	N/A
K2	2011	Stationary	Kirklareli	873.5	1.97	31.14	34.09	41.27	37.02	N/A
K3	2011	Stationary	Kirklareli	1524.4	2.05	33.76	N/A	29.44	N/A	N/A
K4	2011	Stationary	Kirklareli	1753.1	2.04	N/A	38.81	29.21	32.92	N/A
K5	2011	Stationary	Kirklareli	1239.4	2.09	30.27	N/A	N/A	N/A	N/A
K6	2011	Stationary	Kirklareli	1762.8	2.02	37.06	32.91	N/A	32.83	N/A
K7	2011	Stationary	Kirklareli	1554.2	1.97	30.43	34.72	N/A	37.73	N/A
K8	2011	Stationary	Kirklareli	918	1.99	28.09	36.29	N/A	40.11	N/A
K9	2011	Stationary	Kirklareli	1444.6	2	44.58	37.14	36.1	38.89	N/A
K10	2011	Stationary	Kirklareli	1598.1	2.03	30.2	30.5	N/A	37.82	N/A
K11	2011	Stationary	Kirklareli	1119.6	1.98	26.28	31.28	40.92	32.12	N/A
M1	2011	Stationary	Mugla	1418.8	2.06	29.56	22.93	N/A	23.41	N/A
M2	2011	Stationary	Mugla	1682.1	2.01	34.41	35.83	47.12	28.99	N/A
M3	2011	Stationary	Mugla	1647.9	2.11	46.46	N/A	N/A	N/A	N/A
M4	2011	Stationary	Mugla	1535.5	1.94	40.01	40.98	N/A	38.13	N/A
M5	2011	Migratory	Mugla	1553	1.85	36.53	33.27	N/A	34.17	N/A
M6	2011	Migratory	Mugla	1154.3	1.9	40.18	35.09	N/A	36.1	N/A
M7	2011	Stationary	Mugla	1133.3	2.01	30.19	37.42	44.92	42.57	N/A
M8	2011	Migratory	Mugla	1263	1.83	29.89	35.37	30.45	26.41	N/A
M9	2011	Stationary	Mugla	1135.1	1.96	33.63	36.72	32.48	36.87	N/A
M10	2011	Stationary	Mugla	1264.4	1.98	30.06	38.11	N/A	37.03	N/A
M11	2011	Stationary	Mugla	622.5	2.25	29.17	36.2	36.03	34.98	N/A

Table C.1 (Cont'd)

Sample ID	Year	Management	Region	ng/ul	260/280	RPS5 Ct	ABPV Ct	BQCV Ct	DWV Ct	IAPV Ct
Y1	2011	Stationary	Yigilca	1012.8	2.01	32.78	35.17	N/A	38.05	N/A
Y2	2011	Stationary	Yigilca	2052.6	2.03	N/A	27.36	N/A	25.95	N/A
Y3	2011	Stationary	Yigilca	2104.4	2.04	43.43	26.31	N/A	24.64	N/A
Y4	2011	Stationary	Yigilca	1529.2	2.02	31.92	30.24	N/A	33.39	N/A
Y5	2011	Stationary	Yigilca	1857	2.02	31.2	37.59	N/A	35.98	N/A
Y6	2011	Stationary	Yigilca	1484.7	2.01	32.43	34.93	47.36	37.09	N/A
Y7	2011	Stationary	Yigilca	1922.5	2.02	32.24	33.53	N/A	24.72	N/A
Y8	2011	Stationary	Yigilca	1728.7	1.98	32.21	20.82	N/A	24.98	N/A
Y9	2011	Stationary	Yigilca	1792.8	2	33.29	21.44	46.33	26.49	N/A
Y10	2011	Stationary	Yigilca	2477.9	1.94	35.83	36.65	N/A	42.37	N/A
Y11	2011	Stationary	Yigilca	1701.6	2	32.83	32.56	N/A	35.71	N/A
pcr positive						31.7	5.77	5.43	11.52	13
pcr negative						N/A	N/A	N/A	N/A	N/A
ARDH1	2010	Stationary	Ardahan	761.35	2.05	30.78	36.22	N/A	33.68	N/A
ARDH2	2010	Stationary	Ardahan	1235.72	2.04	34.17	N/A	N/A	39.88	N/A
ARDH3	2010	Stationary	Ardahan	772.78	2.06	31.12	39.29	N/A	35.3	N/A
ARDH4	2010	Stationary	Ardahan	730.94	2.06	30.67	N/A	N/A	29.6	N/A
ARDH5	2010	Stationary	Ardahan	634.93	2.14	30.89	36.87	N/A	29.65	N/A
ARDH6	2010	Stationary	Ardahan	859.79	2.07	30.61	34.03	N/A	29.66	N/A
ARDH7	2010	Stationary	Ardahan	661.78	2.08	30.57	35.61	N/A	29.28	N/A

Table C.1 (Cont'd)

Sample ID	Year	Management	Region	ng/ul	260/280	RPS5 Ct	ABPV Ct	BQCV Ct	DWV Ct	IAPV Ct
H1	2010	Migratory	Hatay	1241.19	2.03	32.88	N/A	31.71	31.2	N/A
H2	2010	Migratory	Hatay	957.85	2.01	33.06	N/A	30.8	30.81	N/A
H3	2010	Migratory	Hatay	1098.7	2.05	33.34	N/A	32.37	31.9	N/A
H4	2010	Migratory	Hatay	1022.72	2.02	33.05	N/A	32.14	31.17	N/A
H5	2010	Stationary	Hatay	689.08	1.98	32.12	35.53	N/A	28.18	N/A
H6	2010	Stationary	Hatay	767.59	2.05	32.95	34.92	31.48	29.5	N/A
H7	2010	Stationary	Hatay	531.45	1.87	31.31	36.93	31.14	27.37	N/A
H8	2010	Stationary	Hatay	618.28	1.92	32.31	35.55	31.36	28.31	N/A
M1	2010	Migratory	Mugla	1090.99	2.07	35	35.29	28.27	31.5	N/A
M2	2010	Migratory	Mugla	642.7	2.02	36.99	36.68	30.97	35.73	N/A
M3	2010	Migratory	Mugla	604.86	2.08	33.79	37.78	28.32	30.6	N/A
M4	2010	Migratory	Mugla	994.92	2.05	32.86	35.05	27.5	29.04	N/A
M5	2010	Migratory	Mugla	1086.36	2.03	31.96	N/A	29.1	28.41	N/A
M6	2010	Migratory	Mugla	1165.94	2.07	34.1	36.29	30.56	31.88	N/A
M7	2010	Migratory	Mugla	839.61	2.05	31.84	33.33	29.04	28.92	N/A
M8	2010	Migratory	Mugla	821.2	2.03	33.91	37.39	30.66	30.89	N/A
K1	2010	Stationary	Kırklareli	1026.39	2.06	31.16	N/A	N/A	35.88	N/A
K2	2010	Stationary	Kırklareli	759.82	1.98	30.84	N/A	N/A	35.64	N/A
K3	2010	Stationary	Kırklareli	1362.34	2.07	31.12	N/A	N/A	35.02	N/A
K4	2010	Stationary	Kırklareli	1230.35	2.07	31.88	N/A	N/A	37.85	N/A
K5	2010	Stationary	Kırklareli	984.21	2.06	38.71	N/A	N/A	37.07	N/A
K6	2010	Stationary	Kırklareli	1939.9	2.07	33.16	N/A	N/A	32.17	N/A
K7	2010	Stationary	Kırklareli	1027.26	2.11	30.26	N/A	N/A	28.95	N/A
K8	2010	Stationary	Kırklareli	1258.53	2.07	31.78	N/A	N/A	30.81	N/A

Table C.1 (Cont'd)

Sample ID	Year	Management	Region	ng/ul	260/280	RPS5 Ct	ABPV Ct	BQCV Ct	DWV Ct	IAPV Ct
B1	2010	Migratory	Bitlis	739.68	1.89	31.16	18.02	30.36	26.69	N/A
B2	2010	Migratory	Bitlis	1009.58	1.94	31.99	18.44	29.58	27.16	N/A
B3	2010	Migratory	Bitlis	764.6	1.83	32.07	19.1	30.42	26.16	N/A
B4	2010	Migratory	Bitlis	495.63	1.92	32.68	21.27	36.49	27.89	N/A
B5	2010	Migratory	Bitlis	869.92	1.92	30.25	18.92	33.02	26.6	N/A
B6	2010	Migratory	Bitlis	552.74	1.91	34.16	25.07	38.58	33.23	N/A
B7	2010	Migratory	Bitlis	211.63	1.33	34.3	21.11	37.27	31.36	N/A
H9	2010	Migratory	Hatay	1255.44	1.96	31.17	33.31	29.35	33.25	N/A
H10	2010	Migratory	Hatay	1571.81	1.77	29.56	35.33	27.73	31.09	N/A
H11	2010	Migratory	Hatay	1080.36	2.03	32.85	34.79	30.07	34.68	N/A
H12	2010	Migratory	Hatay	912.67	1.09	31.15	33.22	27.4	31.35	N/A
H13	2010	Migratory	Hatay	1505.96	1.91	31.13	34.16	27.86	31.21	N/A
H14	2010	Migratory	Hatay	1048.09	2.03	29.68	33.85	27.36	31.21	N/A
H15	2010	Stationary	Hatay	889.47	2.01	30.12	N/A	27.44	27.95	N/A
H16	2010	Stationary	Hatay	808.92	2.03	31.04	N/A	28.04	28.5	N/A
H17	2010	Stationary	Hatay	868.13	2.01	30.87	N/A	29.08	28.62	N/A
H18	2010	Stationary	Hatay	672.08	1.98	33.64	N/A	30.56	33.65	N/A
K9	2010	Stationary	Kırklareli	821.49	2.01	33.32	N/A	32.69	N/A	N/A
K10	2010	Stationary	Kırklareli	742.7	1.99	31.64	N/A	32.15	N/A	N/A
K11	2010	Stationary	Kırklareli	800.7	1.98	30.98	N/A	31.18	N/A	N/A
K12	2010	Stationary	Kırklareli	689.72	2.06	38.92	N/A	38.43	N/A	N/A
ED1	2010	Stationary	Edirne	538.72	1.83	33.3	N/A	38.73	38.87	N/A
ED2	2010	Stationary	Edirne	799.2	2.05	36.56	N/A	N/A	N/A	N/A
ED3	2010	Stationary	Edirne	988.49	2.05	33.18	N/A	36.67	37.26	N/A
ED4	2010	Stationary	Edirne	752.38	1.98	34.98	N/A	N/A	39.32	N/A

Table C.1 (Cont'd)

Sample ID	Year	Management	Region	ng/ul	260/280	RPS5 Ct	ABPV Ct	BQCV Ct	DWV Ct	IAPV Ct
ARDH8	2010	Migratory	Ardahan	874.85	2.06	32.34	38.44	28.05	N/A	N/A
ARDH9	2010	Migratory	Ardahan	1030.36	2.02	27.59	33.69	26	N/A	N/A
ARDH10	2010	Migratory	Ardahan	852.36	1.95	29.89	36.58	26.31	N/A	N/A
ARDH11	2010	Migratory	Ardahan	1120.53	2.09	31.09	32.77	27.01	N/A	N/A
ART1	2010	Stationary	Artvin	1367.19	2.03	31.2	35.22	32.81	N/A	N/A
ART2	2010	Stationary	Artvin	711.34	2.07	34.56	N/A	36.64	N/A	N/A
ART3	2010	Stationary	Artvin	769.41	2.05	29.48	38.01	33.21	N/A	N/A
ANK1	2010	Migratory	Ankara	825.31	2.07	31.32	32.04	31.76	N/A	N/A
ANK2	2010	Migratory	Ankara	1069.99	2.1	33.21	35.04	31.76	N/A	N/A
ANK3	2010	Migratory	Ankara	950.96	2.11	30.97	34.15	32.76	N/A	N/A
ANK4	2010	Migratory	Ankara	1034.77	2.04	33.97	39.15	34.6	N/A	N/A
ANK5	2010	Stationary	Ankara	1385.08	2.08	32.05	N/A	27.16	33.69	N/A
ANK6	2010	Stationary	Ankara	978.71	2.05	33.99	36.89	28.19	35.58	N/A
ANK7	2010	Stationary	Ankara	1082.94	2.04	35.35	N/A	29.63	37.3	N/A
ANK8	2010	Stationary	Ankara	1367.33	2.08	33.38	N/A	28.45	36.21	N/A

Table C.1 (Cont'd)

Sample ID	Year	Management	Region	ng/ul	260/280	RPS5 Ct	ABPV Ct	BQCV Ct	DWV Ct	IAPV Ct
EL1	2010	Migratory	Elazığ	610.35	2	31.61	N/A	36.93	37.67	N/A
EL2	2010	Migratory	Elazığ	565.07	2.04	31.36	N/A	36.92	38.54	N/A
EL3	2010	Migratory	Elazığ	473.89	1.98	31.15	N/A	37.83	37.91	N/A
EL4	2010	Migratory	Elazığ	505.07	1.96	31.05	N/A	36.42	36.51	N/A
EL5	2010	Migratory	Elazığ	528.08	2	30.99	N/A	36.65	37.19	N/A
M9	2010	Migratory	Mugla	425.77	1.94	32.4	35.69	34.45	37.51	N/A
M10	2010	Migratory	Mugla	682.97	2.03	31.88	37.49	34.57	36.42	N/A
M11	2010	Migratory	Mugla	430.75	1.98	31.65	36.44	35.14	37.57	N/A
M12	2010	Migratory	Mugla	614.39	1.94	32.98	36.54	34.98	37.94	N/A
M13	2010	Migratory	Mugla	447.19	2	32.18	35.29	34.08	37.54	N/A
M14	2010	Stationary	Mugla	785.73	2.01	33.28	38.16	34.24	33.24	N/A
M15	2010	Stationary	Mugla	1067.94	1.88	36.13	N/A	34.55	33.67	N/A
M16	2010	Stationary	Mugla	752.98	1.99	33.87	N/A	34.29	33	N/A
M17	2010	Stationary	Mugla	707.73	2	33.62	N/A	33.92	33	N/A
M18	2010	Stationary	Mugla	775.01	1.99	33.41	N/A	33.87	31.99	N/A
pcr neg	2010					N/A	N/A	N/A	N/A	N/A
pcr pos	2010					31.26	13.4	10.5	10.3	17.53

Table C.1 (Cont'd)

Sample ID	Year	Management	Region	ng/ul	KBV Ct	SBV Ct	*N. apis* Ct	*N. ceranae* Ct	*P. larvae* Ct	TRYP1 Ct
ARDH1	2011	Stationary	Ardahan	1170.1	N/A	N/A	N/A	27.28	N/A	32.28
ARDH2	2011	Stationary	Ardahan	761.4	N/A	N/A	N/A	35.94	N/A	37.57
ARDH3	2011	Stationary	Ardahan	1155.1	N/A	N/A	33.01	26.24	N/A	25.12
ARDH4	2011	Stationary	Ardahan	1282.1	N/A	N/A	N/A	28.53	N/A	37.03
ARDH5	2011	Stationary	Ardahan	767.4	N/A	N/A	N/A	40.7	N/A	35.15
ARDH6	2011	Stationary	Ardahan	1013.7	N/A	N/A	N/A	40.83	N/A	35.16
ART1	2011	Stationary	Artvin	1637.7	N/A	N/A	N/A	30.28	N/A	22.45
ART2	2011	Stationary	Artvin	1250	N/A	N/A	N/A	27.41	N/A	23.48
ART3	2011	Stationary	Artvin	917.2	N/A	N/A	N/A	24.44	N/A	18.78
ART4	2011	Stationary	Artvin	1754	N/A	N/A	N/A	23.2	N/A	21.82
H1	2011	Migratory	Hatay	1613.3	N/A	N/A	N/A	45.68	N/A	36.78
H2	2011	Migratory	Hatay	1544	N/A	N/A	N/A	37.88	N/A	36.15
H3	2011	Migratory	Hatay	2176.3	N/A	N/A	N/A	N/A	N/A	37.35
H4	2011	Stationary	Hatay	1240.2	N/A	N/A	N/A	N/A	N/A	N/A
H5	2011	Stationary	Hatay	2100.1	N/A	N/A	N/A	N/A	N/A	N/A
H6	2011	Stationary	Hatay	1496	N/A	N/A	N/A	N/A	N/A	N/A
H7	2011	Stationary	Hatay	1747.3	N/A	N/A	N/A	N/A	N/A	41.38
H8	2011	Stationary	Hatay	1246.8	N/A	N/A	N/A	N/A	N/A	49.05

Table C.1 (Cont'd).

Sample ID	Year	Management	Region	ng/ul	KBV Ct	SBV Ct	*N. apis* Ct	*N. ceranae* Ct	*P. larvae* Ct	TRYP1 Ct
K1	2011	Stationary	Kirklareli	1529.2	N/A	N/A	N/A	32.91	N/A	43.4
K2	2011	Stationary	Kirklareli	873.5	N/A	N/A	N/A	29.4	N/A	N/A
K3	2011	Stationary	Kirklareli	1524.4	N/A	N/A	N/A	N/A	N/A	N/A
K4	2011	Stationary	Kirklareli	1753.1	N/A	N/A	N/A	48.26	N/A	39.95
K5	2011	Stationary	Kirklareli	1239.4	N/A	N/A	N/A	43.77	N/A	N/A
K6	2011	Stationary	Kirklareli	1762.8	N/A	N/A	N/A	30.24	N/A	44.9
K7	2011	Stationary	Kirklareli	1554.2	N/A	N/A	N/A	24.54	N/A	29.04
K8	2011	Stationary	Kirklareli	918	N/A	N/A	N/A	23.59	N/A	36.65
K9	2011	Stationary	Kirklareli	1444.6	N/A	N/A	N/A	25.94	N/A	38.43
K10	2011	Stationary	Kirklareli	1598.1	N/A	N/A	N/A	32.8	N/A	40.59
K11	2011	Stationary	Kirklareli	1119.6	N/A	N/A	N/A	33.37	N/A	49.64
M1	2011	Stationary	Mugla	1418.8	N/A	N/A	N/A	31.91	N/A	30.4
M2	2011	Stationary	Mugla	1682.1	N/A	N/A	N/A	N/A	N/A	33.86
M3	2011	Stationary	Mugla	1647.9	N/A	N/A	N/A	43.9	N/A	40.74
M4	2011	Stationary	Mugla	1535.5	N/A	N/A	N/A	N/A	N/A	32.11
M5	2011	Migratory	Mugla	1553	N/A	N/A	N/A	33.98	N/A	26.2
M6	2011	Migratory	Mugla	1154.3	N/A	N/A	N/A	N/A	N/A	30.22
M7	2011	Stationary	Mugla	1133.3	N/A	N/A	N/A	N/A	N/A	N/A
M8	2011	Migratory	Mugla	1263	N/A	N/A	N/A	N/A	N/A	37.95
M9	2011	Stationary	Mugla	1135.1	N/A	N/A	38.37	N/A	N/A	42.09
M10	2011	Stationary	Mugla	1264.4	N/A	N/A	N/A	41.95	N/A	40.09
M11	2011	Stationary	Mugla	622.5	N/A	N/A	N/A	37.6	N/A	36.81
Y1	2011	Stationary	Yigilca	1012.8	N/A	N/A	N/A	36.33	N/A	24.67

Table C.1 (Cont'd)

Sample ID	Year	Management	Region	ng/ul	KBV Ct	SBV Ct	*N. apis* Ct	*N. ceranae* Ct	*P. larvae* Ct	TRYP1 Ct
Y2	2011	Stationary	Yigilca	2052.6	N/A	N/A	N/A	40.91	N/A	33.97
Y3	2011	Stationary	Yigilca	2104.4	N/A	N/A	N/A	40.65	N/A	38.39
Y4	2011	Stationary	Yigilca	1529.2	N/A	N/A	N/A	35.95	N/A	29.14
Y5	2011	Stationary	Yigilca	1857	N/A	N/A	N/A	41.49	N/A	21.3
Y6	2011	Stationary	Yigilca	1484.7	N/A	N/A	N/A	24.37	N/A	23.86
Y7	2011	Stationary	Yigilca	1922.5	N/A	N/A	40.4	34.43	N/A	25.75
Y8	2011	Stationary	Yigilca	1728.7	N/A	N/A	N/A	37.12	N/A	36.83
Y9	2011	Stationary	Yigilca	1792.8	N/A	N/A	N/A	42.13	N/A	33.89
Y10	2011	Stationary	Yigilca	2477.9	N/A	N/A	N/A	39.49	N/A	27.57
Y11	2011	Stationary	Yigilca	1701.6	N/A	N/A	N/A	38.02	N/A	25.47
pcr positive					8.23	26.38	21.03	26.94	32	25.73
pcr negative					N/A	N/A	N/A	N/A	N/A	N/A
ARDH1	2010	Stationary	Ardahan	761.35	N/A	N/A	N/A	N/A	N/A	N/A
ARDH2	2010	Stationary	Ardahan	1235.72	N/A	N/A	N/A	N/A	N/A	N/A
ARDH3	2010	Stationary	Ardahan	772.78	N/A	N/A	N/A	N/A	N/A	N/A
ARDH4	2010	Stationary	Ardahan	730.94	N/A	N/A	N/A	N/A	N/A	N/A
ARDH5	2010	Stationary	Ardahan	634.93	N/A	N/A	N/A	N/A	N/A	N/A
ARDH6	2010	Stationary	Ardahan	859.79	N/A	N/A	N/A	N/A	N/A	N/A
ARDH7	2010	Stationary	Ardahan	661.78	N/A	N/A	N/A	N/A	N/A	N/A

Table C.1 (Cont'd)

Sample ID	Year	Management	Region	ng/ul	KBV Ct	SBV Ct	*N. apis* Ct	*N. ceranae* Ct	*P. larvae* Ct	TRYP1 Ct
H1	2010	Migratory	Hatay	1241.19	N/A	N/A	N/A	N/A	N/A	N/A
H2	2010	Migratory	Hatay	957.85	N/A	N/A	N/A	N/A	N/A	N/A
H3	2010	Migratory	Hatay	1098.7	N/A	N/A	N/A	N/A	N/A	N/A
H4	2010	Migratory	Hatay	1022.72	N/A	N/A	N/A	N/A	N/A	N/A
H5	2010	Stationary	Hatay	689.08	N/A	N/A	N/A	N/A	N/A	N/A
H6	2010	Stationary	Hatay	767.59	N/A	N/A	N/A	N/A	N/A	N/A
H7	2010	Stationary	Hatay	531.45	N/A	N/A	N/A	N/A	N/A	N/A
H8	2010	Stationary	Hatay	618.28	N/A	N/A	N/A	N/A	N/A	N/A
M1	2010	Migratory	Mugla	1090.99	N/A	N/A	N/A	N/A	N/A	20.27
M2	2010	Migratory	Mugla	642.7	N/A	N/A	N/A	N/A	N/A	20.21
M3	2010	Migratory	Mugla	604.86	N/A	N/A	N/A	37.83	N/A	20.22
M4	2010	Migratory	Mugla	994.92	N/A	N/A	N/A	N/A	N/A	20.60
M5	2010	Migratory	Mugla	1086.36	N/A	N/A	N/A	33.21	N/A	37.43
M6	2010	Migratory	Mugla	1165.94	N/A	N/A	N/A	35.07	N/A	37.20
M7	2010	Migratory	Mugla	839.61	N/A	N/A	N/A	34.2	N/A	36.91
M8	2010	Migratory	Mugla	821.2	N/A	N/A	N/A	N/A	N/A	36.25
K1	2010	Stationary	Kırklareli	1026.39	N/A	N/A	N/A	N/A	N/A	N/A
K2	2010	Stationary	Kırklareli	759.82	N/A	N/A	N/A	N/A	N/A	N/A
K3	2010	Stationary	Kırklareli	1362.34	N/A	N/A	N/A	N/A	N/A	46.03
K4	2010	Stationary	Kırklareli	1230.35	N/A	N/A	N/A	N/A	N/A	N/A

Table C.1 (Cont'd)

Sample ID	Year	Management	Region	ng/ul	KBV Ct	SBV Ct	*N. apis* Ct	*N. ceranae* Ct	*P. larvae* Ct	TRYP1 Ct
K5	2010	Stationary	Kırklareli	984.21	N/A	N/A	N/A	N/A	N/A	N/A
K6	2010	Stationary	Kırklareli	1939.9	N/A	N/A	N/A	N/A	N/A	N/A
K7	2010	Stationary	Kırklareli	1027.26	N/A	N/A	N/A	N/A	N/A	N/A
K8	2010	Stationary	Kırklareli	1258.53	N/A	N/A	N/A	36.86	N/A	N/A
B1	2010	Migratory	Bitlis	739.68	N/A	N/A	N/A	N/A	N/A	42.17
B2	2010	Migratory	Bitlis	1009.58	N/A	N/A	N/A	N/A	N/A	N/A
B3	2010	Migratory	Bitlis	764.6	N/A	N/A	N/A	N/A	N/A	N/A
B4	2010	Migratory	Bitlis	495.63	N/A	N/A	N/A	34.96	N/A	30.85
B5	2010	Migratory	Bitlis	869.92	N/A	N/A	N/A	32.02	N/A	29.21
B6	2010	Migratory	Bitlis	552.74	N/A	N/A	N/A	N/A	N/A	28.80
B7	2010	Migratory	Bitlis	211.63	N/A	N/A	N/A	35.18	N/A	28.50
H9	2010	Migratory	Hatay	1255.44	N/A	N/A	N/A	35.26	N/A	25.32
H10	2010	Migratory	Hatay	1571.81	N/A	N/A	N/A	37.54	N/A	25.58
H11	2010	Migratory	Hatay	1080.36	N/A	N/A	N/A	N/A	N/A	25.13
H12	2010	Migratory	Hatay	912.67	N/A	N/A	N/A	35.7	N/A	24.24
H13	2010	Migratory	Hatay	1505.96	N/A	N/A	N/A	36.46	N/A	25.02
H14	2010	Migratory	Hatay	1048.09	N/A	N/A	N/A	36.24	N/A	27.05
H15	2010	Stationary	Hatay	889.47	N/A	N/A	N/A	N/A	N/A	27.80
H16	2010	Stationary	Hatay	808.92	N/A	N/A	N/A	N/A	N/A	26.99
H17	2010	Stationary	Hatay	868.13	N/A	N/A	N/A	N/A	N/A	27.80
H18	2010	Stationary	Hatay	672.08	N/A	N/A	N/A	N/A	N/A	28.07
K9	2010	Stationary	Kırklareli	821.49	N/A	N/A	N/A	N/A	N/A	N/A
K10	2010	Stationary	Kırklareli	742.7	N/A	N/A	N/A	37.08	N/A	42.71
K11	2010	Stationary	Kırklareli	800.7	N/A	N/A	N/A	37.85	N/A	N/A
K12	2010	Stationary	Kırklareli	689.72	N/A	N/A	N/A	N/A	N/A	N/A

Table C.1 (Cont'd)

Sample ID	Year	Management	Region	ng/ul	KBV Ct	SBV Ct	*N. apis* Ct	*N. ceranae* Ct	*P. larvae* Ct	TRYP1 Ct
ED1	2010	Stationary	Edirne	538.72	N/A	N/A	N/A	N/A	N/A	N/A
ED2	2010	Stationary	Edirne	799.2	N/A	N/A	N/A	40	N/A	N/A
ED3	2010	Stationary	Edirne	988.49	N/A	N/A	N/A	N/A	N/A	N/A
ED4	2010	Stationary	Edirne	752.38	N/A	N/A	N/A	N/A	N/A	42.33
ARDH8	2010	Migratory	Ardahan	874.85	N/A	N/A	N/A	N/A	N/A	23.96
ARDH9	2010	Migratory	Ardahan	1030.36	N/A	N/A	N/A	N/A	N/A	23.44
ARDH10	2010	Migratory	Ardahan	852.36	N/A	N/A	N/A	N/A	N/A	23.67
ARDH11	2010	Migratory	Ardahan	1120.53	N/A	N/A	N/A	N/A	N/A	23.96
ART1	2010	Stationary	Artvin	1367.19	N/A	N/A	N/A	N/A	N/A	23.65
ART2	2010	Stationary	Artvin	711.34	N/A	N/A	N/A	N/A	N/A	25.58
ART3	2010	Stationary	Artvin	769.41	N/A	N/A	N/A	N/A	N/A	26.08
ANK1	2010	Migratory	Ankara	825.31	N/A	N/A	N/A	N/A	N/A	43.10
ANK2	2010	Migratory	Ankara	1069.99	N/A	N/A	N/A	N/A	N/A	48.28
ANK3	2010	Migratory	Ankara	950.96	N/A	N/A	N/A	37.17	N/A	N/A
ANK4	2010	Migratory	Ankara	1034.77	N/A	N/A	N/A	N/A	N/A	N/A
ANK5	2010	Stationary	Ankara	1385.08	N/A	N/A	N/A	N/A	N/A	28.41
ANK6	2010	Stationary	Ankara	978.71	N/A	N/A	N/A	N/A	N/A	28.36
ANK7	2010	Stationary	Ankara	1082.94	N/A	N/A	N/A	N/A	N/A	28.53
ANK8	2010	Stationary	Ankara	1367.33	N/A	N/A	N/A	N/A	N/A	28.35

Table C.1 (Cont'd)

Sample ID	Year	Management	Region	ng/ul	KBV Ct	SBV Ct	*N. apis* Ct	*N. ceranae* Ct	*P. larvae* Ct	TRYP1 Ct
EL1	2010	Migratory	Elazığ	610.35	N/A	N/A	N/A	N/A	N/A	38.81
EL2	2010	Migratory	Elazığ	565.07	N/A	N/A	N/A	N/A	N/A	N/A
EL3	2010	Migratory	Elazığ	473.89	N/A	N/A	N/A	N/A	N/A	39.76
EL4	2010	Migratory	Elazığ	505.07	N/A	N/A	N/A	N/A	N/A	49.54
EL5	2010	Migratory	Elazığ	528.08	N/A	N/A	N/A	N/A	N/A	N/A
M9	2010	Migratory	Mugla	425.77	N/A	N/A	N/A	N/A	N/A	24.35
M10	2010	Migratory	Mugla	682.97	N/A	N/A	N/A	35.7	N/A	24.37
M11	2010	Migratory	Mugla	430.75	N/A	N/A	N/A	N/A	N/A	23.29
M12	2010	Migratory	Mugla	614.39	N/A	N/A	N/A	37.74	N/A	25.06
M13	2010	Migratory	Mugla	447.19	N/A	N/A	N/A	37.02	N/A	24.32
M14	2010	Stationary	Mugla	785.73	N/A	N/A	N/A	N/A	N/A	N/A
M15	2010	Stationary	Mugla	1067.94	N/A	N/A	N/A	N/A	N/A	N/A
M16	2010	Stationary	Mugla	752.98	N/A	N/A	N/A	N/A	N/A	N/A
M17	2010	Stationary	Mugla	707.73	N/A	N/A	N/A	37.89	N/A	N/A
M18	2010	Stationary	Mugla	775.01	N/A	N/A	N/A	N/A	N/A	N/A
pcr neg	2010				N/A	N/A	N/A	N/A	N/A	N/A
pcr pos	2010				9.34	20.8	19.8	33.07	25.2	23.04

APPENDIX D

RT-QPCR CT VALUES OF N. CERANAE INFECTED AND CONTRO SAMPLES FOR EACH GENE TARGET

Table D.1 The ratio of 260/280 absorbance and RT-qPCR Ct values of *N. ceranae* infected and control samples for each gene target. Positive and negative control reactions were run on each 96-well plate. (I = infected, C = control, Tm = melting temperature, Ac = Actin, Vg = vitellogenin, Apid = apidaecin, Aba = abaecin). Outliers of the data were removed during statistical analysis.

Subspecies	Colony	Treatment	ng/ul	260/280	Actin	Ac-Tm	N.ceranae	N.cer-Tm	Vg	Vg-Tm	Apidaecin	Apid-Tm	Abaecin	Aba-Tm
Caucasian	1	I	570.6	1.90	28.37	82.50	19.09	80.50	29.50	84.00	26.53	79.50	33.06	80.00
Caucasian	1	I	389.3	1.90	31.32	82.50	14.01	80.50	31.53	84.00	25.74	79.50	27.39	80.00
Caucasian	1	I	498.5	1.86	29.92	82.50	15.11	80.50	32.07	83.50	22.69	79.50	24.96	80.50
Caucasian	1	I	431.1	1.86	30.27	82.50	21.09	80.50	32.60	83.50	19.67	79.50	21.67	80.50
Caucasian	1	I	478.8	1.90	30.66	82.50	27.47	80.50	33.40	83.50	25.66	79.50	30.76	80.50
Caucasian	1	I	450.6	1.89	23.80	82.50	25.21	80.50	28.54	83.50	25.49	79.50	25.76	80.50
Caucasian	1	I	429.4	1.85	27.51	82.50	25.37	80.50	30.50	84.00	29.74	79.50	27.57	80.50
Caucasian	1	I	436.2	1.85	32.95	82.50	18.72	80.50	36.89	83.50	29.76	79.50	30.55	80.50
Caucasian	1	I	349.1	1.92	26.60	82.50	16.51	80.50	32.17	83.50	26.15	79.50	26.79	80.50
Caucasian	1	I	416.1	1.94	32.45	82.50	16.09	80.50	32.11	83.50	28.72	79.50	30.79	80.50
Caucasian	1	I	411.8	1.89	30.41	82.50	16.00	80.50	32.43	83.50	27.84	79.50	29.88	80.50
Caucasian	2	I	527.3	1.97	27.23	82.50	17.31	80.50	36.99	84.00	20.53	79.50	19.42	80.50
Caucasian	2	I	575.3	1.98	29.06	82.50	18.10	80.50	30.88	84.00	24.83	79.50	24.81	80.00
Caucasian	2	I	406.2	1.92	26.97	82.50	14.62	80.50	31.73	83.50	21.70	79.50	25.64	80.00
Caucasian	2	I	600.1	2.00	31.02	82.50	17.13	80.50	33.65	83.50	25.13	79.50	25.77	80.50
Caucasian	2	I	438.2	1.93	28.63	82.50	17.21	80.50	29.77	83.50	24.98	79.50	25.11	80.50
Caucasian	2	I	401.5	1.91	28.68	82.50	16.61	80.50	31.41	83.50	26.35	79.50	26.82	80.50
Caucasian	2	I	439.9	1.94	28.04	82.50	15.25	80.50	33.83	83.50	26.45	79.50	28.34	80.50
Caucasian	2	I	450.8	1.98	26.41	82.50	14.84	80.50	32.04	83.50	28.54	79.50	29.15	80.50
Caucasian	2	I	492.8	1.98	25.22	82.50	11.27	80.50	33.60	83.50	22.81	79.50	21.79	80.50

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Actin	Ac-Tm	N.ceranae	N.cer-Tm	Vg	Vg-Tm	Apidaecin	Apid-Tm	Abaecin	Aba-Tm
Caucasian	**2**	**I**	464.2	1.95	26.12	82.50	13.20	80.50	30.87	83.50	26.15	79.50	24.50	80.50
Caucasian	**2**	**I**	319.8	1.89	31.74	82.50	15.37	80.50	33.83	83.50	28.53	79.50	28.64	80.50
Caucasian	**2**	**I**	461.7	1.92	29.25	82.50	15.57	80.50	32.09	83.50	27.38	79.50	23.97	80.50
Caucasian	**1**	**C**	379.6	1.85	29.99	82.50	50.00	80.50	33.11	84.00	26.30	79.50	27.11	80.50
Caucasian	**1**	**C**	320.1	1.89	30.54	82.50	36.10	80.50	30.30	83.50	32.41	79.50	31.59	80.00
Caucasian	**1**	**C**	355.1	1.87	28.41	82.50	34.19	80.50	34.20	83.50	29.96	79.50	31.18	80.50
Caucasian	**1**	**C**	335.6	1.89	26.28	82.50	50.00	80.50	31.53	83.50	28.83	79.50	27.14	80.50
Caucasian	**1**	**C**	347.6	1.89	26.71	82.50	50.00	80.50	30.04	83.50	27.19	79.50	26.33	80.50
Caucasian	**1**	**C**	487.3	1.97	29.80	82.50	31.69	80.50	35.58	83.50	31.47	79.50	32.31	80.50
Caucasian	**1**	**C**	439.9	1.95	31.17	82.50	39.06	80.50	34.03	83.50	29.92	79.50	29.64	80.50
Caucasian	**1**	**C**	372.1	1.91	26.85	82.50	50.00	80.50	29.35	83.50	24.15	79.50	33.36	80.50
Caucasian	**1**	**C**	462.3	1.95	25.80	82.50	50.00	80.50	33.86	None	21.56	79.50	20.55	80.50
Caucasian	**1**	**C**	478.7	1.95	30.38	82.50	38.51	80.50	28.98	83.50	26.18	79.50	28.15	80.50
Caucasian	**1**	**C**	544.3	2.01	30.18	82.50	50.00	80.50	32.74	83.50	28.32	79.50	31.54	80.50
Caucasian	**1**	**C**	364.5	1.92	29.97	82.50	42.49	80.50	28.74	83.50	26.99	79.50	37.04	80.50
Caucasian	**2**	**C**	376.3	1.91	31.11	82.50	50.00	80.50	34.65	84.00	30.30	79.50	31.74	80.50
Caucasian	**2**	**C**	492.5	1.98	30.45	82.50	32.85	80.50	33.01	83.50	29.31	79.50	35.02	80.00
Caucasian	**2**	**C**	481.8	1.96	28.02	82.50	41.58	80.50	31.68	83.50	26.18	79.50	25.28	80.50
Caucasian	**2**	**C**	324.6	1.91	29.20	82.50	35.35	80.50	34.07	83.50	32.48	79.50	31.22	80.50
Caucasian	**2**	**C**	479.1	1.94	32.67	82.50	50.00	80.50	33.02	83.50	33.98	79.50	34.39	80.50
Caucasian	**2**	**C**	390.0	1.91	24.06	82.50	50.00	80.50	28.60	83.50	26.13	79.50	34.74	80.50

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Actin	Ac-Tm	N.ceranae	N.cer-Tm	Vg	Vg-Tm	Apidaecin	Apid-Tm	Abaecin	Aba-Tm
Caucasian	**2**	**C**	409.1	1.92	27.92	82.50	50.00	80.50	30.69	83.50	25.36	79.50	27.10	80.50
Caucasian	**2**	**C**	410.1	1.91	30.22	82.50	41.45	80.50	32.24	83.50	28.69	79.50	25.73	80.50
Caucasian	**2**	**C**	425.4	1.93	31.76	82.50	50.00	80.50	29.14	83.50	28.47	79.50	29.14	80.50
Caucasian	**2**	**C**	370.3	1.89	25.07	82.50	46.20	80.50	26.99	83.50	24.28	79.50	29.32	80.50
Caucasian	**2**	**C**	472.2	1.94	24.50	82.50	50.00	80.50	27.14	83.50	24.42	79.50	24.16	80.50
Carniolan	**1**	**I**	242.8	1.85	29.03	82.50	16.66	80.50	31.33	84.00	21.66	79.50	34.80	80.50
Carniolan	**1**	**I**	291.3	1.88	29.66	82.50	14.73	80.50	33.76	83.50	27.07	79.50	26.28	80.00
Carniolan	**1**	**I**	270.9	1.88	30.72	82.50	15.58	80.50	31.66	83.50	27.48	79.50	26.85	80.50
Carniolan	**1**	**I**	320.3	1.91	32.01	82.50	13.72	80.50	30.65	83.50	23.92	79.50	25.77	80.50
Carniolan	**1**	**I**	488.3	1.96	27.73	82.50	19.87	80.50	32.09	83.50	25.34	79.50	25.86	80.50
Carniolan	**1**	**I**	473.2	1.99	22.45	82.50	10.95	80.50	25.84	83.50	21.61	79.50	20.55	80.50
Carniolan	**1**	**I**	462.3	1.95	31.30	82.50	18.51	80.50	33.29	83.50	23.58	79.50	23.68	80.50
Carniolan	**1**	**I**	457.3	1.95	31.85	82.50	15.62	80.50	30.68	83.50	29.34	79.50	30.77	80.50
Carniolan	**1**	**I**	439.6	1.95	25.53	82.50	13.89	80.50	32.48	83.50	28.47	79.50	24.57	80.50
Carniolan	**1**	**I**	428.7	1.94	31.07	82.50	17.68	80.50	33.12	83.50	28.91	79.50	29.01	80.50
Carniolan	**1**	**I**	420.9	1.95	25.14	82.50	14.46	80.50	29.82	83.50	17.86	79.50	18.83	80.50
Carniolan	**1**	**I**	442.5	1.94	25.99	82.50	12.78	80.50	34.54	84.00	22.68	79.50	21.00	80.50
Carniolan	**2**	**I**	469.9	1.97	30.92	82.50	15.88	80.50	30.41	84.00	30.84	79.50	37.21	80.50
Carniolan	**2**	**I**	496.6	1.97	29.18	82.50	14.33	80.50	32.45	83.50	28.36	79.50	38.96	80.00
Carniolan	**2**	**I**	453.4	1.94	31.89	82.50	18.96	80.50	32.34	83.50	28.57	79.50	42.15	80.50
Carniolan	**2**	**I**	449.5	1.93	33.00	82.50	15.65	80.50	43.22	83.50	24.18	79.50	34.87	80.50

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Actin	Ac-Tm	N.ceranae	N.cer-Tm	Vg	Vg-Tm	Apidaecin	Apid-Tm	Abaecin	Aba-Tm
Carniolan	**2**	**I**	324.3	1.88	32.81	82.50	18.41	80.50	32.12	83.50	21.72	79.50	32.37	80.50
Carniolan	**2**	**I**	448.4	1.94	30.39	82.50	17.07	80.50	30.37	83.50	27.99	79.50	34.45	80.50
Carniolan	**2**	**I**	447.1	1.96	29.94	82.50	12.49	80.50	33.86	83.50	32.07	79.50	32.72	80.50
Carniolan	**2**	**I**	465.1	1.96	31.45	82.50	16.26	80.50	32.00	None	29.43	79.50	31.89	80.50
Carniolan	**2**	**I**	322.0	1.91	29.33	82.50	16.03	80.50	33.12	83.50	27.36	79.50	28.86	80.00
Carniolan	**2**	**I**	434.0	1.94	32.35	82.50	17.12	80.50	32.55	83.50	28.04	79.50	37.78	80.50
Carniolan	**2**	**I**	325.0	1.91	34.14	82.50	17.67	80.50	32.14	83.50	31.42	79.50	39.67	80.50
Carniolan	**1**	**C**	466.6	1.94	26.32	82.50	50.00	80.50	34.01	84.00	28.47	79.50	27.36	80.00
Carniolan	**1**	**C**	370.4	1.92	29.81	82.50	50.00	80.50	33.26	84.00	31.11	79.50	38.10	80.00
Carniolan	**1**	**C**	297.5	1.89	30.07	82.50	50.00	80.50	37.24	83.50	29.72	79.50	30.71	80.50
Carniolan	**1**	**C**	323.2	1.92	26.93	82.50	50.00	80.50	31.26	83.50	29.89	79.50	37.56	80.50
Carniolan	**1**	**C**	316.7	1.92	30.40	82.50	50.00	80.50	34.16	83.50	28.98	79.50	37.10	80.50
Carniolan	**1**	**C**	422.2	1.98	22.56	82.50	50.00	80.50	28.64	83.50	26.84	79.50	35.76	80.50
Carniolan	**1**	**C**	494.2	1.96	28.39	82.50	50.00	80.50	29.98	83.50	31.38	79.50	43.88	80.50
Carniolan	**1**	**C**	421.9	1.96	26.61	82.50	50.00	80.50	32.32	83.50	27.71	79.50	28.33	80.50
Carniolan	**1**	**C**	478.0	1.94	30.19	82.50	50.00	80.50	36.20	83.50	21.75	79.50	25.95	80.50
Carniolan	**1**	**C**	489.1	1.95	25.11	82.50	50.00	80.50	30.51	83.50	23.82	79.50	36.34	80.50
Carniolan	**1**	**C**	328.1	1.91	30.58	82.50	36.94	80.50	31.85	83.50	30.78	79.50	32.41	80.50
Carniolan	**2**	**C**	418.7	1.93	26.62	82.50	50.00	80.50	33.99	84.00	26.95	79.50	25.53	80.50
Carniolan	**2**	**C**	439.4	1.94	29.77	82.50	50.00	80.50	32.40	84.00	30.28	79.50	30.46	80.00

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Actin	Ac-Tm	N.ceranae	N.cer-Tm	Vg	Vg-Tm	Apidaecin	Apid-Tm	Abaecin	Aba-Tm
Carniolan	2	C	461.7	1.94	26.27	82.50	50.00	80.50	33.39	84.00	25.68	79.50	23.34	80.50
Carniolan	2	C	334.2	1.91	31.90	82.50	50.00	80.50	33.69	84.00	31.62	79.50	30.10	80.50
Carniolan	2	C	454.4	1.96	23.07	82.50	50.00	80.50	31.26	83.50	25.38	79.50	23.01	80.50
Carniolan	2	C	315.6	1.92	27.15	82.50	50.00	80.50	29.37	83.50	27.47	79.50	26.12	80.50
Carniolan	2	C	489.7	1.96	24.58	82.50	50.00	80.50	28.77	83.50	28.85	79.50	26.70	80.50
Carniolan	2	C	683.3	2.01	27.20	82.50	50.00	80.50	31.60	83.50	26.89	79.50	27.62	80.50
Carniolan	2	C	402.6	1.91	25.11	82.50	39.85	80.50	27.50	83.50	25.85	79.50	26.23	80.50
Carniolan	2	C	310.7	1.88	27.14	82.50	50.00	80.50	32.58	83.50	26.33	79.50	23.77	80.50
Carniolan	2	C	643.1	2.04	28.68	82.50	39.44	80.50	29.07	83.50	27.19	79.50	28.19	80.50
Carniolan	2	C	566.7	2.02	27.09	82.50	46.82	80.50	30.58	83.50	27.29	79.50	25.80	80.50
Mugla	1	I	331.3	1.89	28.02	82.50	26.09	80.50	30.17	83.50	27.09	79.50	35.53	80.00
Mugla	1	I	386.4	1.92	29.71	82.50	20.14	80.50	30.04	83.50	25.28	79.50	28.01	80.50
Mugla	1	I	443.2	1.94	30.31	82.50	16.34	80.50	32.58	83.50	27.27	79.50	25.39	80.50
Mugla	1	I	359.1	1.90	27.97	82.50	19.41	80.50	27.43	83.50	24.22	79.50	24.75	80.50
Mugla	1	I	387.4	1.92	25.24	82.50	15.28	80.50	27.17	83.50	24.85	79.50	23.21	80.50
Mugla	1	I	323.5	1.91	29.34	82.50	19.11	80.50	35.58	83.50	30.83	79.50	30.41	80.50
Mugla	1	I	430.2	1.96	27.74	82.50	17.88	80.50	28.57	83.50	27.69	79.50	38.48	80.50
Mugla	1	I	402.8	1.93	33.08	82.50	23.87	80.50	34.62	83.50	33.14	79.50	37.10	80.50
Mugla	1	I	417.3	1.95	31.17	82.50	24.15	80.50	31.63	83.50	21.41	79.50	25.59	80.50
Mugla	1	I	368.9	1.93	30.41	82.50	28.26	80.50	27.49	83.50	28.96	79.50	29.45	80.50

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Actin	Ac-Tm	N.ceranae	N.cer-Tm	Vg	Vg-Tm	Apidaecin	Apid-Tm	Abaecin	Aba-Tm
Mugla	**2**	**I**	499.2	1.97	32.44	82.50	18.19	80.50	31.98	83.50	30.89	79.50	34.60	80.50
Mugla	**2**	**I**	473.2	1.99	27.84	82.50	15.79	80.50	31.98	83.50	25.78	79.50	26.54	80.50
Mugla	**2**	**I**	456.7	1.97	32.08	82.50	18.91	80.50	36.65	83.50	27.64	79.50	37.19	80.50
Mugla	**2**	**I**	473.7	1.98	26.39	82.50	14.10	80.50	30.00	83.50	23.11	79.50	23.54	80.50
Mugla	**2**	**I**	432.9	1.96	27.17	82.50	13.71	80.50	25.09	83.50	22.56	79.50	35.44	80.50
Mugla	**2**	**I**	359.4	1.90	23.52	82.50	13.10	80.50	29.03	83.50	23.69	79.50	24.61	80.50
Mugla	**2**	**I**	425.2	1.96	28.43	82.50	14.81	80.50	30.01	83.50	26.32	79.50	34.38	80.50
Mugla	**2**	**I**	323.0	1.91	27.48	82.50	15.12	80.50	27.45	83.50	26.67	79.50	29.47	80.50
Mugla	**2**	**I**	368.0	1.92	23.56	82.50	11.12	80.50	31.50	83.50	21.39	79.50	31.72	80.50
Mugla	**2**	**I**	350.0	1.92	30.04	82.50	22.53	80.50	30.68	83.50	28.18	79.50	30.91	80.50
Mugla	**2**	**I**	356.7	1.93	26.35	82.50	14.50	80.50	29.83	83.50	23.84	79.50	25.46	80.50
Mugla	**2**	**I**	302.3	1.89	30.40	82.50	16.14	80.50	31.88	83.50	30.17	79.50	28.53	80.50
Mugla	**1**	**C**	371.2	1.92	29.50	82.50	38.49	80.50	32.12	83.50	26.85	79.50	29.78	80.00
Mugla	**1**	**C**	475.8	1.98	25.22	82.50	50.00	80.50	27.31	83.50	23.66	79.50	22.08	80.50
Mugla	**1**	**C**	407.2	1.95	26.74	82.50	34.13	80.50	32.39	84.00	24.09	79.50	24.87	80.50
Mugla	**1**	**C**	457.2	1.99	25.22	82.50	36.38	80.50	32.16	83.50	26.18	79.50	23.77	80.00
Mugla	**1**	**C**	311.8	1.90	30.26	82.50	36.20	80.50	34.73	83.50	29.21	79.50	38.11	80.00
Mugla	**1**	**C**	451.2	1.95	25.24	82.50	37.09	80.50	31.26	83.50	28.22	79.50	25.83	80.00
Mugla	**1**	**C**	436.5	1.94	27.09	82.50	35.80	80.50	25.67	83.50	29.42	79.50	29.96	80.00
Mugla	**1**	**C**	370.3	1.91	28.64	82.50	36.12	80.50	29.53	84.00	27.00	79.50	37.56	80.50

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Actin	Ac-Tm	N.ceranae	N.cer-Tm	Vg	Vg-Tm	Apidaecin	Apid-Tm	Abaecin	Aba-Tm
Mugla	**1**	**C**	373.7	1.91	25.21	82.50	34.15	80.50	27.49	84.00	23.41	79.50	25.88	80.50
Mugla	**1**	**C**	456.8	1.98	24.00	82.50	37.57	80.50	29.61	83.50	21.77	79.50	32.56	80.00
Mugla	**1**	**C**	452.2	1.98	25.40	82.50	38.03	80.50	28.88	84.00	25.97	79.50	25.16	80.50
Mugla	**1**	**C**	424.8	1.96	30.74	82.50	50.00	80.50	34.61	84.00	30.44	79.50	32.31	80.50
Mugla	**1**	**C**	395.1	1.92	30.56	82.50	35.58	80.50	32.08	83.50	25.97	79.50	26.01	80.50
Mugla	**1**	**C**	436.4	1.94	32.12	82.50	35.51	80.50	32.20	83.50	30.28	79.50	33.03	80.50
Mugla	**1**	**C**	485.8	1.98	32.30	82.50	36.46	80.50	31.55	83.50	28.23	79.50	32.00	80.50
Mugla	**1**	**C**	498.1	1.90	33.39	82.50	50.00	80.50	32.34	83.50	32.94	79.50	35.17	80.50
Mugla	**1**	**C**	488.7	1.98	33.95	82.50	35.90	80.50	34.99	83.50	31.23	79.50	38.51	80.50
Mugla	**1**	**C**	459.4	1.96	33.11	82.50	34.16	80.50	32.42	83.50	33.15	79.50	33.89	80.50
Mugla	**1**	**C**	404.2	1.94	29.68	82.50	35.72	80.50	33.47	84.00	31.23	79.50	49.01	80.50
Mugla	**1**	**C**	414.8	1.97	33.57	82.50	38.11	80.50	33.96	83.50	34.80	79.50	32.35	80.50
Mugla	**1**	**C**	359.2	1.93	34.10	82.50	38.20	80.50	33.09	83.50	32.55	79.50	32.28	80.50
Mugla	**1**	**C**	322.1	1.91	31.76	82.50	36.83	80.50	36.68	83.50	34.03	79.50	33.77	80.50
Mugla	**1**	**C**	339.9	1.92	34.39	82.50	50.00	80.50	36.05	83.50	31.32	79.50	34.20	80.50
Mugla	**1**	**C**	308.5	1.89	33.02	82.50	37.03	80.50	33.12	83.50	32.22	79.50	43.18	80.50
Mugla	**1**	**C**	270.8	1.89	34.01	82.50	34.34	80.50	33.20	83.50	35.22	79.50	50.00	80.50
Yigilca	**1**	**I**	442.5	1.99	29.04	82.50	18.35	80.50	35.03	83.50	34.40	79.50	33.44	80.50
Yigilca	**1**	**I**	452.7	1.96	28.62	82.50	25.11	80.50	30.11	83.50	23.31	79.50	24.58	80.50
Yigilca	**1**	**I**	472.3	1.97	30.73	82.50	18.10	80.50	33.08	83.50	26.38	79.50	36.62	80.50
Yigilca	**1**	**I**	331.3	1.91	25.37	82.50	17.13	80.50	29.05	83.50	24.50	79.50	23.74	80.50

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Actin	Ac-Tm	N.ceranae	N.cer-Tm	Vg	Vg-Tm	Apidaecin	Apid-Tm	Abaecin	Aba-Tm
Yigilca	**1**	**I**	322.6	1.91	24.26	82.50	17.33	80.50	27.58	83.50	25.33	79.50	34.59	80.50
Yigilca	**1**	**I**	480.3	1.95	24.60	82.50	28.76	80.50	31.09	83.50	27.88	79.50	25.36	80.50
Yigilca	**1**	**I**	390.6	1.91	28.57	82.50	17.18	80.50	31.27	83.50	28.34	79.50	38.82	80.50
Yigilca	**1**	**I**	480.1	1.99	25.57	82.50	23.11	80.50	28.03	83.50	23.63	79.50	23.71	80.50
Yigilca	**1**	**I**	683.8	2.01	24.07	82.50	14.09	80.50	26.70	83.50	24.06	79.50	35.87	80.50
Yigilca	**1**	**I**	391.1	1.90	29.60	82.50	17.11	80.50	37.54	83.50	29.97	79.50	32.86	80.50
Yigilca	**1**	**I**	382.4	1.90	28.98	82.50	25.23	80.50	32.37	83.50	27.79	79.50	28.14	80.50
Yigilca	**2**	**I**	442.3	1.98	27.52	82.50	16.18	80.50	35.13	83.50	26.90	79.50	33.83	80.50
Yigilca	**2**	**I**	411.8	1.97	31.50	82.50	19.07	80.50	35.35	83.50	30.39	79.50	32.58	80.50
Yigilca	**2**	**I**	369.3	1.94	29.15	82.50	16.80	80.50	36.49	83.50	26.14	79.50	35.18	80.50
Yigilca	**2**	**I**	483.2	1.99	28.40	82.50	19.33	80.50	35.63	83.50	26.01	79.50	26.99	80.50
Yigilca	**2**	**I**	403.9	1.98	32.66	82.50	18.85	80.50	34.39	83.50	33.12	79.50	31.33	80.50
Yigilca	**2**	**I**	494.8	1.96	32.47	82.50	18.11	80.50	36.64	83.50	31.52	79.50	32.05	80.50
Yigilca	**2**	**I**	368.3	1.93	30.11	82.50	16.21	80.50	35.88	83.50	28.80	79.50	35.10	80.50
Yigilca	**2**	**I**	551.0	2.00	36.96	82.50	16.67	80.50	32.78	83.50	28.02	79.50	26.18	80.50
Yigilca	**2**	**I**	390.9	1.94	30.07	82.50	29.82	80.50	32.17	83.50	22.45	79.50	23.48	80.50
Yigilca	**2**	**I**	511.0	2.02	30.96	82.50	20.06	80.50	33.99	83.50	26.43	79.50	27.76	80.50
Yigilca	**2**	**I**	641.2	2.03	28.15	82.50	18.20	80.50	35.71	83.50	30.67	79.50	27.74	80.50
Yigilca	**2**	**I**	398.9	1.95	33.78	82.50	18.72	80.50	35.82	83.50	26.98	79.50	35.05	80.50
Yigilca	**1**	**C**	369.1	1.96	27.63	82.50	50.00	80.50	31.57	84.00	26.78	79.50	28.09	80.00
Yigilca	**1**	**C**	359.5	1.94	31.15	82.50	50.00	80.50	35.24	84.00	31.98	79.50	37.51	80.50

Table D.1 (Cont'd

Subspecies	Colony	Treatment	ng/ul	260/280	Actin	Ac-Tm	N.ceranae	N.cer-Tm	Vg	Vg-Tm	Apidaecin	Apid-Tm	Abaecin	Aba-Tm
Yigilca	**1**	**C**	371.6	1.94	23.51	82.50	50.00	80.50	27.83	84.00	24.31	79.50	22.93	80.50
Yigilca	**1**	**C**	489.7	1.96	25.53	82.50	39.45	80.50	25.85	84.00	27.04	79.50	27.50	80.00
Yigilca	**1**	**C**	563.5	2.04	31.56	82.50	50.00	80.50	32.07	84.00	30.00	79.50	31.82	80.00
Yigilca	**1**	**C**	372.1	1.95	30.64	82.50	50.00	80.50	33.88	83.50	31.25	79.50	35.10	80.00
Yigilca	**1**	**C**	501.7	2.04	30.80	82.50	50.00	80.50	27.99	83.50	27.77	79.50	30.90	80.50
Yigilca	**1**	**C**	663.4	2.03	29.54	82.50	50.00	80.50	25.94	83.50	28.68	79.50	30.60	80.50
Yigilca	**1**	**C**	660.1	2.05	27.27	82.50	50.00	80.50	29.88	83.50	25.61	79.50	26.93	80.50
Yigilca	**1**	**C**	300.1	1.91	23.04	82.50	50.00	80.50	26.45	83.50	24.97	79.50	24.86	80.50
Yigilca	**1**	**C**	308.6	1.91	25.01	82.50	50.00	80.50	29.62	83.50	24.79	79.50	26.64	80.50
Yigilca	**2**	**C**	317.5	1.92	26.97	82.50	42.14	80.50	32.20	83.50	27.30	79.50	35.12	80.50
Yigilca	**2**	**C**	338.2	1.93	27.06	82.50	40.31	80.50	30.71	83.50	25.46	79.50	36.07	80.50
Yigilca	**2**	**C**	396.8	1.98	27.31	82.50	37.57	80.50	34.20	83.50	29.91	79.50	26.89	80.50
Yigilca	**2**	**C**	389.1	1.97	24.63	82.50	37.24	80.50	27.23	84.00	28.22	79.50	33.77	80.50
Yigilca	**2**	**C**	381.1	1.96	28.81	82.50	37.59	80.50	32.50	84.00	30.11	79.50	29.76	80.50
Yigilca	**2**	**C**	319.7	1.91	24.22	82.50	37.19	80.50	29.19	83.50	28.15	79.50	25.67	80.50
Yigilca	**2**	**C**	303.1	1.90	28.39	82.50	36.98	80.50	30.94	83.50	28.15	79.50	38.08	80.00
Yigilca	**2**	**C**	360.9	1.93	26.47	82.50	50.00	80.50	31.77	83.50	25.54	79.50	35.11	80.50
Yigilca	**2**	**C**	447.4	1.98	29.62	82.50	42.67	80.50	33.18	83.50	27.95	79.50	26.22	80.50
Yigilca	**2**	**C**	438.1	1.97	31.97	82.50	50.00	80.50	36.53	83.50	32.02	79.50	29.97	80.50
Yigilca	**2**	**C**	424.8	1.98	25.46	82.50	50.00	80.50	30.26	83.50	25.04	79.50	26.23	80.50
Syrian	**1**	**I**	505.5	2.01	28.76	82.50	17.09	80.50	25.71	83.50	24.37	79.50	35.63	80.50

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Actin	Ac-Tm	N.ceranae	N.cer-Tm	Vg	Vg-Tm	Apidaecin	Apid-Tm	Abaecin	Aba-Tm
Syrian	**1**	**I**	543.3	1.98	28.11	82.50	15.89	80.50	25.71	83.50	24.90	79.50	35.42	80.50
Syrian	**1**	**I**	450.2	1.99	30.33	82.50	14.01	80.50	27.36	83.50	26.14	79.50	37.23	80.50
Syrian	**1**	**I**	593.6	2.02	29.55	82.50	13.20	80.50	25.30	83.50	25.76	79.50	36.04	80.50
Syrian	**1**	**I**	534.7	2.03	31.56	82.50	15.58	80.50	27.28	83.50	26.83	79.50	37.14	80.50
Syrian	**1**	**I**	409.8	1.94	30.97	82.50	16.45	80.50	24.34	83.50	26.78	79.50	38.67	80.50
Syrian	**1**	**I**	335.9	1.91	26.43	82.50	13.10	80.50	25.78	83.50	23.92	79.50	22.63	80.50
Syrian	**1**	**I**	362.9	1.93	26.10	82.50	15.09	80.50	25.47	83.50	24.82	79.50	35.86	80.50
Syrian	**1**	**I**	431.9	1.91	30.07	82.50	16.53	80.50	29.07	83.50	28.10	79.50	31.35	80.50
Syrian	**1**	**I**	394.4	1.93	28.08	82.50	16.46	80.50	29.18	83.50	26.26	79.50	37.07	80.50
Syrian	**1**	**I**	505.5	2.00	23.88	82.50	14.78	80.50	33.49	83.50	18.10	79.50	29.26	80.50
Syrian	**1**	**I**	405.4	1.91	32.05	82.50	20.50	80.50	33.25	83.50	26.13	79.50	36.53	80.50
Syrian	**2**	**I**	443.3	1.96	26.97	82.50	15.44	80.50	30.66	83.50	24.17	79.50	26.22	80.50
Syrian	**2**	**I**	350.2	1.93	26.68	82.50	18.06	80.50	28.68	83.50	25.44	79.50	25.78	80.50
Syrian	**2**	**I**	493.6	1.95	26.22	82.50	15.05	80.50	28.76	83.50	24.86	79.50	33.74	80.50
Syrian	**2**	**I**	454.7	1.95	32.92	82.50	20.52	80.50	30.75	83.50	25.08	79.50	33.10	80.50
Syrian	**2**	**I**	449.8	1.94	28.23	82.50	15.78	80.50	28.10	83.50	26.84	79.50	35.93	80.50
Syrian	**2**	**I**	275.9	1.91	26.48	82.50	15.87	80.50	30.09	83.50	23.81	79.50	32.01	80.50
Syrian	**2**	**I**	262.9	1.93	31.10	82.50	18.14	80.50	29.29	83.50	30.06	79.50	37.26	80.50
Syrian	**2**	**I**	531.9	2.03	30.72	82.50	18.02	80.50	29.16	83.50	29.81	79.50	32.53	80.50
Syrian	**2**	**I**	349.4	1.95	29.49	82.50	16.68	80.50	31.13	83.50	29.11	79.50	35.44	80.50
Syrian	**2**	**I**	585.7	2.02	28.58	82.50	17.52	80.50	36.87	83.50	30.11	79.50	36.43	80.50
Syrian	**2**	**I**	323.3	1.92	26.31	82.50	14.25	80.50	24.42	83.50	25.04	79.50	33.28	80.50
Syrian	**2**	**I**	585.7	1.99	25.39	82.50	14.98	80.50	28.30	83.50	25.99	79.50	34.06	80.50

Table D.1 (Cont'd) T

Subspecies	Colony	Treatment	ng/ul	260/280	Actin	Ac-Tm	N.ceranae	N.cer-Tm	Vg	Vg-Tm	Apidaecin	Apid-Tm	Abaecin	Aba-Tm
Syrian	1	C	289.1	1.90	26.04	82.50	37.58	80.50	29.73	84.00	21.60	79.50	32.60	80.50
Syrian	1	C	480.0	1.97	25.73	82.50	40.28	80.50	29.97	83.50	25.04	79.50	34.84	80.50
Syrian	1	C	482.3	1.97	25.38	82.50	37.13	80.50	28.83	83.50	26.68	79.50	25.58	80.50
Syrian	1	C	419.8	1.93	27.30	82.50	50.00	80.50	27.33	83.50	26.12	79.50	27.64	80.50
Syrian	1	C	290.2	1.91	25.75	82.50	37.37	80.50	28.19	83.50	25.90	79.50	36.28	80.50
Syrian	1	C	514.3	2.00	25.77	82.50	50.00	80.50	33.29	83.50	25.46	79.50	35.48	80.50
Syrian	1	C	487.2	1.97	28.09	82.50	35.30	80.50	32.52	83.50	28.18	79.50	38.92	80.50
Syrian	1	C	291.5	1.91	23.96	82.50	34.00	80.50	25.43	83.50	24.12	79.50	32.52	80.50
Syrian	1	C	303.1	1.98	25.02	82.50	35.45	80.50	23.65	83.50	23.07	79.50	31.85	80.50
Syrian	1	C	310.9	1.93	25.79	82.50	35.25	80.50	28.37	84.00	22.36	79.50	29.21	80.50
Syrian	1	C	330.8	1.94	27.76	82.50	33.87	80.50	25.34	83.50	24.89	79.50	33.92	80.50
Syrian	1	C	456.3	1.95	28.96	82.50	33.31	80.50	27.40	83.50	26.85	79.50	36.14	80.50
Syrian	1	C	306.2	1.92	30.18	82.50	37.60	80.50	29.23	83.50	28.31	79.50	36.93	80.50
Syrian	1	C	434.2	2.01	25.81	82.50	33.79	80.50	25.20	83.50	23.73	79.50	25.70	80.50
Syrian	1	C	417.4	1.98	27.55	82.50	35.87	80.50	27.25	83.50	26.23	79.50	35.16	80.50
Syrian	1	C	294.0	1.99	28.87	82.50	34.28	80.50	29.73	83.50	28.54	79.50	39.98	80.50
Syrian	1	C	374.4	1.96	24.58	82.50	33.98	80.50	24.65	83.50	24.90	79.50	34.00	80.50
Syrian	1	C	285.6	1.92	28.95	82.50	35.64	80.50	32.66	83.50	28.81	79.50	30.82	80.50
Syrian	1	C	528.2	2.00	23.54	82.50	33.51	80.50	29.83	83.50	20.74	79.50	27.32	80.50
Syrian	1	C	432.4	2.01	24.07	82.50	34.49	80.50	25.45	83.50	24.17	79.50	24.78	80.50
Syrian	1	C	328.7	1.93	28.09	82.50	37.13	80.50	29.52	84.00	26.33	79.50	32.05	80.50
Syrian	1	C	336.5	1.96	26.76	82.50	38.22	80.50	25.19	84.00	24.76	79.50	24.87	80.50
pcr positive						82.50		80.50		83.50		79.50		80.50
pcr negative					N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Defensin	Def-Tm	Hymeno	Hymeno-Tm	Eater	Eater Tm
Caucasian	**1**	**I**	570.6	1.90	19.65	81.50	45.35	81.50	35.81	83.50
Caucasian	**1**	**I**	389.3	1.90	27.10	81.50	30.65	81.50	37.39	83.50
Caucasian	**1**	**I**	498.5	1.86	21.93	81.50	30.98	81.50	40.16	83.50
Caucasian	**1**	**I**	431.1	1.86	18.47	81.50	23.42	81.50	38.41	84.00
Caucasian	**1**	**I**	478.8	1.90	24.84	81.50	32.45	81.50	38.32	83.50
Caucasian	**1**	**I**	450.6	1.89	19.81	81.50	28.83	81.50	34.98	83.50
Caucasian	**1**	**I**	429.4	1.85	31.44	81.50	36.63	81.50	35.81	83.50
Caucasian	**1**	**I**	436.2	1.85	32.04	81.50	35.27	81.50	50.00	83.50
Caucasian	**1**	**I**	349.1	1.92	23.37	81.50	31.91	81.00	37.12	83.50
Caucasian	**1**	**I**	416.1	1.94	30.10	81.50	35.79	81.50	39.58	83.50
Caucasian	**1**	**I**	411.8	1.89	28.64	81.50	39.83	81.50	38.87	83.50
Caucasian	**2**	**I**	527.3	1.97	18.24	81.50	43.63	81.50	36.64	83.50
Caucasian	**2**	**I**	575.3	1.98	23.97	81.50	33.29	81.50	36.93	83.50
Caucasian	**2**	**I**	406.2	1.92	23.99	81.50	27.66	81.50	35.38	83.50
Caucasian	**2**	**I**	600.1	2.00	18.80	81.50	29.63	81.50	37.25	83.50
Caucasian	**2**	**I**	438.2	1.93	28.17	81.50	34.97	81.50	37.47	83.50
Caucasian	**2**	**I**	401.5	1.91	26.00	81.50	31.94	81.50	37.87	83.50
Caucasian	**2**	**I**	439.9	1.94	22.22	81.50	28.86	81.50	37.89	83.00
Caucasian	**2**	**I**	450.8	1.98	27.23	81.50	34.56	81.50	37.68	83.50
Caucasian	**2**	**I**	492.8	1.98	19.49	81.50	22.13	81.50	34.80	83.50
Caucasian	**2**	**I**	464.2	1.95	24.05	81.50	29.18	81.50	32.57	83.50
Caucasian	**2**	**I**	319.8	1.89	26.94	81.50	32.29	81.50	40.13	84.00
Caucasian	**2**	**I**	461.7	1.92	27.76	81.50	31.03	81.50	37.06	84.00
Caucasian	**1**	**C**	379.6	1.85	23.33	81.50	31.50	81.50	32.08	83.50

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Defensin	Def-Tm	Hymeno	Hymeno-Tm	Eater	Eater Tm
Caucasian	1	C	320.1	1.89	30.10	81.50	34.61	81.50	32.67	83.50
Caucasian	1	C	355.1	1.87	30.57	81.50	34.02	81.50	33.40	83.50
Caucasian	1	C	335.6	1.89	26.71	81.50	30.93	81.50	33.49	83.50
Caucasian	1	C	347.6	1.89	28.66	81.50	31.78	81.50	34.29	83.50
Caucasian	1	C	487.3	1.97	27.89	81.50	33.32	81.50	39.80	84.00
Caucasian	1	C	439.9	1.95	27.18	81.50	30.58	81.50	34.37	83.50
Caucasian	1	C	372.1	1.91	23.41	81.50	30.21	81.50	31.59	84.00
Caucasian	1	C	462.3	1.95	15.80	81.50	20.79	81.50	33.09	83.50
Caucasian	1	C	478.7	1.95	29.06	81.50	34.19	81.50	35.36	83.50
Caucasian	1	C	544.3	2.01	28.86	81.50	35.03	81.50	36.50	83.50
Caucasian	1	C	364.5	1.92	27.40	81.50	36.77	81.50	34.03	83.50
Caucasian	2	C	376.3	1.91	26.42	81.50	35.26	81.50	30.80	83.50
Caucasian	2	C	492.5	1.98	30.52	81.50	36.81	81.50	32.87	83.50
Caucasian	2	C	481.8	1.96	25.71	81.50	29.88	81.50	35.03	83.50
Caucasian	2	C	324.6	1.91	29.99	81.50	34.72	81.50	33.17	83.50
Caucasian	2	C	479.1	1.94	31.43	81.50	35.15	81.50	37.57	84.00
Caucasian	2	C	390	1.91	26.52	81.50	32.03	81.50	33.51	83.00
Caucasian	2	C	409.1	1.92	21.30	81.50	28.75	81.50	32.85	83.50
Caucasian	2	C	410.1	1.91	27.25	81.50	34.13	81.50	36.16	84.00
Caucasian	2	C	425.4	1.93	29.81	81.50	35.93	81.50	35.66	83.00
Caucasian	2	C	370.3	1.89	20.19	81.50	23.36	81.50	32.86	83.50
Caucasian	2	C	472.2	1.94	24.73	81.50	28.51	81.50	30.43	83.50
Carniolan	1	I	242.8	1.85	22.37	81.50	23.27	81.50	35.46	84.00

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Defensin	Def-Tm	Hymeno	Hymeno-Tm	Eater	Eater Tm
Carniolan	**1**	**I**	291.3	1.88	27.75	81.50	34.06	81.50	36.42	83.50
Carniolan	**1**	**I**	270.9	1.88	31.70	81.50	36.01	81.00	38.90	83.00
Carniolan	**1**	**I**	320.3	1.91	28.17	81.50	32.20	81.50	35.09	84.00
Carniolan	**1**	**I**	488.3	1.96	29.33	81.50	36.09	81.50	38.01	83.00
Carniolan	**1**	**I**	473.2	1.99	22.61	81.50	24.09	81.50	31.49	84.50
Carniolan	**1**	**I**	462.3	1.95	25.73	81.50	28.33	81.00	34.09	83.00
Carniolan	**1**	**I**	457.3	1.95	33.00	81.50	37.56	81.00	44.78	83.50
Carniolan	**1**	**I**	439.6	1.95	26.17	81.50	30.86	81.50	36.14	84.00
Carniolan	**1**	**I**	428.7	1.94	29.19	81.50	36.78	81.00	36.95	83.50
Carniolan	**1**	**I**	420.9	1.95	17.02	81.50	21.24	81.50	34.60	83.00
Carniolan	**1**	**I**	442.5	1.94	25.41	81.50	25.57	81.50	34.20	83.50
Carniolan	**2**	**I**	469.9	1.97	31.12	81.50	37.18	81.50	38.26	84.00
Carniolan	**2**	**I**	496.6	1.97	29.18	81.50	33.23	81.50	37.04	84.00
Carniolan	**2**	**I**	453.4	1.94	29.65	81.50	33.60	81.50	38.84	83.50
Carniolan	**2**	**I**	449.5	1.93	28.42	81.50	33.74	81.00	36.13	82.50
Carniolan	**2**	**I**	324.3	1.88	27.57	81.50	30.53	81.00	38.52	83.50
Carniolan	**2**	**I**	448.4	1.94	29.02	81.50	34.11	81.50	46.82	82.50
Carniolan	**2**	**I**	447.1	1.96	31.98	81.50	33.89	81.00	38.02	83.50
Carniolan	**2**	**I**	465.1	1.96	29.91	81.50	36.69	81.50	38.20	83.50
Carniolan	**2**	**I**	322	1.91	29.82	81.50	33.08	81.50	37.94	82.50
Carniolan	**2**	**I**	434	1.94	27.28	81.50	34.35	81.50	50.00	83.50

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Defensin	Def-Tm	Hymeno	Hymeno-Tm	Eater	Eater Tm
Carniolan	**2**	**I**	325	1.91	30.17	81.50	34.10	81.50	39.13	83.50
Carniolan	**1**	**C**	466.6	1.94	25.03	81.50	30.35	81.50	33.16	83.50
Carniolan	**1**	**C**	370.4	1.92	30.69	81.50	36.60	81.39	33.15	84.00
Carniolan	**1**	**C**	297.5	1.89	29.65	81.50	34.20	81.40	38.36	84.00
Carniolan	**1**	**C**	323.2	1.92	31.35	81.50	33.20	81.40	35.07	83.00
Carniolan	**1**	**C**	316.7	1.92	32.30	81.50	36.16	81.40	38.47	83.50
Carniolan	**1**	**C**	422.2	1.98	26.81	81.50	29.85	81.41	29.79	84.00
Carniolan	**1**	**C**	494.2	1.96	31.55	81.50	36.74	81.41	37.04	84.00
Carniolan	**1**	**C**	421.9	1.96	26.13	81.50	30.31	81.41	32.47	84.00
Carniolan	**1**	**C**	478	1.94	20.97	81.50	24.13	81.42	35.01	84.00
Carniolan	**1**	**C**	489.1	1.95	23.67	81.50	26.80	81.42	31.10	84.00
Carniolan	**1**	**C**	328.1	1.91	29.33	81.50	36.18	81.50	35.84	84.00
Carniolan	**2**	**C**	418.7	1.93	25.33	81.50	33.47	81.50	33.46	83.50
Carniolan	**2**	**C**	439.4	1.94	30.23	81.50	35.49	81.50	33.69	84.00
Carniolan	**2**	**C**	461.7	1.94	27.11	81.50	28.35	81.50	34.17	84.00
Carniolan	**2**	**C**	334.2	1.91	33.45	81.50	35.36	81.50	38.45	83.50
Carniolan	**2**	**C**	454.4	1.96	24.71	81.50	29.89	81.50	32.78	83.50
Carniolan	**2**	**C**	315.6	1.92	25.19	81.50	30.30	81.50	37.16	84.00
Carniolan	**2**	**C**	489.7	1.96	28.31	81.50	34.29	81.50	33.44	83.50
Carniolan	**2**	**C**	683.3	2.01	28.32	81.50	28.51	81.50	34.05	84.00
Carniolan	**2**	**C**	402.6	1.91	24.97	81.50	29.67	81.50	30.21	83.50

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Defensin	Def-Tm	Hymeno	Hymeno-Tm	Eater	Eater Tm
Carniolan	**2**	**C**	310.7	1.88	26.17	81.50	31.24	81.50	34.17	84.00
Carniolan	**2**	**C**	643.1	2.04	28.97	81.50	33.60	81.50	35.28	84.00
Carniolan	**2**	**C**	566.7	2.02	27.16	81.50	31.99	81.50	34.26	84.00
Mugla	**1**	**I**	331.3	1.89	28.65	81.50	31.19	81.50	37.36	84.00
Mugla	**1**	**I**	386.4	1.92	29.63	81.50	32.23	81.50	36.77	83.50
Mugla	**1**	**I**	443.2	1.94	28.44	81.50	31.53	81.50	37.07	83.00
Mugla	**1**	**I**	359.1	1.90	27.86	81.50	32.25	81.50	37.55	83.00
Mugla	**1**	**I**	387.4	1.92	25.32	81.50	29.94	81.50	35.03	84.00
Mugla	**1**	**I**	323.5	1.91	30.73	81.50	35.09	81.50	36.90	84.00
Mugla	**1**	**I**	430.2	1.96	27.54	81.50	33.56	81.50	39.91	83.50
Mugla	**1**	**I**	402.8	1.93	33.60	81.50	50.00	81.00	40.39	83.50
Mugla	**1**	**I**	417.3	1.95	27.24	81.50	26.68	81.50	38.29	83.50
Mugla	**1**	**I**	368.9	1.93	30.29	81.50	38.29	81.50	39.22	83.50
Mugla	**2**	**I**	499.2	1.97	33.29	81.50	37.69	81.50	38.47	83.50
Mugla	**2**	**I**	473.2	1.99	22.37	81.50	28.57	81.00	35.64	84.00
Mugla	**2**	**I**	456.7	1.97	26.65	81.50	30.99	81.50	38.15	83.50
Mugla	**2**	**I**	473.7	1.98	27.16	81.50	29.95	81.00	33.19	83.50
Mugla	**2**	**I**	432.9	1.96	28.81	81.50	29.77	81.50	35.20	83.50
Mugla	**2**	**I**	359.4	1.90	25.73	81.50	26.57	81.50	34.12	83.50
Mugla	**2**	**I**	425.2	1.96	23.17	81.50	28.61	81.50	40.78	83.50
Mugla	**2**	**I**	323	1.91	29.06	81.50	32.46	81.50	36.25	84.00

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Defensin	Def-Tm	Hymeno	Hymeno-Tm	Eater	Eater Tm
Mugla	**2**	**I**	368	1.92	25.39	81.50	25.72	81.50	34.09	84.00
Mugla	**2**	**I**	350	1.92	28.07	81.50	34.09	81.50	45.81	83.50
Mugla	**2**	**I**	356.7	1.93	24.89	81.50	32.48	81.50	34.70	83.50
Mugla	**2**	**I**	302.3	1.89	29.59	81.50	31.98	81.00	44.28	83.50
Mugla	**1**	**C**	371.2	1.92	26.22	81.50	32.92	81.50	36.27	84.50
Mugla	**1**	**C**	475.8	1.98	23.39	81.50	28.50	81.50	37.50	83.50
Mugla	**1**	**C**	407.2	1.95	28.46	81.50	29.46	81.50	38.55	83.50
Mugla	**1**	**C**	457.2	1.99	26.27	81.50	29.03	81.50	33.12	83.50
Mugla	**1**	**C**	311.8	1.90	34.29	81.50	34.65	81.50	34.79	83.50
Mugla	**1**	**C**	451.2	1.95	27.17	81.50	30.09	81.50	33.37	84.00
Mugla	**1**	**C**	436.5	1.94	29.82	81.50	36.12	81.50	35.32	83.50
Mugla	**1**	**C**	370.3	1.91	30.59	81.50	35.16	81.50	36.47	84.00
Mugla	**1**	**C**	316.5	1.90	30.29	81.50	48.78	81.50	38.53	84.00
Mugla	**1**	**C**	373.7	1.91	25.04	81.50	30.66	81.50	34.25	84.00
Mugla	**1**	**C**	456.8	1.98	25.48	81.50	27.10	81.50	30.70	84.00
Mugla	**1**	**C**	452.2	1.98	25.78	81.50	31.85	81.50	33.42	84.00
Mugla	**1**	**C**	424.8	1.96	30.44	81.50	36.91	81.50	49.63	83.50
Mugla	**1**	**C**	395.1	1.92	26.25	81.50	30.42	81.50	37.75	83.50
Mugla	**1**	**C**	436.4	1.94	30.58	81.50	34.28	81.50	37.05	83.50
Mugla	**1**	**C**	485.8	1.98	31.59	81.50	32.35	81.50	38.05	83.50
Mugla	**1**	**C**	498.1	1.90	30.88	81.50	36.54	81.50	40.47	84.00

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Defensin	Def-Tm	Hymeno	Hymeno-Tm	Eater	Eater Tm
Mugla	**1**	**C**	488.7	1.98	29.03	81.50	33.99	81.50	38.15	83.50
Mugla	**1**	**C**	459.4	1.96	29.68	81.50	36.42	81.50	39.01	83.50
Mugla	**1**	**C**	404.2	1.94	32.22	81.50	36.74	81.50	36.26	83.50
Mugla	**1**	**C**	414.8	1.97	31.60	81.50	36.91	81.50	38.51	84.00
Mugla	**1**	**C**	359.2	1.93	29.22	81.50	35.41	81.50	44.50	83.50
Mugla	**1**	**C**	322.1	1.91	31.57	81.50	36.68	81.50	36.08	83.50
Mugla	**1**	**C**	339.9	1.92	36.02	81.50	37.05	81.50	50.00	83.50
Mugla	**1**	**C**	308.5	1.89	32.43	81.50	35.62	81.50	38.41	83.50
Mugla	**1**	**C**	270.8	1.89	32.41	81.50	38.01	81.50	42.17	83.50
Yigilca	**1**	**I**	442.5	1.99	31.65	81.50	36.20	81.00	37.26	83.50
Yigilca	**1**	**I**	452.7	1.96	23.03	81.50	28.35	81.50	35.77	84.00
Yigilca	**1**	**I**	472.3	1.97	26.29	81.50	34.08	81.50	39.45	83.50
Yigilca	**1**	**I**	331.3	1.91	27.54	81.50	29.06	81.50	34.22	83.50
Yigilca	**1**	**I**	322.6	1.91	25.34	81.50	28.94	81.50	34.04	83.50
Yigilca	**1**	**I**	480.3	1.95	26.14	81.50	29.62	81.50	35.95	83.50
Yigilca	**1**	**I**	390.6	1.91	31.10	81.50	35.97	81.50	40.43	83.50
Yigilca	**1**	**I**	480.1	1.99	21.21	81.50	27.73	81.00	35.54	83.50
Yigilca	**1**	**I**	683.8	2.01	25.62	81.50	31.56	81.50	34.37	83.50
Yigilca	**1**	**I**	391.1	1.90	28.53	81.50	31.56	81.50	36.66	83.50
Yigilca	**1**	**I**	382.4	1.90	25.40	81.50	26.83	81.50	34.78	83.50
Yigilca	**2**	**I**	442.3	1.98	26.32	81.50	29.71	82.00	34.45	83.50

Table D.1 (Cont'd).

Subspecies	Colony	Treatment	ng/ul	260/280	Defensin	Def-Tm	Hymeno	Hymeno-Tm	Eater	Eater Tm
Yigilca	**2**	**I**	411.8	1.97	27.75	81.50	30.20	82.00	39.17	83.50
Yigilca	**2**	**I**	369.3	1.94	27.68	81.50	28.27	82.00	37.75	83.50
Yigilca	**2**	**I**	483.2	1.99	22.46	81.50	27.11	82.00	34.60	83.50
Yigilca	**2**	**I**	403.9	1.98	28.83	81.50	37.66	82.00	40.31	83.50
Yigilca	**2**	**I**	494.8	1.96	30.78	81.50	34.96	82.00	50.00	83.50
Yigilca	**2**	**I**	368.3	1.93	24.13	81.50	33.08	82.00	36.43	83.50
Yigilca	**2**	**I**	551	2.00	27.52	81.50	30.01	82.00	35.34	83.50
Yigilca	**2**	**I**	390.9	1.94	27.07	81.50	26.81	82.00	35.25	83.50
Yigilca	**2**	**I**	511	2.02	25.43	81.50	30.31	82.00	41.26	83.50
Yigilca	**2**	**I**	641.2	2.03	27.43	81.50	34.67	82.00	36.65	83.50
Yigilca	**2**	**I**	398.9	1.95	24.64	81.50	28.69	82.00	36.71	83.50
Yigilca	**1**	**C**	369.1	1.96	21.81	81.50	28.62	81.50	37.55	83.50
Yigilca	**1**	**C**	359.5	1.94	32.26	81.50	35.33	81.50	35.29	83.50
Yigilca	**1**	**C**	371.6	1.94	25.01	81.50	28.21	81.50	31.68	83.50
Yigilca	**1**	**C**	489.7	1.96	25.59	81.50	31.86	81.50	35.10	83.50
Yigilca	**1**	**C**	563.5	2.04	28.51	81.50	35.06	81.50	38.04	83.50
Yigilca	**1**	**C**	372.1	1.95	30.01	81.50	35.08	81.50	37.37	83.50
Yigilca	**1**	**C**	501.7	2.04	30.43	81.50	43.45	81.50	37.99	83.50
Yigilca	**1**	**C**	663.4	2.03	28.75	81.50	37.65	81.50	33.62	83.50
Yigilca	**1**	**C**	660.1	2.05	24.68	81.50	30.83	81.50	33.33	84.00
Yigilca	**1**	**C**	300.1	1.91	26.30	81.50	44.61	81.50	33.13	84.00

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Defensin	Def-Tm	Hymeno	Hymeno-Tm	Eater	Eater Tm
Yigilca	**1**	**C**	308.6	1.91	27.76	81.50	27.85	81.50	32.83	84.00
Yigilca	**2**	**C**	317.5	1.92	33.22	81.50	36.81	81.50	33.33	83.50
Yigilca	**2**	**C**	338.2	1.93	23.53	81.50	27.66	81.50	34.43	84.00
Yigilca	**2**	**C**	396.8	1.98	27.89	81.50	32.63	81.50	33.93	83.50
Yigilca	**2**	**C**	389.1	1.97	27.17	81.50	31.78	81.50	32.57	83.50
Yigilca	**2**	**C**	381.1	1.96	30.17	81.50	35.40	81.50	34.68	83.50
Yigilca	**2**	**C**	319.7	1.91	27.16	81.50	34.46	81.50	32.81	83.50
Yigilca	**2**	**C**	303.1	1.90	28.91	81.50	37.08	81.50	33.40	83.50
Yigilca	**2**	**C**	360.9	1.93	25.40	81.50	29.26	81.50	32.50	83.50
Yigilca	**2**	**C**	447.4	1.98	25.45	81.50	31.21	81.50	33.81	83.50
Yigilca	**2**	**C**	438.1	1.97	29.68	81.50	34.81	81.50	37.03	83.50
Yigilca	**2**	**C**	424.8	1.98	24.06	81.50	31.72	81.50	33.91	83.50
Syrian	**1**	**I**	505.5	2.01	25.95	81.50	31.95	82.00	34.82	83.50
Syrian	**1**	**I**	543.3	1.98	25.03	81.50	30.85	82.00	35.47	83.50
Syrian	**1**	**I**	450.2	1.99	28.73	81.50	34.21	82.00	35.96	83.50
Syrian	**1**	**I**	593.6	2.02	25.97	81.50	31.22	82.00	35.09	83.50
Syrian	**1**	**I**	534.7	2.03	29.18	81.50	35.00	82.00	38.67	83.50
Syrian	**1**	**I**	409.8	1.94	30.92	81.00	35.55	82.00	39.07	83.50
Syrian	**1**	**I**	335.9	1.91	25.24	81.50	29.80	82.00	34.79	83.50
Syrian	**1**	**I**	362.9	1.93	25.31	81.50	34.24	82.00	34.35	83.50
Syrian	**1**	**I**	431.9	1.91	29.83	81.50	33.75	82.00	37.99	83.50

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Defensin	Def-Tm	Hymeno	Hymeno-Tm	Eater	Eater Tm
Syrian	**1**	**I**	394.4	1.93	27.95	81.00	30.36	82.00	36.56	83.50
Syrian	**1**	**I**	505.5	2.00	16.39	81.00	18.70	82.00	33.29	83.50
Syrian	**1**	**I**	405.4	1.91	25.05	81.00	28.55	82.00	39.47	83.50
Syrian	**2**	**I**	443.3	1.96	20.12	81.00	27.95	82.00	36.40	83.50
Syrian	**2**	**I**	350.2	1.93	25.58	81.50	32.04	82.00	35.34	83.50
Syrian	**2**	**I**	493.6	1.95	24.20	81.50	30.81	82.00	34.44	83.50
Syrian	**2**	**I**	454.7	1.95	25.53	81.00	30.62	82.00	35.21	83.50
Syrian	**2**	**I**	449.8	1.94	27.54	81.50	33.51	82.00	34.97	83.50
Syrian	**2**	**I**	275.9	1.91	23.89	81.50	30.07	82.00	34.34	83.50
Syrian	**2**	**I**	262.9	1.93	30.76	81.50	38.07	82.00	37.25	83.50
Syrian	**2**	**I**	531.9	2.03	30.48	81.50	36.63	82.00	38.49	83.50
Syrian	**2**	**I**	349.4	1.95	30.22	81.50	34.34	82.00	36.39	83.50
Syrian	**2**	**I**	585.7	2.02	23.97	81.00	32.72	82.00	36.37	83.50
Syrian	**2**	**I**	323.3	1.92	25.68	81.00	32.18	82.00	32.70	83.50
Syrian	**2**	**I**	585.7	1.99	25.75	81.00	32.79	82.00	33.82	83.50
Syrian	**1**	**C**	289.1	1.90	22.80	81.50	26.13	81.50	31.97	83.50
Syrian	**1**	**C**	480	1.97	26.77	81.00	30.58	81.50	35.60	83.50
Syrian	**1**	**C**	482.3	1.97	28.08	81.00	31.64	81.50	33.17	83.50
Syrian	**1**	**C**	419.8	1.93	30.04	81.50	32.09	81.50	34.33	83.50
Syrian	**1**	**C**	290.2	1.91	26.15	81.50	33.33	81.50	33.72	83.50
Syrian	**1**	**C**	514.3	2.00	25.50	81.50	28.61	81.50	34.91	83.50

Table D.1 (Cont'd)

Subspecies	Colony	Treatment	ng/ul	260/280	Defensin	Def-Tm	Hymeno	Hymeno-Tm	Eater	Eater Tm
Syrian	**1**	**C**	487.2	1.97	28.88	81.50	36.10	81.50	34.03	83.50
Syrian	**1**	**C**	291.5	1.91	24.37	81.00	28.34	81.50	31.71	83.50
Syrian	**1**	**C**	303.1	1.98	25.06	81.50	29.39	81.50	32.16	83.50
Syrian	**1**	**C**	310.9	1.93	24.48	81.50	27.05	81.50	37.96	83.50
Syrian	**1**	**C**	330.8	1.94	27.91	81.00	34.03	81.50	32.30	83.50
Syrian	**1**	**C**	456.3	1.95	32.14	81.00	35.60	81.50	33.79	83.50
Syrian	**1**	**C**	306.2	1.92	30.36	81.50	36.93	81.50	34.29	83.50
Syrian	**1**	**C**	434.2	2.01	25.07	81.50	30.67	81.50	32.60	83.50
Syrian	**1**	**C**	417.4	1.98	29.78	81.50	36.17	81.50	33.19	83.50
Syrian	**1**	**C**	294	1.99	30.56	81.50	37.44	81.50	35.15	83.50
Syrian	**1**	**C**	374.4	1.96	26.21	81.50	32.04	81.50	31.09	83.50
Syrian	**1**	**C**	285.6	1.92	31.17	81.00	36.32	81.50	36.34	83.50
Syrian	**1**	**C**	528.2	2.00	18.86	81.50	21.64	81.50	31.56	83.50
Syrian	**1**	**C**	432.4	2.01	25.37	81.00	28.25	81.50	32.33	83.50
Syrian	**1**	**C**	328.7	1.93	25.05	81.00	28.47	81.50	35.99	83.50
Syrian	**1**	**C**	336.5	1.96	24.73	81.50	29.40	81.50	33.08	83.50
pcr positive						81.50		81.50		83.50
pcr negative					N/A	N/A	N/A	N/A	N/A	N/A

CURRICULUM VITAE

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EDUCATION

Degree	Institution	Year of Graduation
High school	Mugla Anatolian High School	1998
BS	Ankara Uni. Biology Dept.	2003
MS	METU Biology Dept.	2007

ACADEMIC WORK EXPERIENCE

A) Teaching Assistant: In Molecular and Cellular Biology (Gene 104), General and Molecular Genetics Laboratory (Gene 367/Bio 352); General Biology Laboratory (Bio 102),); Plant tissue culture Laboratory (Bio 408). METU Biology Department (2004-present).

B) Research Assistant: In METU Biology Department, Ecology and Evolutionary Genetics Laboratory (2009-present); Plant Genetics and Tissue Culture Laboratory (2004-2008)

C) Visiting Scholar: In USDA-ARS Bee Research Laboratory **(**2013-February - 2014-June)

D) Organizing committee member: In COLOSS (Prevention of honeybee Colony Losses) meeting preceded the Eurbee conference at the Middle East Technical University in Ankara, Turkey in September 2010.

E) Course Attendee: Bal Arısı Islahı ve Yapay Tohumlama Çalıştayı. Orta Doğu Teknik Üniversitesi, Biyoloji Bölümü. 7-8-9 Ağustos 2009.

F) Course Attendee: In the NCBI Discovery Workshops. National Center for Biotechnology Information, U.S. National Library of Medicine, Rockville Pike, Bethesda MD, USA. May 7-8 2013.

B) Project Assistant: Tübitak 2012 yılı ortaöğretim öğrencileri arası araştırma projeleri yarışması. Proje Adı: Nosemosis Hastalığına Neden Olan *Nosema Apis* Sporlarının Arıların Lokomotor Aktivitelerindeki Etkisi.

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