Final Scientific Report

Cover Page

BARD Project Number: IS-4290-10

Date of Submission of the report: 1.12.13

Project Title: Title: Honeybee modulation of infection with the Israeli Acute Paralysis Virus, in asymptomatic, acutely infected and CCD colonies

Investigators

Principal Investigator (PI): Nor Chejanovsky

Co-Principal Investigator (Co-PI): Diana Cox-Foster

Collaborating Investigators:

Soroker, Victoria
Ophir, Ron

Institutions

Entomology
The Volcani Center, POB 6
Bet Dagan, 50250, Israel
Office: 972-3-9683694
Fax: 972-3-9604180
ninar@volcani.agri.gov.il

Department of Entomology
Penn State University
501 ASI Bldg.
University Park, PA 16802
Office phone: 814-865-1022
Dept. Office phone: 814-865-1895
dxcl2@psu.edu

ARO, The Volcani Center
ARO, The Volcani Center

Keywords

RNA interference, small interfering RNA

Abbreviations commonly used in the report, in alphabetical order:

ACI, Acute infections; ASI, asymptomatic infections; BQCV, Black Queen Cell Virus; CCD, Colony collapse disorder; DWV, Deformed wing virus; IAPV, Israeli acute paralysis virus; RNAi, RNA interference; siRNA, small interfering RNA.

Budget: IS: \$ 145000

US: \$ 145000

Total: \$ 290000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Final Scientific Report

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged				
Submitted, in review, in preparation	1		2	3
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts	4		1	5
Not refereed (proceedings, reports, etc.)	4		1	5

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	1		2
Longer Visits (Sabbaticals)				

Description Cooperation:

The cooperation was synergistic: DCF and NC maintained communication throughout the years using e-mail and Skype calls discussing the sequencing and analysis of the CCD colonies. DCF visited NC lab in June 2012 and met with the Israeli partners of the project, Ron Ofir and Victoria Soroker. DCF visited Israeli apiaries where CCD has been detected and followed throughout 2012. Then the PIs put together the scheme of the 2012 report, discussed the experiments to be conducted during the third year of the project and their future projections. Finally, NC and DCF met to discuss the findings and achievements in Pennsylvania US by the end of the project in August 2013. The research findings were presented in several international meetings including two Annual meetings of the Society for Invertebrate Pathology (2012 and 2013, at Buenos Aires, Argentina and Pittsburgh, USA, respectively), a European Conference of Apidology, EURBEE meeting (2013, Halle, Germany), a Federation of European Microbiology Society FEMS meeting (Leipzig, Germany, 2013), and the international network of colony losses COLOSS meeting (Kiev, Ukraine, 2013).

Final Scientific Report

Abstract

Honey bee (*Apis mellifera*) colony losses pose a severe risk to the food chain. The IAPV (Israeli acute paralysis virus) was correlated with CCD, a particular case of colony collapse. Honey bees severely infected with IAPV show shivering wings that progress to paralysis and subsequent death. Bee viruses, including IAPV, are widely present in honey bee colonies but often there are no pathological symptoms. Infestation of the beehive with *Varroa* mites or exposure to stress factors leads to significant increase in viral titers and fatal infections. We hypothesized that the honey bee is regulating/controlling IAPV and viral infections in asymptomatic infections and this control is broken through "stress" leading to acute infections and/or CCD. Our aims were:

1. To discover genetic changes in IAPV that may affect tissue tropism in the host, and/or virus infectivity and pathogenicity. 2. To elucidate mechanisms used by the host to regulate/ manage the IAPV-infection in vivo and in vitro.

To achieve the above objectives we first studied stress-induced virus activation. Our data indicated that some pesticides, including myclobutanil, chlorothalonil and fluvalinate, result in amplified viral titers when bees are exposed at sub lethal levels by a single feeding. Analysis of the level of immune-related bee genes indicated that CCD-colonies exhibit altered and weaker immune responses than healthy colonies. Given the important role of viral RNA interference (RNAi) in combating viral infections we investigated if CCD-colonies were able to elicit this particular antiviral response. Deep-sequencing analysis of samples from CCD-colonies from US and Israel revealed high frequency of small interfering RNAs (siRNA) perfectly matching IAPV, Kashmir bee virus and Deformed wing virus genomes. Israeli colonies showed high titers of IAPV and a conserved RNAi pattern of targeting the viral genome. Our findings were further supported by analysis of samples from colonies experimentally infected with IAPV. Following for the first time the dynamics of IAPV infection in a group of CCD colonies that we rescued from collapse, we found that IAPV conserves its potential to act as one lethal, infectious factor and that its continuous replication in CCD colonies deeply affects their health and survival. Ours is the first report on the dominant role of IAPV in CCD-colonies outside from the US under natural conditions. We concluded that CCD-colonies do exhibit a regular siRNA response that is specific against predominant viruses associated with colony losses and other immune pathways may account for their weak immune response towards virus infection. Our findings: 1. Reveal that preventive measures should be taken by the beekeepers to avoid insecticide-based stress induction of viral infections as well as to manage CCD colonies as a source of highly infectious viruses such as IAPV. 2. Contribute to identify honey bee mechanisms involved in managing viral infections.

Final Scientific Report

1-Achievements

1.1 Significance of main scientific achievements

Our data indicate that some pesticides, including myclobutanil, chlorothalonil and fluvalinate, result in amplified viral titers of honey bee viruses, dicistroviruses (IAPV and BQCV) and iflaviruses (DWV) when bees were exposed at sub lethal levels by a single feeding confirming the working hypothesis. In the US, the elevated titers of multiple viruses occur at least at 24 h after low-level pesticide exposure (well below the acute or sub acute exposure levels). Also, it is clear that the long-term survivorship of the bees is negatively impacted by this one time exposure and concurrent viral increase. Furthermore, it is important to notice that this phenomena occurs with both, summer and winter bees in the US, since the US summer bees are more alike the IS bees in terms of physiology. The US "winter" bees (bees entering into the winter) need to survive from Sept/Oct through to March/April, in order to maintain hive temperature in the cold and to begin foraging in spring for the build-up in brood. We modeled the expected lifetime of the bees exposed to the pesticide with elevated viral titers and found that very few would survive more than 120 days. *This suggests that winter loss might occur under these conditions.*

For the first time we were able to show collapse of honey bee colonies with typical CCD symptoms outside of the US where colonies lose most of the adult bee population and remain with a live queen and no dead honeybees in the hive with plenty of food. Moreover, we achieved the rescue of four CCD colonies to follow the dynamics of IAPV infection in these colonies. The viral titers decreased from April to September and increased from September to December. The health of the colonies was seriously compromised: a rise in IAPV genomic copies was followed by a decline in their brood and adult population, and two of them collapsed. In vitro injection of healthy pupae with IAPV extracted at various times during the year resulted in several fold amplification of the virus genome and production of the virion protein VP3. Thus, *we found that IAPV conserves its potential to act as one lethal, infectious factor and that its continuous replication in CCD colonies deeply affects their health and survival* (see accompanying submitted paper on the subject for details).

Also to elucidate mechanisms used by the host to regulate/ manage the IAPV-infection we studied the level of previously characterized immune-related bee genes, including phenoloxidase (PO), lysozyme (Lys), glucose oxidase (GOX) and FAD glucose

Final Scientific Report

dehydrogenase (GLD), in CCD-colonies indicated and found that CCD-colonies exhibit altered and weaker immune responses. Also, the fact that CCD-colonies show abundance of pathogens reinforced the idea that they have a weakened immune system. Since honey bee viruses are major players in colony collapse and given the important role of viral RNA interference (RNAi) in combating viral infections we investigated if CCD-colonies elicit an RNAi response. Deep-sequencing analysis of samples from CCD-colonies from US and Israel revealed high frequency of small interfering RNAs (siRNA) perfectly matching the IAPV, KBV and DWV genomes. Israeli colonies showed high titers of IAPV and a conserved RNAi pattern of targeting the viral genome. That was further supported by sample analysis from colonies experimentally infected with IAPV. We concluded that CCD-colonies do exhibit a regular robust siRNA response that is specific against predominant viruses associated with colony losses.

In conclusion, CCD-colonies exhibit a robust enhanced siRNA response that is virus specific and targets particular regions of the viral genome, suggesting that the RNAi pathway in these colonies is functional and that other components involved in the innate immune response against viruses are deficient in them. In this respect, possible candidates to look after are phagocytosis and components of the JAK/STAT and *imd* pathways that were shown to be manipulated by dicistroviruses in *Drosophila* (for details see accompanying submitted paper on the subject).

Finally, we found that the most abundant siRNA size observed in the honey bee is 22 nucleotides length, followed by 21 nucleotide length molecules. In this respect it is remarkable that the honey bee genome bears orthologues of *dicer-1*, *d2r2* and *ago-2* and a *dicer*-like gene with low homology to *Drosophila*'s *dicer-2* (1). In *Drosophila* Dicer-1 is involved in the biogenesis of 22 nucleotides' small RNAs and the miRNA pathway (2). Thus, future research of the components of the RNA-dicing machinery in *A. mellifera* is required to clarify the source of the size nucleotide siRNA distribution that we observed in our analysis (for details see accompanying submitted paper).

Our findings in this study enable further identification of honey bee mechanisms involved in managing viral infections and provide additional insight on possible causes of CCD.

Agricultural and/or economic impacts of the research findings

Honey bees (*Apis mellifera*) play a vital role in agriculture by pollinating on a wide variety of crops and flowers. It was estimated that about one third of the agricultural crops in the

Final Scientific Report

world depend on honey bee pollination (3). Since 2006, CCD (Colony Collapse Disorder, was the major cause of sudden colony losses of beekeepers in USA. In Israel, honey bees provide crop pollination of around 300 million USD/year and main beekeepers estimated recent colony losses at about 25%. Colony losses pose a severe risk to the food chain that is reflected in wide media coverage of the subject and by increasing research efforts to solve it.

Our findings reveal that preventive measures should be taken by the beekeepers to avoid insecticide-based stress induction of viral infections as well as to manage CCD colonies as a source of viruses such as IAPV that remain infectious and can be activated to yield high loads of infectious viruses. These measures will assist in ameliorating the rate and extent of colony losses.

References

1. The Honeybee Genome Sequencing Consortium. 2006. Insights into social insects from the genome of the honeybee, *Apis mellifera*. Nature 443:931-949.
2. Karlikow M, Goic B, Saleh M-C. 2014. RNAi and antiviral defense in Drosophila: Setting up a systemic immune response. Dev. Comp. Immun. 42:85-92.
<http://dx.doi.org/10.1016/j.dci.2013.05.004>
3. Gallai N, Salles JM, Settele J, Vaissière BE (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol. Economics 68: 810-821.

2. Details of cooperation

The cooperation was synergistic: DCF and NC maintained communication throughout the years using e-mail and Skype calls to plan and discuss the sequencing and analysis of the small RNA of the CCD colonies. DCF visited NC lab in June 2012 and met with the Israeli partners of the project, Ron Ofir and Victoria Soroker. DCF visited Israeli apiaries where CCD has been detected and followed throughout 2012. Then the PIs put together the scheme of the 2012 report, discussed the experiments to be conducted during the third year of the project and their future projections. Finally, NC and DCF met to discuss the findings and achievements in Pennsylvania US by the end of the project in August 2013. The research findings were presented in several international meetings including two Annual meetings of the Society for Invertebrate Pathology (2012 and 2013, at Buenos Aires, Argentina and Pittsburgh, USA, respectively), a European Conference of Apidology, EURBEE meeting (2013, Halle, Germany), a Federation of European Microbiology Society FEMS meeting (Leipzig, Germany, 2013), and the international network of colony losses COLOSS meeting (Kiev, Ukraine, 2013).

Final Scientific Report

3. List of Publications:

-Nor Chejanovsky, Ron Ophir, Michal Sharabi Schwager , Yossi Slabezki, Smadar Grossman and Diana Cox-Foster (2013). "Characterization of viral siRNA populations in honey bee Colony Collapse Disorder" submitted'

-Hou Chunsheng, Rivkin Hadassah, Slabezki Yossi and Nor Chejanovsky (2013) "Dynamics of the presence of Israeli Acute Paralysis Virus in Honey bee colonies with Colony Collapse Disorder" submitted'