The Influence of Cover Crop Forage on Honey Bee Nutrition and Gut Microbes, and on Colony Growth and Activity

Project No.: **14-POLL14-McFrederick/Carroll/Meikle**

Project Leaders:

Quinn S. McFrederick
Department of Entomology
UC Riverside
900 University Avenue
Riverside, CA 92521
951.827.5817
951.827.3086 (fax)
quinnmc@ucr.edu

Mark Carroll
Carl Hayden Bee Research Center
USDA-ARS, Honey Bee Research
2000 East Allen Road
Tucson AZ 85719
520.670.6380 x124
520.670.6493 (fax)
Mark.Carroll@ars.usda.gov

William G. Meikle
Carl Hayden Bee Research Center
USDA-ARS, Honey Bee Research
2000 East Allen Road
Tucson AZ 85719
520.647-9196
520.670.6493
william.meikle@ars.usda.gov

Project Cooperators and Personnel:

Kirk Anderson, USDA-ARS, Carl Hayden Bee Research Center

Objectives:

In 2012, around 1.5 million honey bee colonies were used to pollinate California's almond orchards (Oliver 2012), resulting in the production of around 2 billion pounds of kernels valued at 4.3 billion dollars (USDA 2013). 60% of all managed colonies in the United States are required for almond pollination, yet overwinter colony losses have remained at about 30% in recent years (USDA 2012). The multi-billion dollar almond crop depends on a slim cushion of honey bee colonies, and increased winter colony losses could lead to a pollination disaster (USDA 2012). Understanding how to reduce colony losses is therefore crucial for continued almond production.

Colonies experience nutritional stress in the times of dearth surrounding almond pollination due to the lack of alternative forage and reliance on supplemental feeds. Bees suffer nutritional deficiencies when limited to supplemental feedings of high fructose corn syrup

(HFCS) in the time of dearth preceding almond bloom (Mao et al. 2013), and from the collection of pollen and nectar from a monoculture system (Oldroyd 2007). Nutritional deficiencies also lead to poor colony strength (fewer bees in the colony) and reduced individual health, which in turn can affect pollination (Crailsheim *et al.*1992; Sagili and Pankiw 2007). Nutritionally stressed colonies also experience higher queen losses and supercedures than well-fed ones, resulting in significant losses in brood production and foraging (Kostarelou-Damianidou et al., 1995). Pollination can be adversely affected either directly through loss of bees or through reduced foraging capacity among the remaining bees. For example, a colony with 8 frames of bees can collect 3 times as much pollen as a 4 frame colony (Sheesley and Poduska 1970).

With the exception of bee feed supplements containing some pollen, the composition of bee feeds do not contain nutritional components that bees commonly encounter while foraging (Sheesley and Poduska 1968). Supplemental diets are not stored in cells and converted to bee bread, thus, while supplements are necessary to keep bees rearing brood and for colony growth when plants are not in bloom, they probably do not provide the necessary nutrition needed for long-term colony health (Mao et al. 2013). Indeed, bees that are fed high fructose corn syrup (HFCS) compared to honey show a lower potential for immune and detoxification functions (Mao et al. 2013) and protein metabolism (Wheeler and Robinson 2014).

Alternate food sources, such as rapini (*Brassica rapa*), that can bloom prior to pollination may increase colony strength and health during almond bloom (Keller et al. 2005), thus increasing pollination rates, which is a direct benefit to growers and beekeepers. Determining whether this supplemental forage can increase honey bee colony is in the interest of the members of the Almond Board of California (ABC), as growers that plant cover crops may be able to negotiate lower pollination fees through the reduced costs of lower supplemental feedings of HFCS and pollen substitutes or possibly from using fewer colonies per acre if colony strength is increased. In addition, the oil seeds of these cover crops may be harvested for additional economic return to the grower.

In addition to affecting honey bee nutrition and health, supplemental feeds may also affect the gut microbial community (=gut microbiome) of honey bees. Sucrose and HFCS lack the natural plant chemicals found in honey (Johnson et al. 2012), and the main bacteria that form the honey bee gut microbiome vary in the different carbon sources that they utilize and their enzymatic abilities (Kwong and Moran 2013), (Engel et al. 2013b), (Olofsson et al. 2014). Differing diets are also known to affect the composition of the human gut microbiome (Turnbaugh et al. 2009), and conversely, human gut microbiomes are known to affect obesity (Turnbaugh et al. 2008). Diet is therefore likely to affect the bee gut microbiome, and, in turn, bee health.

Altering the composition of gut microbes likely has consequences for honey bee health. Honey bees host a simple, yet distinctive gut microbiome that is consistent worldwide (Martinson et al. 2011; Moran et al. 2012). These microbes have characteristics beneficial to honey bee health (Engel et al. 2012; Martinson et al. 2012). For example, worker honey bee gut microbes protect larvae when they are challenged with *Paenibacillus larvae* (Forsgren et al. 2010; Oliver 2012) or *Melissococcus plutonius* (Vásquez et al. 2012; USDA 2013). The bumble bee and honey bee gut microbiome comprise closely related microbes, and in bumble

bees these microbes protect their hosts from the trypanosome pathogen *Crithidia bombi* (Koch and Schmid-Hempel 2011; USDA 2012; Koch and Schmid-Hempel 2012).

As only certain strains of the honey bee gut microbiome secrete enzymes that degrade pectin (Engel et al. 2012), if feeding bees' sugar syrup or HFCS instead of providing winter forage lowers the abundance of microbes that aid in pollen digestion, honey bee health is likely to be affected. To test this hypothesis, we are comparing the composition of honey bee gut communities from colonies fed high fructose corn syrup to colonies foraging on different cover crops. Bee colonies in the study were placed on electronic scales powered by solar cells to measure colony weight changes over time, daily forager activity and internal colony temperature, as well as measuring the adult and brood populations and food reserves. Treatment effects on bee nutrition were examined by measuring the nutrient content of corbicular pollen and bee bread. The nutritional status of the colony was monitored for bees and for queens. By relating changes in microbial communities to measures of honey bee nutrition, and continuous measurements of colony size, health and activity, our goal is to make honey bee management recommendations that will directly benefit beekeepers and almond growers. In addition, we are developing continuous monitoring both as research tools and as potential monitoring tools for beekeepers and growers.

We made regular assessments of colony pollen collection and adult and brood populations to link events detected by continuous monitoring to changes in colony productivity by. As measures of overall colony health, we examined incoming pollen flow from pollen traps and estimated adult and brood populations (Sheesley et al. 1968). Samples of pollen from pollen traps (Ontario Agricultural College traps) were taken weekly to determine pollen species composition in order to determine where bees have been foraging.

We hypothesize that diet affects the nutritional status, productivity, and gut microbiome of honey bee colonies as there are being built up for the almond pollination. Our prediction was that colonies fed artificial diets would exhibit poorer nutrition and colony productivity (measured as brood and food resources in the colony). Additionally, we expected that our treatments would alter the composition of the adult worker gut microbiome. Our overarching goal is to relate nutrition to the microbiome and overall colony productivity. To achieve this goal, we are addressing the following objectives:

1) Develop methods to exploit data from continuous monitoring of bee colonies in the field.
2) Determine the effect of strategically planted oilseed cover crops that bloom prior to, and shortly after the almond pollination, on honey bee nutrition, health, and queen quality.
3) Determine if cover crops affect the honey-bee gut microbiota when compared to bees fed high fructose corn syrup.
4) Understand the interplay between cover crops, honey bee nutrition, health, queen quality, and microbes by synthesizing the results from objectives 1-3.

Interpretive Summary:

We are still in the middle of analyzing the data we collected during the 2015 almond pollination, and it is therefore too early for us to draw conclusions from our study. We predict that colonies fed artificial diets will exhibit poorer nutrition, colony productivity, and an unhealthy gut bacterial community when compared to colonies allowed to forage on rapini in

January and February. If so, we will be able to recommend providing rapini forage before the almond pollination occurs. The benefit to the bees is expected to amount in healthier and more reliable honey bee colonies for almond production.

One limitation to the work we did in 2015 was that we were unable to locate almond orchards suitable for our experimental design. We had to apply the forage treatment in Arizona, and move the colonies into almonds once the bloom began. We were therefore unable to assess how rapini planted near almond orchards affected honey bee colonies. We have already begun to plan for the 2016 pollination, and are currently discussing plans with Gordy Wardell of Wonderful Orchards, in hopes that we can set up the experiment in California almond orchards as originally planned.

Materials and Methods:

Design of field component

Colonies were fed either a forage-supplemented or unsupplemented diets during pre-almond placements at apiaries around Tucson, Arizona. A total of 40 colonies were first brought to the Carl Hayden Bee Research Center (CHBRC) apiary in early December, 2014. To control for genetics, all queens were from the same line. The colonies were evaluated and equalized to comparable size, then fed either BeePro patties (colonies at sites lacking supplemental forage) or a mixture of BeePro patties and pollen patties (colonies at sites with supplemental forage) as protein supplements during the mid-winter dearth. The colonies were fed protein twice more in late December and early January. By mid-January, the colonies were moved in equal numbers to one of four sites: two sites with no supplemental forage and limited wild forage (CHBRC and MAC), although some outside pollen was collected by these colonies)) or two sites that were planted with two acres of rapini each (Red Rock and CG, AZ). The rapini bloomed at the supplemental forage sites from mid-January to early February and provided detectable stores of rapini pollen in forage-supplemented colonies. The colonies were fed supplemental sugar syrup and protein again in late January and early February and evaluated immediately before transportation to California. On February 17, 2015, the colonies were transported to California, and placed in an almond orchard owned by Wonderful Orchards located in Lost Hills, CA (35°38’37.3”N, 119°54’54.3”W). The colonies remained in the field until after the peak almond bloom (March 6, 2015), at which point they were evaluated and moved to a pistachio orchard and wildflower site also owned by Wonderful Orchards (35°40’10.3”N,120°06’21.4”W). Natural forage was restricted at this site due to the high density of colonies such that the colonies relied heavily on food stores and reserves. The colonies remained at this site until a final evaluation was conducted in early April 2015 and the experiment was terminated.

Objective 1: Develop methods to exploit data from continuous monitoring of bee colonies in the field.

We tracked changes in colony performance using continuous monitoring of colony weight and internal temperature to provide round-the-clock detection of colony events, and to provide information about colony growth and activity. As a tool, continuous monitoring has been robustly linked to observations of colony performance and individual bee health as the bees’ progress through different forage environments. Sixteen bee colonies were kept on electronic balances (TEKFA model 2418 bee scales, max. capacity: 100 kg, precision: ±10g) with 2

temperature sensors (Thermochron iButton) placed in the brood box. The balances were linked to 12-bit dataloggers (HOBO dataloggers, from Onset Computer Corp.), and the weighing systems were solar- and battery-powered (National Solar Technologies). The dates and locations of any crop management practices were recorded and correlated with changes observed in the continuous data.

On 15 December WGM colonies were evaluated using methods described by Meikle et al. (2008, 2015a). Each colony frame was weighed separately on a portable balance and digital photographs were taken of each side of each frame and the area of brood, capped honey and pollen per frame was estimated using image analysis software, as described in (Meikle et al. 2015b). Weight of the adult bees was estimated by subtracting the weight of the colony parts from the total colony weight as measured from the bee scale prior to disturbing the colony, and then data from the photo analysis was used to estimate brood mass and honey. Colonies were inspected for diseases and parasites as well. On 19 December colonies were placed in two groups: the "artificial diet" group fed BeePro patties and which were fitted with pollen traps; and the "natural forage" group fed both BeePro patties and pollen patties (these colonies that would eventually be placed at the winter forage sites) and which had pollen traps set to "open" and so did not collect pollen. All colonies were given 2 kg sugar syrup (1:1 ratio). The colonies were fed protein twice more in December and early January. In addition to the initial colony evaluation, they were evaluated 3 more times, in February, March and April (January was avoided because of the adverse effects of cold temperatures on bees).

Continuous temperature and weight data were divided into the 25-hour running average and the hourly detrended data. Running average colony weight data was compared to data from colony inspections to allocate weight changes to the brood population, the adult bee population or food reserves. Changes in adult and brood populations and amount of stored food from the colony visits will be compared between the groups of colonies and correlated with data on worker nutritional status. The focus of inferential statistics is on relative growth and activity. A similar analysis will be conducted with temperature data. Pre-treatment data will be used as covariates to help control for within-group differences among replicate colonies.

<u>Objective 2: Effect of cover crops on honey bee nutrition, health and queen quality.</u>
Changes in colony performance were linked to changes in individual bees by monitoring worker nutrition, foraging, and queen performance over time. The effects of supplemental forage on bee nutritional health were determined by analyzing the nutritional content of stored pollen and the key nutrient reserves of bees. Nutritional deficiencies can be detected as nutrient shortages or imbalances in both the insect and its food. Nutrients in both bees and their food can be analyzed at a microscale (mg or µL amounts) level using subsamples of homogenized tissues (see methods below). Some tissues were partitioned to provide subsamples for both nutritional and microbial analyses, for example queen guts were removed, and the rest of the queens are therefore available for nutritional analyses. Approximately 500 mg each of corbicular pollen and bee bread (stored pollen) was sampled from each colony when available. Corbicular pollen was collected by pollen trap as previously described, while bee bread samples were obtained by coring multiple stored pollen cells with a modified pipette tip. Pollen samples were analyzed for total protein and total lipid contents as well as amino acid, fatty acid, sterol, carbohydrate, water soluble vitamin, and fat soluble vitamin compositions. When supplemental forage was not available, nectar and pollen substitutes

were evaluated for nutrient contents. Five individual capping (5th instar) larvae, emerging adult bees, nurse bees, and incoming foragers were collected from each colony. The former two developmental stages represent key stages in the nutritional development of bees (i.e. at the end of larval feeding and at the beginning of adult feeding), while the latter two perform critical nutritional functions. In the event that brood was not present, frame bees were substituted for nurse bees. Larvae were analyzed for fresh mass, total protein content, and total lipid content. Adult bees were characterized for fresh mass, total protein content of the hypopharyngeal gland, and total lipid content of the fat body.

All nutrients were identified and quantified by comparison against known internal and external standards. To breach the exine coat, pollen samples were ruptured by osmotic shock and vibration before chemical extraction (Human and Nicolson 2003). All tissues were homogenized by BeadBeater or grinding in liquid nitrogen before chemical analyses. Lipids were extracted by Folch extraction (Folch et al. 1957) and total lipids quantified by a chromic acid oxidation assay (Amenta 1970). Proteins and amino acids were digested by heated acid hydrolysis and neutralized before analysis (Otter 2012). Total protein content of neutralized hydrolyzed proteins was determined by a Pierce BCA assay (Thermo-Fisher, Rockford, IL). Hydrolyzed amino acids were derivatized and characterized by EI GC-MS analysis (Kaspar et al. 2008). Fatty acid, sterol, and carbohydrate components were derivatized by silylation and characterized by EI GC-MS analysis (modified after Aliferis et al. 2012). Water soluble vitamins and antioxidants were extracted under acid hydrolysis and analyzed by HPLC (Lebiedzinska et al, 2007). Fat soluble vitamins and antioxidants were extracted, saponified, and separated by HPLC based on the methods of Wang et al. (2007) and Slavin and Yu (2012). Body masses and nutrient contents were compared across treatments and sampling occasions with a mixed-model repeated-measures ANOVA with treatment, time and their interaction as fixed effects and colony within treatment as a random effect.

The effects of supplemental forage on queen productivity and queen retention were periodically assessed through the forage and almond pollination periods. Since queen losses are often initiated by workers, we examined queen emissions of queen mandibular pheromone (QMP), a pheromone complex that signals queen right quality to the workers (Keeling et al. 2003; Slessor et al. 2005). We have recently developed methods to estimate QMP emissions from live queens that are a marked improvement over current methods, which extract QMP contents from dissected queens (Niño et al. 2012). Our non-destructive methods can be used to monitor individual queens through a treatment period. QMP emissions were collected after a brief isolation of the queen and her retinue workers on brood comb and characterized by unpublished analytical chemistry methods. Queen attractiveness to workers was quantified by recording the average size of the queen retinue during two observations (Niño et al. 2012).

Queens were assessed for QMP emissions and retinue formation during the pre-forage bloom, the pre-almond bloom, the end of almond bloom, and the end of the post-bloom forage crop. After the last assessment of live queens, queens were removed and sacrificed to assess fecundity metrics and gut microbes. Ovariole development and spermatheca contents will be quantified in dissected queens (Niño et al. 2012). To allow comparisons with previous studies, we will also perform conventional estimates of mandibular gland QMP contents (Niño et al. 2012).

Retinue sizes were compared across treatments and sampling occasions with a mixed-model repeated-measures ANOVA with treatment, time and their interaction as fixed effects and colony within treatment as a random effect. QMP emissions sampled sequentially from individual queens were normalized against the initial baseline value, arcsine transformed, and compared across treatments and sampling occasions with mixed-model repeated-measures ANOVA. All other queen metrics sampled on a single occasion were compared across treatments by a one-way ANOVA. QMP emissions and retinue size were compared separately against brood production (estimated from frame photographs as described earlier) to determine if pheromone emissions or queen attractiveness are correlated with queen/worker reproductive productivity (brood production).

Objective 3: Effects of cover crops on gut microbiota.

To test the hypothesis that artificial diet versus forage will influence the composition of the honey bee gut microbiome, we are characterizing the gut microbiome from our experimental colonies. We collected five worker bees (when available) from each colony at four different time points. We surface sterilized each sample by rinsing each sample in a 10% bleach solution, followed by three rinses in sterilized, deionized water. We then dissected the gut from each sample, using sterilized dissection trays and tools.

To quantify bacterial communities found in the workers and the queens, we will use a combination of next-generation sequencing and quantitative PCR. We have extensive experience with these methods (USDA 2012; McFrederick et al. 2012; 2013; 2014), and recently co-authored a paper creating standard methods for analyses of the honey bee gut microbiota as part of the COLOSS BEEBOOK (Kwong and Moran 2013; Engel et al. 2013a). To identify gut bacteria and determine their relative abundances, we will sequence a gene found in all bacteria, using modern sequencing technology that allows us to generate thousands of DNA sequences from a single sample (Illumina DNA sequencing). We will then compare the sequences that we obtain to sequences from known bacteria and thereby determine what species of bacteria are present in our samples. To determine the absolute number of bacteria in each gut sample, we will additionally use quantitative PCR, which is a technique that allows us to determine the starting number of copies of a gene in a sample. Combined with the DNA sequence data, this will allow us to determine what microbes are present, their abundance relative to each other, and the overall size of the gut microbiota.

We will analyze the next generation sequencing data using standardized methods (Engel et al. 2013). We will use the QIIME pipeline (Martinson et al. 2011; Kuczynski et al. 2011; Moran et al. 2012) and UniFrac analyses (Hamady and Lozupone 2009; Engel et al. 2012; Martinson et al. 2012) to determine if the gut microbiota differ by treatment. To determine if the absolute abundance of bacteria differ by treatment, we will use standard Analysis of Variance.

Objective 4: Understand the interplay between cover crops, honey bee nutrition, health, queen quality, and gut microbes.

Through the proposed collaboration of researchers from different fields, we will be able to build a complete picture of how cover crops in almond orchards affect honey bee nutrition, health, queen quality, and gut microbes. In objectives 1-3 we will use cutting-edge technology to measure the response of these individual variables to cover crop treatment, and in objective 4 we will synthesize the results from objectives 1-3. Using individual correlation analyses and

correcting for multiple tests, we will determine if variation in honey bee nutrition, health, queen quality and colony-level growth and activity significantly correlates with variation in gut communities. To use the gut microbial community data in these analyses, we will use principal coordinates analysis to summarize microbial community composition into a single continuous variable (Ramette 2007). All bees that we sample for gut microbes will also be used for nutrient analyses, and we will therefore be able to determine if nutrient levels correlate with gut microbial community composition. We will also quantify and correlate gut microbial communities and queen mandibular pheromone production from the queen samples. Finally, we will use running average and detrended colony weight data from the period coinciding with our sampling of bees for gut microbial analysis to determine if colony growth and forager activity correlates with gut microbial community composition. As this synthesis is a novel approach, we will explore further analysis options as well. While the ability to determine if cover crop affects honey bee nutrition, health, queen quality, and gut microbes by themselves is a powerful approach, this synthesis will allow us to further determine if gut microbes underlie significant effects of cover crop treatments. If so, gut microbes may be the mechanism by which cover crops affect bee health. We will therefore be able to make recommendations of cover crop plantings that will maximize the beneficial properties of the honey bee gut microbiota. The proposed research may help secure low cost pollination services for future almond crops.

Results and Discussion:

We are still processing and analyzing our data, but have made substantial progress on Objectives 1-3. Objective 4 is dependent on the completion of objectives 1-3, and will be conducted once those objectives are complete.

Objective 1: Develop methods to exploit data from continuous monitoring of bee colonies in the field

The continuous weight and temperature data has been downloaded and is currently being analyzed with respect to average values and within-day variability (detrended analysis), and with respect to the colony evaluation data.

Objective 2: Effect of cover crops on honey bee nutrition, health and queen quality.

We are currently processing workers and food materials to quantify nutrient reserves in bees and the nutrient quality of food materials. We will characterize queen pheromone emissions from queens once the dissection of queens is completed for Objective 3. We will complete approximately 4,200 chemical analyses of the workers, queens, queen retinues, and food materials as well as frame analysis of colony productivity by October 31, 2015.

Objective 3: Effects of cover crops on gut microbiota.

We have currently dissected 768 worker honey bees. All dissections will be completed by August 1, 2015. DNA extraction, library preparation and sequencing of nearly 200 samples will be completed by October 1, 2015. Data analysis and a final report will be completed by December 1, 2015.

Objective 4: Understand the interplay between cover crops, honey bee nutrition, health, queen quality, and gut microbes.

Objective four will be completed when the separate components (Objectives 1-3) are finished and available for synthesis.

Research Effort Recent Publications:

None yet to report.

References Cited:

Engel, P., R. R. James, R. Koga, W. K. Kwong, Q. S. McFrederick, and N. A. Moran. 2013a. Standard methods for research on Apis mellifera gut symbionts. Journal of Apicultural Research 52:1–24.

Engel, P., V. G. Martinson, and N. A. Moran. 2012. Functional diversity within the simple gut microbiota of the honey bee. Proceedings of the National Academy of Sciences 109:11002–11007.

Engel, P., W. K. Kwong, and N. A. Moran. 2013b. Frischella perrara gen. nov., sp. nov., a gammaproteobacterium isolated from the gut of the honey bee, Apis mellifera. International Journal of Systematic and Evolutionary Microbiology 63:3646–3651.

Forsgren, E., T. C. Olofsson, A. Vásquez, and I. Fries. 2010. Novel lactic acid bacteria inhibiting Paenibacillus larvae in honey bee larvae. Apidologie 41:99–108.

Hamady, M., and C. Lozupone. 2009. Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. The ISME Journal 4:17–27.

Human H., S.W. Nicolson, and V. Dietemann. 2006. Do honeybees, *Apis mellifera scutellata,* regulate humidity in their nest? Naturwissenschaften 93: 397–401.

Johnson, R. M., W. Mao, H. S. Pollock, G. Niu, M. A. Schuler, and M. R. Berenbaum. 2012. Ecologically Appropriate Xenobiotics Induce Cytochrome P450s in Apis mellifera. PLoS One 7:e31051–e31051. Public Library of Science.

Koch, H., and P. Schmid-Hempel. 2012. Gut microbiota instead of host genotype drive the specificity in the interaction of a natural host-parasite system. Ecology Letters 15:1095–1103.

Koch, H., and P. Schmid-Hempel. 2011. Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. Proceedings of the National Academy of Sciences 108:19288–19292.

Kuczynski, J. J., J. J. Stombaugh, W. A. W. Walters, A. A. González, J. G. J. Caporaso, and R. R. Knight. 2011. Using QIIME to analyze 16S rRNA gene sequences from microbial communities. Curr Protoc Bioinformatics Chapter 10:Unit10.7.

Kwong, W. K., and N. A. Moran. 2013. Cultivation and characterization of the gut symbionts of honey bees and bumble bees: Snodgrassella alvi gen. nov., sp. nov., a member of the Neisseriaceae family of the Betaproteobacteria; and Gilliamella apicola gen. nov., sp. nov., a member of Orbaceae fam. nov., Orbales ord. nov., a sister taxon to the Enterobacteriales order of the Gammaproteobacteria. International Journal of Systematic and Evolutionary Microbiology 63:2008–2018.

Mao, W., M. A. Schuler, and M. R. Berenbaum. 2013. Honey constituents up-regulate detoxification and immunity genes in the western honey bee Apis mellifera. PNAS 110:8842–8846.

Martinson, V. G., B. N. Danforth, R. L. Minckley, O. Rueppell, S. Tingek, and N. A. Moran. 2011. A simple and distinctive microbiota associated with honey bees and bumble bees. Mol Ecol 20:619–628.
Martinson, V. G., J. Moy, and N. A. Moran. 2012. Establishment of characteristic gut bacteria during development of the honeybee worker. Appl Environ Microbiol 78:2830–2840.
McFrederick, Q. S., J. J. Cannone, R. R. Gutell, K. Kellner, R. M. Plowes, and U. G. Mueller. 2013. Host specificity between Hymenoptera and lactobacilli is the exception rather than the rule. Appl Environ Microbiol 79:1803–1812.
McFrederick, Q. S., W. T. Wcislo, D. R. Taylor, H. D. Ishak, S. E. Dowd, and U. G. Mueller. 2012. Environment or kin: whence do bees obtain acidophilic bacteria? Mol Ecol 21:1754–1768.
McFrederick, Q., U. Mueller, and R. R. James. 2014. Interactions between fungi and bacteria influence microbial community structure in the Megachile rotundata larval gut. Proceedings of the Royal Society B-Biological Sciences In Press. 10.1007/s13592-015-0370-1.
Meikle, W.G. and N. Holst. 2015a. Application of continuous monitoring of honey bee colonies. Apidologie 46:10–22 DOI: 10.1007/s13592-014-0298-x.
Meikle, W.G., M. Weiss and A.R. Stilwell. 2015b. Monitoring colony phenology using within-day variability in continuous weight and temperature of honey bee hives. *Apidologie* DOI:
Meikle, W.G., B.G. Rector, G. Mercadier and N. Holst. 2008. Within-day variation in continuous colony weight data as a measure of honey bee colony activity. Apidologie 39: 694-707. DOI: 10.1051/apido:2008055
Meikle, W.G., M. Weiss and A.R. Stilwell. 2015. Monitoring colony phenology using within-day variability in continuous weight and temperature of honey bee colonies. Apidologie DOI: 10.1007/s13592-015-0370-1.
Moran, N. A., A. K. Hansen, J. E. Powell, and Z. L. Sabree. 2012. Distinctive gut microbiota of honey bees assessed using deep sampling from individual worker bees. PLoS One 7:e36393.
Oliver, R. 2012. 2012 Almond Pollination Update. American Bee Journal 152:315–322.
Olofsson, T. C., M. Alsterfjord, B. Nilson, É. Butler, and A. Vásquez. 2014. Lactobacillus apinorum sp. nov., Lactobacillus mellifer sp. nov., Lactobacillus mellis sp. nov., Lactobacillus melliventris sp. nov., Lactobacillus kimbladii sp. nov., Lactobacillus helsingborgensis sp. nov. and Lactobacillus kullabergensis sp. nov., isolated from the honey stomach of the honeybee Apis mellifera. International Journal of Systematic and Evolutionary Microbiology 64:3109–3119.
Turnbaugh, P. J., V. K. Ridaura, J. J. Faith, F. E. Rey, R. Knight, and J. I. Gordon. 2009. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med 1:6ra14. NIH Public Access.
Turnbaugh, P., M. Hamady, and T. Yatsunenko. 2008. A core gut microbiome in obese and lean twins. Nature.
USDA. 2013. Noncitrus Fruits and Nuts. USDA.
USDA. 2012. Report on the National Stakeholders Conference on Honey Bee Health. USDA.
Vásquez, A., E. Forsgren, I. Fries, R. J. Paxton, E. Flaberg, L. Szekely, and T. C. Olofsson. 2012. Symbionts as major modulators of insect health: lactic acid bacteria and honeybees. PLoS One 7:e33188.
Wheeler, M. M., and G. E. Robinson. 2014. Diet-dependent gene expression in honey bees: honey vs. sucrose or high fructose corn syrup. Sci. Rep. 4.