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CHARACTERIZING THE MATERNALLY INHERITED ENDOSYMBIONTS OF SOLITARY BEES

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
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ABSTRACT OF THESIS

CHARACTERIZING THE MATERNALLY INHERITED ENDOSYMBIONTS OF SOLITARY BEES

Solitary bees are important pollinators of crops, with species in the family Megachilidae (mason bees) being used for orchard pollination. Commercial movement of these bees also moves their microbiota, including bacterial endosymbionts capable of reproductive manipulation. To test for presence of these bacteria, I screened commercially available species of US orchard pollinators and locally captured solitary bees from Kentucky. I also set up mason bee boxes in five apple orchards to examine recruitment of local pollinators. I conducted 454-pyrosequencing to determine bacterial diversity within four species followed by diagnostic PCR of 30 collected species (184 individuals) to determine infection frequency of selected endosymbionts. Consistent with literature, *Wolbachia* was abundant in these bees. I also found two other endosymbiotic bacteria, *Sodalis* (previously undetected in Hymenoptera), and *Arsenophonus.* Diagnostic screening demonstrated that *Sodalis* was present at moderate frequency in *Osmia aglaia*, whereas *Arsenophonus* was present at low frequency in *Lasioglossum pilosum*. Neither was found in other bees, but three bee species were infected with *Sodalis*-like endosymbionts. Although recruitment of bees to bee boxes was ineffective, I was able to independently collect native orchard pollinating Andrenidae species. My results demonstrate that other endosymbionts capable of reproductive manipulation, besides *Wolbachia*, are present in bees.

KEYWORDS: *Arsenophonus*, *Lasioglossum pilosum*, *Osmia aglaia*, *Sodalis*, *Wolbachia*

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DEDICATION

For my father and my hero, Asim Nadeem Saeed.

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Frequency and Diversity of Solitary Bee Endosymbionts

Introduction

Solitary bees play an important role as pollinators of crops and native flora. Due to the ongoing decline of managed *Apis mellifera* populations, solitary bees also act as a buffer to protect worldwide crop pollination operations (Winfree et al., 2007). There are approximately 20,000 bee species in the world (Michener, 2007), many of which provide a valuable economic service to a variety of essential crops (Kremen et al., 2002; Klein et al., 2003a,b,c; Kremen et al. 2004; Ricketts 2004; Morandin and Winston, 2005; Greenleaf and Kremen, 2006a). In the United States of America, the value of these pollinator services has been placed at over $1.25 billion per year (Buchmann and Nabhan, 1997).

Despite the economic and ecological importance of solitary bees, relatively little is known about the microbial associations of this agriculturally important group of insects. The majority of research has been focused on pathogens and gut microbiota, comparing the gut biota of honey bees and their more solitary cousins (Martinson et al. 2011). Spread of pathogens has been an ongoing concern in honey bees, since pathogens have been linked with colony collapse disorder (Martin, 2001; Cox-Foster et al., 2007; vanEngelsdorp et al., 2009; Higes et al., 2009). Although some pathogens, such as Deformed Wing Virus, appear to be primarily found in honey bees and bumblebees, other microorganisms such as *Ascosphaera* fungi and Microsporidia appear to also have a significant presence in solitary bees (Evison et al., 2012).

Solitary bees are also particularly prone to infection by the maternally inherited endosymbiont *Wolbachia* (Gerth et al., 2011; Evison et al., 2012). Surveys have estimated maternally inherited endosymbionts to be present in approximately 30% of arthropods (Duron et al., 2008a). With respect to bees in particular, *Wolbachia* has been shown to infect approximately 66% of surveyed species (Gerth et al. 2011). Most described strains of *Wolbachia* are reproductive manipulators, able to influence host reproduction through mechanisms such as cytoplasmic incompatibility (Stouthamer at al., 1999), feminization (Hiroki et al., 2002), parthenogenesis (Stouthamer, 1997), and male killing (Hurst et al., 1997). Other strains of *Wolbachia* have been found to provide benefits to their hosts, including increased resistance to pathogens (Hedges et al., 2008; Teixeira et al., 2008; Walker et al., 2011), and nutritional mutualisms (Hosokawa et al., 2010). The role(s) of *Wolbachia* have not yet been explored in solitary bees.

Wolbachia is not the only maternally inherited bacterium in arthropods. Other similar endosymbionts include *Arsenophonus* (Gherna et al., 1991), *Cardinium* (Zchori-Fein and Perlman 2004), *Rickettsia* (Hagimori et al., 2006), and *Spiroplasma* (Hurst et al., 1999), many of which are capable of similar reproductive manipulations as *Wolbachia*. These endosymbionts are less prevalent than *Wolbachia* overall, but can be common in certain groups of arthropods (Duron et al 2008a). For example: *Cardinium*, although uncommon in most insects, is present in 22% of surveyed spiders (Duron et al., 2008b). With the dominant presence of the maternally-inherited *Wolbachia* in bees, one might wonder if they are prone to infection by other maternally-inherited endosymbionts as well. While over a hundred bee species have been screened for *Wolbachia* (Jeong et al., 2009; Gerth et al., 2011; Evison et al., 2012), the frequency of other maternally

inherited endosymbionts remains largely unexplored; less than two dozen bee species have ever been screened for any endosymbionts other than *Wolbachia* (Weeks et al., 2003; Jeong et al., 2009; Weinert et al., 2009; Martinson et al., 2011).

Knowledge of the bacterial associations of solitary bees could be vital when considering their commercial use. Bees in the family Megachilidae are utilized in crop and orchard settings (Bosch and Kemp, 2002; Gruber et al., 2011). These bees are sold on many pollinator supply websites, and are available for purchase throughout the United States. This results in movement of solitary bees from state to state to supply commercial pollination demands, and also means movement of their associated microbiota. Translocated bee populations with different endosymbiont infections may differ in fitness and reproductive compatibility with one another (Ryan and Saul 1968; Breeuwer and Werren 1990; Breeuwer 1997; Vavre et al. 2000), with potentially negative impacts on population dynamics and pollinator efficacy.

In this chapter, I explore the maternally inherited endosymbiont infections in solitary and semi-social bees. I surveyed several species of commercially available as well as wild-caught solitary and semi-social bees for various endosymbionts through (i) 454-pyrosequencing and (ii) diagnostic screening. In addition, I compared *Wolbachia* strains among infected bees, to understand strain diversity within the solitary bee community.

Specific Objectives

1) Use 454-pyrosequencing to assess diversity of bacterial endosymbionts within four species of solitary bees.
2) Use diagnostic screening (PCR) to determine frequency of endosymbiont infection across a wider array (30 species) of solitary bees.
3) Compare *Wolbachia* strains among solitary bee species.

Materials and Methods

Specimen collection

Specimens were collected in and around Lexington, Kentucky between 2011 and 2013 (Table 1.1). Bees were collected free-hand (by capturing them directly into vials), with nets, and with bee bowls (filled with a soapy water solution containing 30 mL of Blue Dawn Dishwashing soap (Procter & Gamble, Cincinnati Ohio) mixed with 1 L of water). Collected individuals were placed in 95% ethanol and stored at -20°C until identification and DNA extraction. Bees were identified morphologically using the Discoverlife IDnature guides for Apoidea (http://www.discoverlife.org/20/q?search=Apoidea) and/or molecularly using CO1 and EF1-alpha sequences (see below).

Additionally, eight species of commercially available solitary bees were obtained from a variety of suppliers (Table 1.2). To determine whether endosymbiont frequency or diversity differed geographically, multiple bee populations were examined per species when possible. Two incidental species, *Osmia taurus* and *Osmia caerulescens* were included in the dataset, as they were mixed in within requested samples. Bees were shipped as cocooned adults in diapause, except for *Megachile rotundata*, which was in a larval state within the cocoons. Upon arrival, bees were removed from the cocoons and stored using the same protocol as the locally captured bees. Bees that were visibly diseased were excluded from further processing.

Individual bees were surface sterilized using a 5% bleach solution (for 60 seconds); followed by three 95% ethanol rinses (60 seconds each), and finally a deionized

(DI) water rinse (60 seconds). For smaller bees (<10mm) the entire abdomen was removed using a sterile blade, and macerated. For larger bees, the ventral side of the abdomen was longitudinally sliced, and the contents were excavated using sterile forceps. Larval bees (*M. rotundata*) were macerated in entirety. DNA was extracted using DNeasy kits (Qiagen) following manufacturer's instructions with a 3 hour incubation time. Extraction quality was evaluated by screening for positive DNA presence through CO1 or EF1-alpha. Unsuccessful extractions (3/184 = 1.6% extracted individuals) were discarded from the dataset.

Specimen screening

To characterize the bacterial community composition of solitary bees, the bacterial metagenome of 4 species was evaluated using 454-pyrosequencing. Two commercially supplied species (*Osmia aglaia* and *Osmia lignaria*), and two abundant locally captured species (*Halictus ligatus* and *Lasioglossum pilosum*) were examined. Extracted DNA of 8-10 individuals was pooled into a single sample of DNA for each species, at a concentration of 20ng/µL DNA. Samples were submitted to Research and Testing Laboratory (Lubbock, TX), for bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) using 28F (5′-GAGTTTGATCNTGGCTCAG-3′) and 519R (5′-GWNTTACNGCGGCKGCTG-3′) primers for a segment of bacterial 16S rRNA (Dowd et al., 2008; Medina et al., 2011; Ishak et al., 2011; Brady and White, 2013). Low quality sequences (length of <250bp) were discarded. Remaining sequences were classified using an NCBI-derived database as part of Research and Testing Laboratory's standard data analysis pipeline (Dowd et al. 2008) and allocated to appropriate taxonomic

levels based on percent similarity to the closest match in the database using the criteria described in Brady and White (2013).

Each collected bee was individually screened using previously published diagnostic primers (Table 1.3) to detect the endosymbionts *Wolbachia*, *Sodalis* and *Arsenophonus*, based on identification of these bacteria from the pyrosequencing data. PCR reactions were carried out in a total volume of 10µL, containing: 2.0 µl of DNA template, 1.0 µl of Invitrogen 10X buffer ($MgCl_2$ free), 10 mM dNTP mixture, 1.0 µl of 25mM $MgCl_2$, 1.0 µl of 5.0 pmole μl^{-1} of each primer, 0.1 µl of 5 U/ µl Invitrogen Taq polymerase and ddH_2O up to 10 µl. Positive controls contained DNA from specimens with confirmed infection of the targeted endosymbiont. Negative controls contained 2µL of ddH_2O instead of DNA template. A representative individual of each species that tested positive was validated by Sanger sequencing at the Advanced Genetic Technologies Center (University of Kentucky). Sequences were edited in Geneious Pro (v. 5.6.4, Biomatters Ltd.), and compared to the NCBI nr database using the Blastn algorithm. Endosymbiont infection was confirmed if the sequence matched endosymbiont taxa within the database at >97%. All negative samples were screened twice for each endosymbiont to confirm lack of infection.

Wolbachia differentiation

Species testing positive for *Wolbachia* were further investigated to determine strain variation. Initial screening efforts focused on *Wolbachia* surface protein (*wsp*), a highly variable gene with great sensitivity for detecting strain differentiation. *Wolbachia* from thirteen solitary bee species were sequenced using the *wsp* gene, and categorized as

different strains if the sequences differed. Four of these *Wolbachia* strains (from hosts spanning 4 different genera, and three families) were chosen for subsequent multi-locus strain typing (MLST), to assess phylogenetic relationships among the strains. Four housekeeping genes, *gatB, hcpA, ftsZ* and *fbpA* (as described in Baldo et al., 2006; Gerth et al., 2013) were amplified, sequenced, edited and aligned in Geneious Pro v. 5.6.4, (Biomatters Ltd.) using templates from the *Wolbachia* PubMLST website (http://pubmlst.org/wolbachia/; Jolley et al., 2004; Gerth et al., 2013). Gene sequences were then compared among strains for percent similarity.

Results and Discussion

Through pyrosequencing, I found two bacteria (in addition to *Wolbachia*) known to be maternally inherited endosymbiotic associates of insects. This included a novel endosymbiont within Hymenoptera, *Sodalis*, as well as the endosymbiont *Arsenophonus*. In addition, I diagnostically confirmed a dominant presence of *Wolbachia* in solitary bees, especially within the family Halictidae.

In the pyrosequenced sample of the commercial bee *O. aglaia*, 95.6% of bacterial reads came from *Sodalis* (Table 1.4), a genus of bacteria that is known to be maternally-transmitted in insect hosts (Cheng and Aksoy, 1999). Through subsequent diagnostic screening, we found 7 out of the 11 *O. aglaia* individuals from this population were infected (Table 1.6). Two additional *O. aglaia* populations were also screened for *Sodalis*. The population from the same location in the following year (population 2) had 3/18 infected individuals, whereas a population from a different location (population 3) had 0/10 infected individuals (Table 1.1 and 1.6). The various *Sodalis* primers (Table 1.3) returned appropriately-sized fragments in 7 more individuals (Table 1.5). Through subsequent Sanger sequencing, *Ceratina calcarata* was determined to have a '*Sodalis*-like' endosymbiont based on its groEL bacterial chaperonin gene (Table 1.3), which resulted in a closest match (92.2%) with the *Sodalis*-like primary endosymbiont of *Sitophilus oryzae* (accession number: CP006568). Based on a smaller fragment from the rp1B1 *Sodalis* specific ribosomal protein gene, the bacteria from *Augochlora pura* and *Augochlorella aurata* also best matched the *Sodalis* HS1 complete genome (P006569) at 90% similarity. The other four individuals (*Halictus ligatus*, *Halictus paralellus*,

Lasioglossum pilosum and *Lasioglossum pruinosum*) best matched *Gluconobacter cerinus* (80.2-80.8%; FN391717), and thus were discarded as potential *Sodalis* infections. However, the low percentage similarity to any GenBank accessions leaves the identity of these bacteria unclear.

Sodalis is a gram-negative bacteria associated with various groups of insects. It is best known for its endosymbiotic association with the tsetse fly (*Glossina* spp. Aksoy et al., 1997; Dale and Maudlin, 1999), and other blood-sucking flies (Novakova and Hypsa, 2007). Within the tsetse fly, *Sodalis glossinidius* behaves as a mutualist of *Trypanosoma brucei rhodesiense* (Dale and Maudlin, 1999; Dale and Welburn, 2001), the causative agent of trypanosomiasis (African Sleeping Sickness), which is vectored by the tsetse fly (Maudlin, 2006). Other strains of *Sodalis* have been found to have an obligate (potentially nutritional) relationship within some species of weevils (Heddi et al., 1999), and unknown roles in chewing lice (Fukatsu et al., 2007). This is the first record of this genus of endosymbiotic bacteria within the order Hymenoptera. The relatively high infection frequency of this endosymbiont within *O. aglaia* as well as detection of a *Sodalis*-like strain in 3 other bee species calls for further research into the potential role of this bacteria in infected populations of bees, and may provide insight into the health of these important pollinators.

Pyrosequencing of *L. pilosum* demonstrated the presence of the maternally-inherited endosymbiont *Arsenophonus*, which dominated the sequence reads from this sample (87.7% of reads; Table 1.4). Diagnostic screening, however, showed this endosymbiont to only be present in 1 out of the 11 screened individuals of *L. pilosum* (Table 1.6). *Arsenophonus* was not convincingly found in any of the other screened

solitary bee species (Table 1.6); however, the low frequency of infected individuals in *L. pilosum* suggests it is possible for infection to be missed in host species without large sample sizes. *Arsenophonus* is a bacterial genus that contains arthropod-associated bacteria with a variety of functions. *Arsenophonus nasoniae*, a species present in the parasitoid wasp *Nasonia vitripennis*, is a reproductive manipulator that demonstrates 'son killing' (male killing) (Taylor et al., 2011; Darby et al., 2010; Ferree et al., 2008; Skinner, 1985). Other strains of *Arsenophonus* have been documented to act as plant pathogens transmitted by hemipterans (Bressan et al., 2008; Semetey et al., 2007; Danet et al., 2003 Zreik et al., 1998), and as potentially obligate nutritional endosymbionts in some blood-feeding hemipterans and dipterans (Novakova et al., 2009). The low infection frequency within *L. pilosum* is similar to the reported frequency of infection in *N. vitripennis* (Skinner, 1983; Balas et al., 1996).

Wolbachia was detected within the pooled pyrosequenced specimens from both *H. ligatus* and *L. pilosum*, at relatively low prevalence of reads (2.4 and 1.6% of reads per sample, respectively) (Table 1.4). Diagnostic screening, however, showed this endosymbiont to be present in each individual of the pyrosequenced samples of these two species (Table 1.6). *Wolbachia* was additionally found in 17 other species, including all screened species within the family Halictidae (Table 1.6). Each infected species showed a 100% infection frequency when multiple individuals were available for screening (Table 1.6).

Wolbachia is well-known for the various reproductively manipulative roles it plays within many infected taxa (Werren et al., 2008). Some *Wolbachia* strains can also play beneficial roles such as increased resistance to pathogens (Hedges et al., 2008;

Teixeira et al., 2008; Walker et al., 2011), and nutritional mutualisms (Hosokawa et al., 2010) in some host species. In more general surveys of arthropods, *Wolbachia* has been shown to be present at varying frequencies among taxa (Hughes et al., 2011; Arthofer et al., 2009a; 2009b). Bees, particularly within the Halictidae, appear to be a group with a high frequency of *Wolbachia* infection across species (Evison et al., 2012; Gerth et al., 2011), however the endosymbiont's role in this group remains unknown.

In addition to examining variation in *Wolbachia* presence/absence, I also examined potential strain type variation among infected species. The *wsp* gene was sequenced for 13 species and 7 different *wsp* sequences were found (Table 1.7). Base and percentage differences of sequences between these groups can be found in Table 1.8. I found that 6 bee species (across 2 families) were all infected by a common *Wolbachia* strain (strain 1), whereas all the other *Wolbachia* strains were restricted to 1 or 2 bee species. Strains 2 and 3 were very similar to strain 1, with minimal (1-2bp or <0.5%) differences (Table 1.8). Strain 5 showed a greater (26-27bp or 6-7%) difference from strain 1, and strains 4, 6, and 7 were very different (57-107bp or 13-26%) from all other groups (Table 1.8). MLST typing confirmed the similarity among strains 1through 3; as well as their difference from strain 7 (Table 1.9). Two species were determined to have multiple *Wolbachia* infections through visual inspection of sequence chromatograms, and were therefore not included in the *wsp* strain groupings. These species will be further examined to determine the strains they harbor.

The commonality of *Wolbachia* strains among host species spanning multiple families, as well as the presence of different *Wolbachia* strains in closely related host genera is indicative of horizontal transmission among species. Horizontal transmission

has previously been inferred as a mode of interspecific *Wolbachia* transfer, primarily based on phylogenetic inconsistencies between bacterial strains and arthropod hosts (O'Neill et al., 1992; Schilthuizen and Stouthamer, 1997; Vavre et al., 1999; Baldo et al., 2008; Raychoudhury et al., 2009). MLST comparisons in bees have been conducted to determine the mechanism of horizontal *Wolbachia* transmission (Gerth et al., 2013). The mechanism remains unclear; however the authors implied the possibility of *Wolbachia* transfer from bee hosts to their kleptoparasites through movement from salivary glands to pollen provisions for offspring, and subsequent movement from the gut into the ovaries for infection establishment (Gerth et al., 2013). *Wolbachia* can also show geographic structuring, which has been observed on a large scale between old world and new world Lycaenid butterflies (Russell et al., 2009). Preliminary comparisons between *Wolbachia* strains in the present study versus the European species of Gerth et al. (2013) support the hypothesis of geographic structuring of *Wolbachia* infection among Halictidae. Further MLST sequencing of strains 4 through 6 will allow direct comparison of *Wolbachia* from these two bee communities.

In addition to the set of focal facultative endosymbionts, I was able to look into the entire bacterial community within four chosen bee species (*O. aglaia*, *O. lignaria*, *L. pilosum*, and *H. ligatus*). The *H. ligatus* sample was largely composed of *Lactobacillus* (~93.6% of reads; Table 1.4), which is frequently associated with bees (Mohr and Tebbe 2006; Martinson et al. 2011; McFrederick et al. 2012). The other three species also contained *Lactobacillus* (Table 1.4), although at lower prevalence within the pyrosequenced sample (Table 1.4). *Lactobacillus* is a well-known component of invertebrate as well as vertebrate gut fauna and has beneficial associations in microbial

defense within the gut (Cross 2002; Walter et al., 2011). It can also be coevolved with the host (Koch and Schmid-Hempel, 2011; Martinson et al., 2011; Vasquez et al., 2012). Within hymenoptera, honey bees and bumble bees have been shown to have host-specific *Lactobacillus* strains, whereas sweat bees (such as those within the family Halictidae) have diverse *Lactobacilli* that are not taxon specific (McFrederick et al., 2013). Our results further supported this lack of host specificity, as *H. ligatus* appeared to have two strains of *Lactobacillus*, whereas *L. pilosum* had only one, which was identical to one in *H. ligatus*. Lack of host specificity does not necessarily indicate lack of importance, as environmentally acquired bacteria have been known to play important roles in microbial defense and pesticide detoxification (Kikuchi et al., 2012) within their hosts.

In contrast with the other three pyrosequenced species, apparent endosymbionts in the *O. lignaria* sample could not be confirmed by diagnostic PCR. *Arsenophonus*, *Sodalis*, and *Wolbachia* were all present in the pyrosequenced sample, but at low to very low prevalence. All three of these symbionts were highly represented in other barcoded samples that shared the same lane (some from other studies not presented here), and these sequences may have been erroneously allocated to *O. lignaria* due to barcoding errors (Balzer et al., 2010; 2011).

Osmia lignaria also showed the most diverse bacterial community. *O. lignaria* had 33 bacterial genera represented (Table 1.4 and 1.5), 23 of which each represented <1% of the total number of reads. In contrast, the other 3 bee species had only 3 (*H. ligatus*), 3 (*L. pilosum*), and 5 (*O. aglaia*) low prevalence bacterial genera represented (Table 1.4 and 1.5). This apparent difference in bacterial community diversity likely results from the overshadowing dominant bacterial fauna in the other three samples,

which may have reduced sensitivity for detecting bacterial diversity in pyrosequenced samples. Balzer et al. (2011) outlined error sources through various steps in the pyrosequencing process, from erroneous reads, quality trimming and filtering sequences through algorithms (Balzer et al., 2010; 2011). Thus, pyrosequencing results may not accurately estimate the diversity of bacteria in the sample. Additionally, percentage composition within a sample may not reflect overall bacterial titer and importance. In *L. pilosum, Arsenophonus* represented a disproportionate 87.7% of the total bacterial reads, but was only present in 1 out of the 8 individuals within that sample, despite equal volumes of DNA utilized from each specimen. Conversely, *Wolbachia* showed a low number of reads within *H. ligatus* (2.4%) and *L. pilosum* (1.6%) (Table 1.4), but was present within every individual of these species (Table1.6). These apparent discrepancies further reinforce the strength of a combined approach of both 454-pyrosequencing and diagnostic screening, to account for the shortcomings of each approach used on their own.

Conclusions

The focus of bee health has grown to include an interest in their bacterial fauna and other microflora. With honeybee decline, solitary bees are becoming an increasing topic of interest, as potential compensatory providers of pollinator services. Maternally inherited bacterial endosymbionts can show reproductive manipulation capabilities that may have potentially devastating effects on population size and sex ratios. Screening of these endosymbionts within bees has been limited almost exclusively to *Wolbachia*. My survey of commercially supplied, as well as locally captured bees from Kentucky, has demonstrated the presence of a novel maternally inherited endosymbiont within hymenoptera, *Sodalis,* within the commercially available pollinator, *Osmia aglaia*. This survey has also shown the presence of *Arsenophonus* in the species *Lasioglossum pilosum*. In addition, these screening efforts have confirmed a high frequency of *Wolbachia* infection in this group (aligned with previous findings), and support the possibility of geographic structuring of *Wolbachia* infection among Halictidae communities. Further study is required to assess the roles of these endosymbionts within this important group of pollinators.

Table 1.1: Local bee collection dates and locations: (species collected within Central Kentucky)

Population	Family	Species	Specimens Collected	Location[3]	Collection Method	Date
1	Apidae	*Epeolus bifasciatus*	1	Spindletop Farm	Free-hand[1]	11-May-12
2	Halictidae	*Agapostemon texanus*	1	Spindletop Farm	Free-hand	11-May-12
3	Halictidae	*Agapostemon virescens*	1	Spindletop Farm	Free-hand	5-Aug-11
4	Halictidae	*Agapostemon virescens*	1	South Farm	Free-hand	16-Aug-11
5	Halictidae	*Agapostemon virescens*	1	Spindletop Farm	Free-hand	11-May-12
6	Halictidae	*Agapostemon virescens*	1	University Club Golf Course	Bee-bowl[2]	21-Aug-12
7	Halictidae	*Agapostemon virescens*	1	Spindletop Farm	Bee-bowl	24-Aug-12
8	Halictidae	*Augochlora pura*	1	Spindletop Farm	Free-hand	11-May-12
9	Halictidae	*Augochlorella aurata*	1	Shaker Village	Free-hand	16-Aug-11
10	Halictidae	*Augochloropsis metallica*	1	Shaker Village	Free-hand	16-Aug-11
11	Halictidae	*Ceratina calcarata*	1	Spindletop Farm	Bee-bowl	23-Aug-12
12	Halictidae	*Halictus ligatus*	9	Spindletop Farm	Free-hand	14-Jun-12
13	Halictidae	*Halictus ligatus*	5	Shaker Village	Free-hand	16-Aug-11
14	Halictidae	*Halictus ligatus*	1	Spindletop Farm	Free-hand	16-Aug-11
15	Halictidae	*Halictus ligatus*	2	Kearney Hill Golf Links	Bee-bowl	12-Aug-12
16	Halictidae	*Halictus ligatus*	2	University Club Golf Course	Bee-bowl	21-Aug-12
17	Halictidae	*Halictus ligatus*	13	Spindletop Farm	Bee-bowl	23-Aug-12
18	Halictidae	*Halictus ligatus*	4	Spindletop Farm	Bee-bowl	24-Aug-12
19	Halictidae	*Halictus parallelus*	2	Spindletop Farm	Bee-bowl	24-Aug-12
20	Halictidae	*Lasioglossum hitchensi*	1	Griffin's Gate Golf Course	Bee-bowl	21-Aug-12
21	Halictidae	*Lasioglossum hitchensi*	1	University Club Golf Course	Bee-bowl	21-Aug-12
22	Halictidae	*Lasioglossum hitchensi*	1	Shaker Village	Free-hand	16-Aug-11
23	Halictidae	*Lasioglossum imitatum*	6	Shaker Village	Free-hand	16-Aug-11
24	Halictidae	*Lasioglossum paradmirandum*	1	Shaker Village	Free-hand	16-Aug-11

Table 1.1 (Cont.): Local bee collection dates and locations: (species collected within Central Kentucky)

Population	Family	Species	Specimens Collected	Location	Collection Method	Date
25	Halictidae	*Lasioglossum pilosum*	8	Spindletop Farm	Free-hand	5-Aug-11
26	Halictidae	*Lasioglossum pilosum*	1	Spindletop Farm	Bee-bowl	24-Aug-12
27	Halictidae	*Lasioglossum pruinosum*	3	Spindletop Farm	Bee-bowl	24-Aug-12
28	Halictidae	*Lasioglossum tegulare*	1	Griffin's Gate Golf Course	Bee-bowl	21-Aug-12
29	Halictidae	*Lasioglossum trigeminum*	1	Kearney Hill Golf Links	Bee-bowl	12-Aug-12
30	Halictidae	*Lasioglossum zephyrum*	1	Spindletop Farm	Free-hand	16-Aug-11
31	Andrenidae	*Andrena barbara*	1	Ayre's Orchard (Owington)	Free-hand	24-Apr-13
32	Andrenidae	*Andrena forbesii*	1	Ayre's Orchard (Owington)	Free-hand	24-Apr-13
33	Andrenidae	*Andrena imitatrix*	4	Ayre's Orchard (Owington)	Free-hand	24-Apr-13
34	Andrenidae	*Andrena nasonii*	5	Ayre's Orchard (Owington)	Free-hand	24-Apr-13
35	Andrenidae	*Andrena sp. 1*	1	Ayre's Orchard (Owington)	Free-hand	24-Apr-13

[1]Free-hand: bees obtained through active collection using nets and containers

[2]Bee-bowls: bees obtained through passive collection in white, yellow and blue bowls filled with a soapy water solution

[3]Location latitudes and longitudes (Spindletop farm: 38.12985, -84.50770; South Farm: 37.97593, -84.53329; University Club Golf Course: 38.11482, -84.60995; Shaker Village: 37.81702, -84.74222; Kearney Hill Golf Links: 38.12373, -84.53657; Griffin’s Gate Golf Course: 38.08898, -84.48781; Ayre’s Orchard: 38.43285, -84.85863)

Table 1.2: Collection origins and years of commercially available solitary bees

Population	Species	Number of Individuals	Origin (State)	Year Obtained
1	*Osmia aglaia*	11	Washington	2012
2	*Osmia aglaia*	18	Washington	2013
3	*Osmia aglaia*	10	Oregon	2013
4	*Osmia caerulescens*	1	Washington	2012
5	*Osmia californica*	6	Washington	2013
6	*Osmia cornifrons*	9	Washington	2012
7	*Osmia lignaria*	20	Utah	2012
8	*Osmia lignaria*	3	Washington[1]	2012
9	*Osmia lignaria*	10	Ohio	2012
10	*Osmia lignaria*	2	Washington[2]	2012
11	*Osmia taurus*	2	Virginia	2012
12	*Megachile pugnata*	8	Utah	2012
13	*Megachile rotundata*	1	Utah	2012

[1] and [2] indicate two different suppliers for *O. lignaria* from Washington in 2012

Table 1.3: Primers and cycling conditions of targeted endosymbionts or DNA in diagnostic PCR

Target Symbiont or DNA	Target Gene	Primer name	Primer sequence 5' to 3'	References	PCR cycling conditions
Arsenophonus	23S	Ars23sF Ars23sR	CGTTTGATGAATTCATAGTCAAA GGTCCTCCAGTTAGTGTTACCCAAC	Thao and Baumann 2004	95°C for 2 min, then 35 cycles consisting of 92°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec.
Sodalis	16S	Sodalis370F 16S Sod590R	CGRTRGCGTTAAYAGCGC AACAGACCGCCTGCGTACG	Toju et al 2010	94°C for 3 min, then 35 cycles consisting of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min.
Sodalis	16S	GroEL Sod 200F GroEL Sod 500R	GAACATGGGCGCCCAGATGGTG CCSGAACCCTCTTCCACGGTGATG	Toju et al 2010	94°C for 3 min, then 35 cycles consisting of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min.
Sodalis	rplB	SodrplB1 F SodrplB1 R	TGCTGGAAACTCTCAGCAAAT CTCCAGACGTTCTACCACTGC	Smith et al 2013	95°C for 2 min, then 35 cycles consisting of 92°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec.
Wolbachia	wsp	Wsp F Wsp R	GTCCAATARSTGATGARGAAAC CYGCACCAAYAGYRCTRTAAA	Baldo et al 2005	94°C for 2 min, then 38 cycles consisting of 94°C for 30 sec, 55°C for 45 sec, and 72°C for 90 sec.
CO1	CO1	LCO1490 HCO700	GGTCAACAAATCATAAAGATATTGG TCAGGGTGACCAAAAAATCA	Folmer et al 1994 Breton et al 2006	94°C for 3 min, then 35 cycles consisting of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 1 min.
EF1-alpha	EF1- α	For1deg Rev2	GYATCGACAARCGTACSATYG YTCSACYTTCCATCCCTTGTACC	Danforth, Conway and Ji 2003 Brady and Danforth 2004	94°C for 2 min, then 35 cycles consisting of 94°C for 1 min, 52°C for 1 min, and 72°C for 1.5 min.

Table 1.4: 454-Pyrosequencing reads and percentages of high prevalence (>1% of total reads per species) bacteria

	Prevalence			
Bacterial genera	***H. ligatus* (N=10)[1]**	***L. pilosum* (N=8)**	***O. lignaria* (N=10)**	***O. aglaia* (N=8)**
Acidovorax/ Diaphorobacter	8 (0.2%)	0	1233 (69.9%)	10 (0.2%)
Acinetobacter	0	0	26 (1.5%)	0
***Arsenophonus*[2]**	1 (0.2%)	**5980 (87.7%)**	63 (3.5%)	5 (0.1%)
Enterobacteriaceae (unknown genus)	0	123 (1.8%)	0	90 (2.1%)
Enterococcus	0	0	81 (4.6%)	0
Hafnia	98 (2.4%)	0	0	0
Lactobacillus	3773 (93.6%)	584 (8.5%)	9 (0.5%)	5 (0.1%)
Riemerella	0	0	64 (3.6%)	1 (0.02%)
Sodalis	0	0	2 (0.1%)	**4035 (95.6%)**
Staphylococcus	0	0	112 (6.4%)	0
Streptococcus	0	0	22 (1.2%)	0
Wolbachia	**96 (2.4%)**	**108 (1.6%)**	7 (0.4)	0
Xenorhabdus	0	0	0	72 (1.7%)
Other*	58 (1.4%)	25 (0.4%)	143 (8.3%)	17 (0.4%)
Total number of bacterial reads	**4033**	**6820**	**1762**	**4219**

***** indicates bacterial genera that comprised <1% of total bacterial reads

[1]N= number of specimens from which DNA was pooled

[2]Bacterial genera in bold represent known maternally inherited bacterial endosymbionts which were targeted in subsequent diagnostic screening

Table 1.5: 454-Pyrosequencing reads and percentages of low prevalence (<1% of total reads per species) bacteria

	Prevalence			
Bacterial Genera	***H. ligatus* (N=10)**	***L. pilosum* (N=8)**	***O. lignaria* (N=10)**	***O. aglaia* (N=8)**
Acidobacterium			2 (0.1%)	
Anaerococcus			6 (0.3%)	
Arcobacter			1 (0.1%)	
Bacillus			1 (0.1%)	
Bergeyella			8 (0.5%)	
Chloroflexus			1 (0.1%)	
Chryseobacterium			13 (0.7%)	
Comamonas			3 (0.2%)	
Corynebacterium			9 (0.5%)	
Empedobacter		7 (0.1%)		
Escherichia				4 (0.1%)
Flavobacterium			1 (0.1%)	
Haemophilus			4 (0.2%)	
Janthinobacterium			7 (0.4%)	
Legionella			11 (0.6%)	
Marmoricola			1 (0.1%)	
Methylobacterium		5 (0.1%)	3 (0.2%)	
Myroides		4 (0.1%)		
Nocardioides			14 (0.8%)	
Novosphingobium			6 (0.3%)	
Pediococcus	9 (0.2%)			
Prevotella			4 (0.2%)	
Propionibacterium			15 (0.9%)	
Rahnella			1 (0.1%)	
Rickettsiella			5 (0.3%)	
Serratia			11 (0.6%)	
Sphingobium			6 (0.3%)	
Spiroplasma	13 (0.3%)	4 (0.1%)		
Tatumella	33 (0.8%)			

*Bacteria that represented <1% of total number of reads but were not identified to a genus level distinction were excluded

Table 1.6: Maternally inherited endosymbiont frequency screening of solitary bee species

Family	Species	# Screened	*Wolbachia*	*Arsenophonus*	*Sodalis*
Apidae	*Epeolus bifasciatus*	1	0	0	0
Halictidae	*Agapostemon texanus*	1	1	0	0
Halictidae	*Agapostemon virescens*	5	5	0	0
Halictidae	*Augochlora pura*	1	1	0	1[1]
Halictidae	*Augochlorella aurata*	1	1	0	1[1]
Halictidae	*Augochloropsis metallica*	1	1	0	0
Halictidae	*Ceratina calcarata*	1	1	0	1[1]
Halictidae	*Halictus ligatus*	36	36	0	1[2]
Halictidae	*Halictus parallelus*	2	2	0	1[2]
Halictidae	*Lasioglossum hitchensi*	3	3	0	0
Halictidae	*Lasioglossum imitatum*	6	6	0	0
Halictidae	*Lasioglossum paradmirandum*	1	1	0	0
Halictidae	*Lasioglossum pilosum*	9	9	1	1[2]
Halictidae	*Lasioglossum pruinosum*	3	3	0	1[2]
Halictidae	*Lasioglossum tegulare*	1	1	0	0
Halictidae	*Lasioglossum trigeminum*	1	1	0	0
Halictidae	*Lasioglossum zephyrum*	1	1	0	0
Andrenidae	*Andrena barbara*	1	0	0	0
Andrenidae	*Andrena forbesii*	3	0	0	0
Andrenidae	*Andrena imitatrix*	4	0	0	0
Andrenidae	*Andrena nasonii*	4	4	0	0
Andrenidae	*Andrena sp. 1*	1	0	0	0
Megachilidae	*Osmia aglaia*	39	0	0	10
Megachilidae	*Osmia caerulescens*	1	1	0	0
Megachilidae	*Osmia californica*	6	0	0	0
Megachilidae	*Osmia cornifrons*	9	9	0	0
Megachilidae	*Osmia lignaria*	33	0	0	0
Megachilidae	*Osmia taurus*	1	0	0	0
Megachilidae	*Megachile pugnata*	8	0	0	0
Megachilidae	*Megachile rotunda*	1	0	0	0

[1] denotes the detection of a *Sodalis*-like endosymbiont (with ~90% best match to *Sodalis* or *Sodalis*-like endosymbiont)

[2] denotes the detection of *Gluconobacter* (~80% best match) picked up using *Sodalis* primers

Table 1.7: *Wolbachia* strain groupings, based on identical *Wolbachia* surface protein (*wsp*) sequence

Family	Species	*Wolbachia* Group #
Halictidae	*Agapostemon texanus*	1
Halictidae	*Agapostemon virescens*	1
Halictidae	*Augochlora pura*	4
Halictidae	*Augochlorella aurata*	4
Halictidae	*Augochloropsis metallica*	N/A
Halictidae	*Ceratina calcarata*	1
Halictidae	*Halictus ligatus*	3
Halictidae	*Halictus parallelus*	3
Halictidae	*Lasioglossum hitchensi*	6
Halictidae	*Lasioglossum imitatum*	1
Halictidae	*Lasioglossum paradmirandum*	M
Halictidae	*Lasioglossum pilosum*	2
Halictidae	*Lasioglossum pruinosum*	1
Halictidae	*Lasioglossum tegulare*	5
Halictidae	*Lasioglossum trigeminum*	M
Halictidae	*Lasioglossum zephyrum*	N/A
Andrenidae	*Andrena nasonii*	1
Megachilidae	*Osmia caerulescens*	7

[1]M = infected with multiple *Wolbachia* strains, N/A = *wsp* not sequenced

Table 1.8: *Wsp* gene distance matrix (# bases/percentage difference) among *Wolbachia* strains

	wsp group 1	*wsp* group 2	*wsp* group 3	*wsp* group 4	*wsp* group 5	*wsp* group 6	*wsp* group 7
wsp group 1 (411 bp)		1 (0.2%)	1 (0.2%)	73 (17.8%)	26 (6.4%)	57 (13.8%)	94 (22.8%)
wsp group 2 (411 bp)			2 (0.5%)	74 (18.1%)	27 (6.6%)	58 (14.1%)	95 (23%)
wsp group 3 (411 bp)				74 (18.1%)	27 (6.6%)	58 (14.1%)	95 (23%)
wsp group 4 (395 bp)					83 (20.3%)	75 (18.5%)	101 (24.8%)
wsp group 5 (406 bp)						71 (17.4%)	100 (24.3%)
wsp group 6 (396 bp)							107 (25.9%)
wsp group 7 (408 bp)							

*Shaded areas denote <1% difference between bases

Table 1.9: *Wolbachia* MLST gene distance matrix (# bases /percentage difference) of four standard housekeeping genes among sequenced species

	Andrena nasonii				*Halictus ligatus*				*Lasioglossum pilosum*				*Osmia caerulescens*			
	fbpA	*ftsZ*	*gatB*	*hcpA*	*fbpA*	*ftsZ*	*gatB*	*hcpA*	*fbpA*	*ftsZ*	*gatB*	*hcpA*	*fbpA*	*ftsZ*	*gatB*	*hcpA*
A. nasonii					1 (0.2%)	1 (0.2%)	0	3 (0.7%)	0	1 (0.2%)	0	1 (0.2%)	51 (12.5%)	54 (11.7%)	53 (13.6%)	51 (11.9%)
H. ligatus									1 (0.2%)	0	0	2 (0.5%)	51 (12.5%)	53 (11.7%)	53 (13.6%)	53 (12.4%)
L. pilosum													51 (12.5%)	53 (11.7%)	53 (13.6%)	52 (12.1%)
O. caerulescens																

*Shaded areas denote <1% difference between species

Chapter 2

An Exploratory Trial of Mason Bee Recruitment and Assessment of Orchard Bee Prevalence

Introduction

Orchard crops make up an $18 billion dollar per year industry within the United States (USDA, 2012), often relying on large volumes of pollinators for successful yield. Honey bees have been the historic pollinator of choice within these systems, and are valued at $5-$15 billion per year in the United States alone (Southwick and Southwick, 1992; Morse and Calderone, 2000; Calderone, 2012). With ongoing reduction of honey bees due to disease, habitat loss and insecticide related declines (Nabhan and Buchmann, 1997; Allen-Wardell et al., 1998; Daberkow et al., 2009; vanEngelsdorp and Meixner, 2010), there is concern whether this heavily used pollinator species will be able to supply demands from the various economically important crops where they are used. Researchers are starting to look into the use of alternative pollinators to offset some of these demands.

Solitary bees within the family Megachilidae, and particularly those in the genus *Osmia*, may be one of the possible solutions. Certain species within this genus, commonly referred to as mason bees, are being closely examined for their pollinator efficiency within orchard crops (Bosch and Kemp, 2000; Bosch and Kemp, 2002). Apples are one of the largest orchard crops in the Unites States, valued at $3.1 billion (NASS, 2013). These crops are also known for large scale use of honey bees for pollination, making successful pollination a large financial investment. Solitary bees can

sometimes be a more efficient alternative to honey bees, requiring fewer individuals to accomplish efficient pollination (Strickler, 1979; Heard, 1999). The species *Osmia lignaria* has been one of these solitary species that has been known be effective in pollination of several orchard plant species (Torchio, 1982a; 1982b; 1985). Thus, purchasing this pollinator in place of renting hundreds of bee hives could result in a reduction of pollination costs.

An alternative to purchasing these *Osmia* species is to attract your own population. The value of native pollinators and their conservation is becoming more and more important, especially with the destruction of prairie and wildflower habitats (Kevan et al., 1990; Allen-Wardell et al., 1998; Kearns et al., 1998; Kremen and Ricketts, 2000). These bees are generally cavity-nesting, finding refuge in tree holes, pith and other similar locations (Cane et al., 2007). Sampling of pollinators for various studies have shown recruitment of cavity nesting species of bees to trap nests (Frankie et al., 1998; Steffan-Dewenter, 2003; Tylianakis et al., 2005; Buschini et al., 2006; Westphal et al., 2008), with varying levels of recruitment. Some solitary bee suppliers encourage pollinator recruitment using nest boxes to attract mason bees (*Osmia* sp.). These "Mason Bee Homes" or "Mason Bee Boxes" can be readily purchased from a variety of sources (including online) that encourage the recruitment of these pollinators to homes and gardens (e.g., www.crownbees.com ; www.masonbeehomes.com, etc.). These Mason Bee Boxes not only provide mason bees nesting sites, to complement available nectar and pollen resources, but also have been promoted to provide better pollination of gardens (www.masonbeehomes.com , etc).

If one could attract these pollinators to a home or garden, it should be possible to attract these pollinators to small scale orchards, thereby potentially increasing native pollinators within the orchard, and simultaneously reducing the need for honey bees. The objective of my research was to attempt to recruit mason bees to nesting boxes, to determine the available mason bee diversity within orchards around Central Kentucky. In addition, I wanted to collect solitary bees found in the orchards during the apple bloom period, in order to determine the presence of native pollinators and to incorporate them into the endosymbiont survey in the previous chapter.

Specific Objectives

1) Set up mason bee boxes in apple orchards in Lexington, KY to assess natural mason bee recruitment.
2) Capture additional solitary pollinators to determine native bee presence during bloom period of apple trees.

Materials and Methods

Orchards

Five apple orchards were selected within Central Kentucky. Each orchard was located within a different county. The names and locations for the orchards can be found in Table 2.1.

Experimental design/ setup

Ready-to-assemble Mason Bee nesting boxes were purchased from Crown Bees (www.crownbees.com) in addition to reeds for placing within the boxes. Both cardboard and traditional bamboo reed varieties were used, with three different internal diameters (7mm, 8mm, and 9mm), to recruit the greatest possible diversity of mason bees. The nesting boxes were set up within orchards 1 week prior to the first bloom of the apple trees in 2013. Two boxes were placed at each of the orchards, facing South/South East, between a height of 3-5 feet from the ground (Table 2.2), as per supplier recommendations of mason bee nesting preferences. Each nest box contained 3 reeds of each size of each material (for a total of 18 reeds within a nesting box) (Figure 2.1), arranged randomly to ensure equal exposure of each type of nesting material to potential parasitism along outer edges. Thus, there were a total of 180 reeds (distributed amongst 10 nesting boxes) in place for the recruitment of native mason bees. The nesting boxes were checked 1-2 times per week for the first month of apple blooms, and 2 times per month thereafter until the end of August.

Additional collection and identification

In addition to nesting boxes, bees were collected from the orchards during the time the trees were in bloom at a frequency of 1-2 times per week. Collections were primarily conducted by free-hand and/or net, however, bee bowls of three colors (white, yellow, and blue) were placed in sets for a total of 6 sets per orchard. Three of those sets were placed within the blooming apple trees, balanced atop branches within the tree or tied with string, and the other three sets were placed on the ground in between apple trees. The bee bowls were filled with a soapy water solution of 30 mL Blue Dawn Dishwashing soap (Procter & Gamble, Cincinnati Ohio) mixed with 1 L of water. Bee bowls were left in the orchard over a 24 hour period, after which any bees within the bowls were collected and placed within a 95% ethanol solution and stored in a freezer at -20ºC. Bees were identified morphologically using the Discoverlife IDnature guides for Apoidea (http://www.discoverlife.org/20/q?search=Apoidea) and/or molecularly using CO1 and EF1-alpha sequences.

Results

No bees were found to have nested in the mason bee boxes through the course of the apple season. However, bee bowls and free-hand/net collections yielded 9 species of bees, all within the family Andrenidae (Table 2.3). The endosymbiont screening results of the first set of these species can be found in Chapter 1 (Table 1.6). The complete results will be incorporated in manuscript.

Discussion

The lack of recruitment and nesting in the bee boxes may have been due to a variety of reasons. Three out of the five orchards actively owned/rented honeybee hives during the apple pollination season (orchards 2, 3 and 4) (Table 2.1). One orchard had an adjacent farm that had honeybee hives on the property (orchard 1). Only one out of the five orchards did not rent/own nor had any adjacent properties with honeybee hives (orchard 5). It is possible that competition for resources due to an introduction of managed pollinators may have played a role in the general lack of mason bees present within the orchards (Thomson, 2006). This may be due to large overlaps in plant use among bee species (Matsumura et al., 2004; Thomson, 2006). Additionally these mason bee boxes were small, and their location may have been cryptic to bees looking for nesting locations. Consequently, mason bee homes may not be the ideal strategy for large scale recruitment of mason bees, as these bees may not be present in large numbers within these locations. Westphal et al. (2008) showed far fewer numbers of bees collected from these nest box set-ups than other bee collection methods in their evaluation of sampling methods.

Andrenidae is a family of solitary bees, also referred to as mining bees. These are common ground-nesting pollinators, and are often abundant in apple orchards (Gardner and Ascher, 2006; Park et al., 2010). All solitary bees collected from the orchards were in the family Andrenidae, and all came from the two orchards that did not rent/own honeybee hives (orchard 1 and orchard 5). There were very few observations of *Apis mellifera* in orchard 5. The owner of this orchard stated that he has not used honeybee

hives for orchard pollination in the last 12 years, and has still experienced dependable pollination and successful fruit yield.

The ability to achieve natural pollination through available bee species is a promising indication for native bee preservation. It has the potential to create a minimal interference style of apple orchard management, whereby farmers have the chance to depend on local bees for pollination of their crop. However, native pollinator recruitment would be dependent on the orchard itself, as well as the surrounding landscape, climate, and availability of nearby resources. Diversity of landscapes will have varying effects on pollinator communities. Orchards with greater habitat connectivity and spanning larger areas tend to have greater pollinator diversity (Steffan-Dewenter, 2003). In contrast, orchards surrounded by urban areas may also act as a refuge for pollinators, thereby increasing diversity within the orchard. Studies in New York urban gardens have shown these urbanized areas to have high pollinator diversity (Matteson et al., 2008).

As more is understood about native bee biology and pollinator efficiency, people are starting to make a push away from the reliance on honeybee pollination. Use of bees from local sources may mediate a reliably pollinated crop system with minimal required intervention and economic input. The promotion of native bees in orchard systems may also provide further incentives towards their conservation. Despite the benefits of promoting a sustainable and ecologically responsible system for pollination, recruitment of mason bees into an orchard in which they may not have been previously present is likely not the most sound solution. A more effective means of increasing mason bee diversity may be through the purchase of bees from local suppliers, and the subsequent propagation of released populations in order to allow continued population growth. In

addition, promotion of bee diversity to facilitate orchard pollination can potentially be as simple as increasing floral/habitat diversity in order to provide a more attractive habitat for native bee species (Isaacs et al., 2008). Future studies should focus on the most efficient mechanisms for increasing native bee diversity within orchard systems, and recruiting and maintaining local and sustainable populations of mason bees.

Table 2.1: Surrounding landscape composition and managed honey bee use within orchards

Orchard	Orchard Name	County	Surrounding Landscape	Honey Bee Use
1	Ayre's Orchard	Owen	Wooded area (forest), hilly with few farms	Neighboring farm
2	Evan's Orchard	Scott	Surrounded by farmland, little wooded area	Brought in
3	Reed Valley Orchard	Bourbon	Surrounded by farmland, little wooded area	Owned
4	Bramble Ridge Orchard	Montgomery	Surrounded by farms as well as houses nearby (minimal tree cover)	Owned
5	Boyd's Orchard	Woodford	Surrounded by farms	N/A

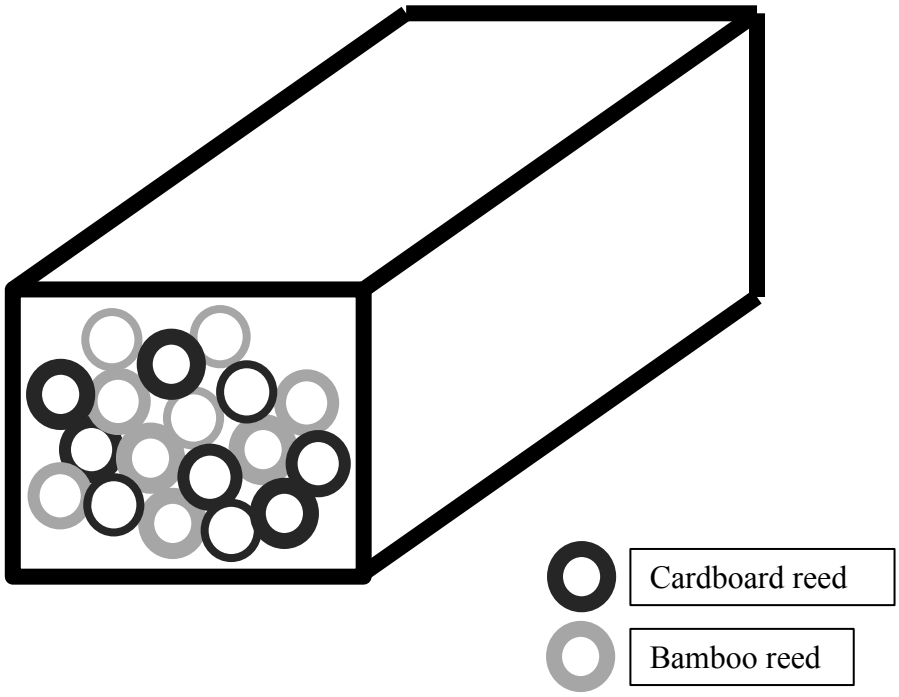
Table 2.2: Mason bee nest box installation date and placement information

Box	Orchard	Date Installed	Directional Orientation	Height	Mounted on
1	Ayre's Orchard	March 20th/2013	South	5 ft	White metal shed
2	Ayre's Orchard	March 20th/2013	East	3.5 ft	White metal shed
3	Evan's Orchard	March 22nd/2013	South east	4.5 ft	Wooden fence
4	Evan's Orchard	March 22nd/2013	South east	4.5 ft	Wooden fence
5	Reed Valley Orchard	March 22nd/2013	South east	4 ft	Large poplar tree
6	Reed Valley Orchard	March 22nd/2013	South east	5 ft	Large poplar tree
7	Bramble Ridge Orchard	March 27th/2013	South east	5 ft	large tree/fence on perimeter
8	Bramble Ridge Orchard	March 27th/2013	South east	5 ft	large tree/fence on perimeter
9	Boyd's Orchard	March 31st/2013	South east	4.5 ft	apple sign post
10	Boyd's Orchard	March 31st/2013	South east	4.5 ft	apple sign post

Table 2.3: Native bee prevalence within apple orchards that were not dominated by honey bees

Family	Species	# Collected	Date Collected	Method	Orchard
Andrenidae	*Andrena forbesii*	1	April 24th/2013	Free-hand	Ayre's Orchard
Andrenidae	*Andrena barbara*	1	April 24th/2013	Bee-bowl	Ayre's Orchard
Andrenidae	*Andrena imitatrix*	4	April 24th/2013	Free-hand	Ayre's Orchard
Andrenidae	*Andrena nasonii*	5	April 24th/2013	Free-hand	Ayre's Orchard
Andrenidae	*Andrena sp. 1*	1	April 24th/2013	Free-hand	Ayre's Orchard
Andrenidae	*Andrena bisalicis*	1	April 24th/2013	Bee-bowl	Ayre's Orchard
Andrenidae	*Andrena bisalicis*	1	April 29th/2013	Free-hand	Boyd Orchard
Andrenidae	*Andrena wilkella*	5	May 1st/2013	Free-hand	Boyd Orchard
Andrenidae	*Andrena confederata*	2	May 1st/2014	Free-hand	Boyd Orchard
Andrenidae	*Andrena cressonii*	1	May 1st/2015	Bee-bowl	Boyd Orchard

Figure 2.1: Cardboard and Bamboo reed layout in mason bee nest box (front view)



Chapter 3

Patterns, Conclusions and Future Directions

This exploratory study looked at the maternally inherited endosymbiont diversity and frequencies within commercially supplied (throughout the United States) and locally captured solitary bees (from central Kentucky). Through 454-pyrosequencing, coupled with diagnostic screening for selected endosymbiotic bacteria, I was able to demonstrate the presence of a maternally inherited endosymbiont novel to Hymenoptera, *Sodalis*, present at a moderate frequency within a commercially sold solitary bee species (*Osmia aglaia*). In addition, *Sodalis*-like endosymbionts were detected from three other species (*Augochlora pura*, *Augochlorella aurata* and *Ceratina calcarata*). The endosymbiont *Arsenophonus* was found to be present at low frequency within *Lasioglossum pilosum*. In addition, *Wolbachia* was shown to be present in many species, and at high frequencies within infected species. In particular, all tested individuals within the sweat bee family, Halictidae, were infected with this endosymbiont. Further examination through assessment of standard *Wolbachia* housekeeping genes showed conserved similarities amongst sequences of strains within Andrenidae and Halictidae. In contrast, the *Wolbachia* within the Megachilidae species *Osmia caerulescens* was dissimilar from the other sequenced strains. Future studies should assess the role of *Wolbachia*, *Arsenophonus*, and *Sodalis* in these solitary bee communities, as they may provide insight into the microbial associations and overall health of these important pollinators.

In addition to characterizing the maternally inherited bacterial endosymbionts of solitary bees, this study also examined the potential recruitment of native mason bees to

apple orchards, and tested mason bee box effectiveness. No mason bees were recruited to the nest boxes, however, several species of native solitary bees were independently captured from two out of five orchards. Future studies should look at the differences between solitary bee diversity within orchards that import honey bees, versus those that do not. Studies should also consider the efficiency of establishing local mason bee recruitment through release of purchased bees, and efficiency of subsequent retention of individuals in the following seasons. Use of pheromone lures may also facilitate mason bee recruitment to nest boxes, but this needs to be studied as well.

References

Aksoy, S., Chen, X. A., and Hypsa, V. (1997). Phylogeny and potential transmission routes of midgut-associated endosymbionts of tsetse (Diptera: Glossinidae). *Insect Molecular Biology, 6*, 183-190.

Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., et al. (1998). The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology, 12*, 8-17.

Arthofer, W., Riegler, M., Avtzis, D.V., and Stauffer, C. (2009a). Evidence for low-titre infections in insect symbiosis: *Wolbachia* in the bark beetle *Pityogenes chalcographus* (Coleoptera, Scolytinae). *Environmental Microbiology, 11,* 1923-1933.

Arthofer, W., Riegler, M., Schneider, D., Krammer, M., Miller, W. J., and Stauffer, C. (2009b). Hidden *Wolbachia* diversity in field populations of the European cherry fruit fly, *Rhagoletis cerasi* (Diptera, Tephritidae). *Molecular Ecology, 18*, 3816-3830.

Balas, M., Lee, M. H., and Werren, J. H. (1996). Distribution and fitness effects of the son-killer bacterium in *Nasonia*. *Evolutionary Ecology, 10*, 593-607.

Baldo, L., Lo, N., and Werren, J. H. (2005). Mosaic nature of the *Wolbachia* surface protein. *Journal of Bacteriology, 187,* 5406–5418.

Baldo, L., Dunning Hotopp, J. C., Jolley, K. A., Bordenstein, S. R., Biber, S. A., Choudhury, R. R., Hayashi, C., Maiden, M. C., Tettelin, H. & Werren, J. H. (2006). Multilocus sequence typing system for the endosymbiont *Wolbachia* pipientis. *Appl Environmental Microbiology, 72,* 7098–7110.

Baldo, L., Ayoub, N. A., Hayashi, C., Russell, J. A., Stahlut, J. K., and Werren, J. H. (2008). Insight into the routes of Wolbachia invasion: high levels of horizontal transfer in the spider genus *Agelenopsis* revealed by *Wolbachia* strain and mitochondrial DNA diversity. *Molecular Ecology, 17,* 557–569.

Balzer, S., Malde, K., Lanzén, A., Sharma, A., and Jonassen, I. (2010). Characteristics of 454 pyrosequencing data—enabling realistic simulation with flowsim. *Bioinformatics, 26*, i420-i425.

Balzer, S., Malde, K., and Jonassen, I. (2011). Systematic exploration of error sources in pyrosequencing flowgram data. *Bioinformatics, 27*, i304-i309.

Bosch, J., and Kemp, W. P. (2000). Development and emergence of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). *Environmental Entomology, 29*, 8-13.

Bosch, J., and Kemp, W. P. (2002). Developing and establishing bee species as crop pollinators: the example of *Osmia* spp. (Hymenoptera: Megachilidae) and fruit trees. *Bulletin of Entomological Research 92*, 3-16.

Brady, C. M., and White, J. A. (2013). Cowpea aphid (*Aphis craccivora*) associated with different host plants has different facultative endosymbionts. *Ecological Entomology, 38*, 433-437.

Brady, S. G., and Danforth, B. N. (2004). Recent intron gain in elongation factor-1α of colletid bees (Hymenoptera: Colletidae). *Molecular Biology and Evolution, 21*, 691-696.

Breeuwer, J. A. J. (1997). Wolbachia and cytoplasmic incompatibility in the spider mites *Tetranychus urticae* and *T. turkestani*. *Heredity, 79*, 41–47.

Breeuwer, J. A. J., and Werren, J. H. (1990). Microorganism associated with chromosome destruction and reproductive isolation between two insect species. *Nature, 346*, 558–560.

Bressan, A., Semetey, O., Nusillard, B., Clair, D., and Boudon-Padieu, E. (2008). Insect vectors (Hemiptera: Cixiidae) and pathogens associated with the disease syndrome "basses richesses" of sugar beet in France. *Plant Disease, 92*, 113-119.

Breton, S., Burger, G., Stewart, D. T., and Blier, P. U. (2006). Comparative analysis of gender-associated complete mitochondrial genomes in marine mussels (Mytilus spp.). *Genetics, 172*, 1107-1119.

Buchmann, S. L., and Nabhan, G. P. (1997). The forgotten pollinators. Island Press.

Buschini, M. L. T. (2006). Species diversity and community structure in trap-nesting bees in Southern Brazil. *Apidologie, 37*, 58.

Calderone, N. W. (2012). Insect pollinated crops, insect pollinators and US agriculture: Trend analysis of aggregate data for the period 1992–2009. *PLoS One, 7*, e37235.

Cane, J. H., Griswold, T., and Parker, F. D. (2007). Substrates and materials used for nesting by North American *Osmia* bees (Hymenoptera: Apiformes: Megachilidae). *Annals of the Entomological Society of America, 100*, 350-358.

Cheng, Q., and Aksoy, S. (1999). Tissue tropism, transmission and expression of foreign genes *in vivo* in midgut symbionts of tsetse flies. *Insect Molecular Biology, 8*, 125-132.

Cox-Foster, D. L., Conlan, S., Holmes, E. C., Palacios, G., Evans, J. D., Moran, N. A., Quan, P. L., Briese, T., Hornig, M., Geiser, D. M., Martinson, V., vanEngelsdorp, D., Kalkstein, A. L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchinson, S. K., Simons, J. F., Engholm, M., Pettis, J. S., and Lipkin, W. I. (2007). A metagenomic survey of microbes in honey bee colony collapse disorder. *Science, 318*, 283-287.

Cross, M. L. (2002). Microbes versus microbes: immune signals generated by probiotic lactobacilli and their role in protection against microbial pathogens. *FEMS Immunology and Medical Microbiology, 34*, 245–253.

Daberkow, S., Korb, P., and Hoff, F. (2009). Structure of the US beekeeping industry: 1982-2002. *Journal of Economic Entomology, 102*, 868-886.

Dale, C., and Maudlin, I. (1999). *Sodalis* gen. nov. and *Sodalis glossinidius* sp. nov., a microaerophilic secondary endosymbiont of the tsetse fly *Glossina morsitans morsitans*. *International Journal of Systematic Bacteriology, 49*, 267-275.

Dale, C., and Welburn, S. C. (2001). The endosymbionts of tsetse flies: manipulating host–parasite interactions. *International Journal for Parasitology, 31*, 628-631.

Danet, J. L., Foissac, X., Zreik, L., Salar, P., Verdin, E., Nourisseau, J. G., and Garnier, M. (2003). "*Candidatus* Phlomobacter fragariae" is the prevalent agent of marginal chlorosis of strawberry in French production fields and is transmitted by the planthopper *Cixius wagneri* (China). *Phytopathology, 93*, 644-649.

Danforth, B. N., L. Conway, and Ji, S. (2003). Phylogeny of eusocial *Lasioglossum* reveals multiple losses of eusociality within a primitively eusocial clade of bees (Hymenoptera: Halictidae). *Systematic Biology, 52*, 23-36.

Darby, A. C., Choi, J. H., Wilkes, T., Hughes, M. A., Werren, J. H., Hurst, G. D., and Colbourne, J. K. (2010). Characteristics of the genome of *Arsenophonus nasoniae*, son-killer bacterium of the wasp *Nasonia*. *Insect Molecular Biology, 19* Suppl 1, 75-89.

Dowd, S. E., Callaway, T. R., Wolcott, R. D., Sun, Y., McKeehan, T., Hagevoort, R. G., and Edrington, T. S. (2008). Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). *BMC Microbiology, 8*, 125.

Duron, O., Bouchon, D., Boutin, S., Bellamy, L., Zhou, L. Q., Engelstadter, J., and Hurst, G. D. D. (2008a). The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biology, 6*, 27.

Duron, O., Hurst, G. D. D., Hornett, E. A., Josling, J. A., and Engelstaedter, J. (2008b). High incidence of the maternally inherited bacterium *Cardinium* in spiders. *Molecular Ecology, 17*, 1427-1437.

Evison, S. E. F., Roberts, K. E., Laurenson, L., Pietravalle, S., Hui, J., Biesmeijer, J. C., Smith, J. E., Budge, G., and Hughes, W. O. H. (2012). Pervasiveness of parasites in pollinators. *Plos One 7*, e30641.

Ferree, P. M., Avery, A., Azpurua, J., Wilkes, T., and Werren, J. H. (2008). A bacterium targets maternally inherited centrosomes to kill males in *Nasonia*. *Current Biology, 18*, 1409-1414.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*, 294-299.

Frankie, G. W., Thorp, R. W., Newstrom-Lloyd, L. E., Rizzardi, M. A., Barthell, J. F., Griswold, T. L., Jong-Yoon, K., and Kappagoda, S. (1998). Monitoring solitary bees in modified wildland habitats: implications for bee ecology and conservation. *Environmental Entomology, 27*, 1137-1148.

Fukatsu, T., Koga, R., Smith, W. A., Tanaka, K., Nikoh, N., Sasaki-Fukatsu, K., Yoshizawa, K., Dale, C., and Clayton, D. H. (2007). Bacterial endosymbiont of the slender pigeon louse, *Columbicola columbae*, allied to endosymbionts of grain weevils and tsetse flies. Applied *Environmental Microbiology, 73*, 6660-6668.

Gardner, K. E., and Ascher, J. S. (2006). Notes on the native bee pollinators in New York apple orchards. *Journal of New York Entomological Society, 114*(1-2), 86-91.

Gerth, M., Geissler, A., and Bleidorn, C. (2011). *Wolbachia* infections in bees (Anthophila) and possible implications for DNA barcoding. *Systematics and Biodiversity, 9*, 319-327.

Gerth, M., Röthe, J., and Bleidorn, C. (2013). Tracing horizontal *Wolbachia* movements among bees (Anthophila): a combined approach using multilocus sequence typing data and host phylogeny. *Molecular ecology, 22*, 6149-6162.

Gherna, R. L., Werren, J. H., Weisburg, W., Cote, R., Woese, C. R., Mandelco, L., and Brenner, D. J. (1991). *Arsenophonus nasoniae* gen. nov., sp. nov., the causative agent of the Son-killer trait in the parasitic wasp *Nasonia vitripennis*. *International Journal of Systematic Bacteriology, 41*, 563-565.

Greenleaf, S. S., and Kremen, C. (2006a). Wild bee species increase tomato production and respond differently to surrounding land use in Northern California. *Biological Conservation, 133,* 81-87.

Greenleaf, S. S., and Kremen, C. (2006b). Wild bees enhance honey bees' pollination of hybrid sunflower. *Proceedings of the National Academy of Sciences of the United States of America, 103,* 13890-13895.

Gruber, B., Eckel, K., Everaars, J., and Dormann, C. F. (2011). On managing the red mason bee (*Osmia bicornis*) in apple orchards. *Apidologie, 42,* 564-576.

Hagimori, T., Abe, Y., Date, S., and Miura, K. (2006). The first finding of a *Rickettsia* bacterium associated with parthenogenesis induction among insects. *Current Microbiology, 52,* 97-101.

Heard, T. A. (1999). The role of stingless bees in crop pollination. *Annual Review of Entomology, 44,* 183-206.

Heddi, A., Grenier, A. M., Khatchadourian, C., Charles, H., and Nardon, P. (1999). Four intracellular genomes direct weevil biology: nuclear, mitochondrial, principal endosymbiont, and *Wolbachia*. *Proceedings of the National Academy of Sciences of the United States of America, 96,* 6814-6819.

Hedges, L. M., Brownlie, J. C., O'Neill, S. L., and Johnson, K. N. (2008). *Wolbachia* and virus protection in insects. *Science, 322,* 702-702.

Higes, M., Martín-Hernández, R., Garrido-Bailón, E., González-Porto, A. V., García-Palencia, P., Meana, A., Del Nozal, M. J., Mayo, R., and Bernal, J. L. (2009). Honeybee colony collapse due to Nosema ceranae in professional apiaries. Environmental *Microbiology Reports, 1,* 110-113.

Hiroki, M., Kato, Y., Kamito, T., and Miura, K. (2002). Feminization of genetic males by a symbiotic bacterium in a butterfly, *Eurema hacabe* (Lepidoptera: Pieridae). *Naturwissenschaften, 89,* 167-170.

Hosokawa, T., Koga, R., Kikuchi, Y., Meng, X. Y., and Fukatsu, T. (2010). Wolbachia as a bacteriocyte-associated nutritional mutualist. *Proceedings of the National Academy of Sciences, 107,* 769-774.

Hughes, G. L., Allsopp, P. G., Brumbley, S. M., Woolfit, M., McGraw, E. A., and O'Neill, S. L. (2011). Variable infection frequency and high diversity of multiple strains of Wolbachia pipientis in Perkinsiella planthoppers. *Applied and Environmental Microbiology, 77,* 2165-2168.

Hurst, G. D. D., Hurst, L. D., and Majerus, M. E. (1997). Cytoplasmic sex-ratio distorters. In: O'Neill, S. L., Hoffmann, A. A., and Werren, J. H. (eds) Influential Passengers- Inherited Microorganisms and Arthropod Reproduction, pp. 125-154. Oxford University Press, Oxford.

Hurst, G. D. D., Jiggins, F. M., von der Schulenburg, J. H. G., Bertrand, D., West, S. A., Goriacheva, I. I., Zakharov, I. A., Werren, J. H., Stouthamer, R., and Majerus, M. E. (1999). Male-killing *Wolbachia* in two species of insect. *Proceedings of the Royal Society of London B, 266,* 735-740.

Isaacs, R., Tuell, J., Fiedler, A., Gardiner, M., and Landis, D. (2008). Maximizing arthropod-mediated ecosystem services in agricultural landscapes: the role of native plants. *Frontiers in Ecology and the Environment, 7,* 196-203.

Ishak, H.D., Plowes, R., Sen, R., Kellner, K., Meyer, E., Estrada, D.A., Dowd, S.E., and Mueller, U.G. (2011). Bacterial diversity in *Solenopsis invicta* and *Solenopsis*

geminata ant colonies characterized by 16S amplicon 454 pyrosequencing. *Microbial Ecology, 61*, 821-831.

Jeong, G., Lee, K., Choi, J., Hwang, S., Park, B., Kim, W., Choi, Y., Park, I., and Kim, J. (2009). Incidence of *Wolbachia* and *Cardinium* endosymbionts in the *Osmia* community in Korea. *Journal of Microbiology, 47*, 28-32.

Jolley, K. A., Chan, M. S., and Maiden, M. C. (2004). mlstdbNet–distributed multi-locus sequence typing (MLST) databases. *BMC Bioinformatics, 5*, 86.

Koch, H., and Schmid-Hempel, P. (2011). Bacterial communities in Central European bumblebees: low diversity and high specificity. *Microbial Ecology 62*, 121–133.

Kearns, C. A., Inouye, D. W., and Waser, N. M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics*, *29*, 83-112.

Kevan, P. G., Clark, E. A., and Thomas, V. G. (1990). Insect pollinators and sustainable agriculture. *American Journal of Alternative Agriculture, 5*, 13-22.

Kikuchi, Y., M. Hayatsu, T. Hosokawa, A. Nagayama, K. Tago, and Fukatsu, T. (2012). Symbiont-mediated insecticide resistance. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 8618-8622.

Klein, A. M., Steffan-Dewenter, I., and Tscharntke, T. (2003a). Bee pollination and fruit set of *Coffea arabica* and *C-canephora* (Rubiaceae). *American Journal of Botany, 90*, 153-157.

Klein, A. M., Steffan-Dewenter, I., and Tscharntke, T. (2003b). Fruit set of highland coffee increases with the diversity of pollinating bees. *Proceedings of the Royal Society of London Series B- Biological Sciences, 270*, 955-961.

Klein, A. M., Steffan-Dewenter, I., and Tscharntke, T. (2003c). Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *Journal of Applied Ecology 40*, 837-845.

Kremen, C., and Ricketts, T. (2000). Global perspectives on pollination disruptions. *Conservation Biology*, *14*, 1226-1228.

Kremen, C., Williams, N. M., Bugg, R. L., Fay, J. P., and Thorp, R. W. (2004). The area requirements of an ecosystem service: crop pollination by native bee communities in California. *Ecology Letters, 7*, 1109-1119.

Kremen, C., Williams, N. M., and Thorp, R. W. (2002). Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 16812-16816.

Martin, S. J. (2001). The role of *Varroa* and viral pathogens in the collapse of honeybee colonies: a modelling approach. *Journal of Applied Ecology, 38*, 1082-1093.

Martinson, V. G., Danforth, B. N., Minckley, R. L., Rueppell, O., Tingek. S., and Moran, N. A. (2011). A simple and distinctive microbiota associated with honey bees and bumble bees. *Molecular Ecology, 20*, 619–628.

Matsumura, C., Yokoyama, J., and Washitani, I. (2004). Invasion status and potential ecological impacts of an invasive alien bumblebee, *Bombus terrestris* L. (Hymenoptera: Apidae) naturalized in Southern Hokkaido, Japan. *Global Environmental Research- English Edition 8*, 51-66.

Matteson, K. C., Ascher, J. S., and Langellotto, G. A. (2008). Bee richness and abundance in New York city urban gardens. *Annals of the Entomological Society of America*, *101*, 140-150.

Maudlin, I. (2006). African trypanosomiasis. *Annals of Tropical Medicine and Parasitology, 100*, 679-701.

McFrederick, Q. S., Wcislo, W. T., Taylor, D. R., Ishak, H. D., Dowd, S. E., and Mueller, U. G. (2012). Environment or kin: whence do bees obtain acidophilic bacteria? *Molecular Ecology, 21*, 1754–1768.

McFrederick, Q. S., Cannone, J. J., Gutell, R. R., Kellner, K., Plowes, R. M., and Mueller, U. G. (2013). Specificity between Lactobacilli and Hymenopteran hosts is the exception rather than the rule. *Applied and Environmental Microbiology, 79*, 1803-1812.

Medina, R. F., Nachappa, P., and Tamborindeguy, C. (2011). Differences in bacterial diversity of host-associated populations of *Phylloxera notabilis* Pergande (Hemiptera: Phylloxeridae) in pecan and water hickory. *Journal of Evolutionary Biology, 24*, 761-771.

Michener, C. D. (2007). *The bees of the world, 2nd edn*. John Hopkins University Press, Baltimore, Maryland.

Mohr, K. I., and Tebbe, C. C. (2006). Diversity and phylotype consistency of bacteria in the guts of three bee species (Apoidea) at an oilseed rape field. *Environmental Microbiology, 8*, 258-272.

Morandin, L. A., and Winston, M. L. (2005). Wild bee abundance and seed production in conventional, organic, and genetically modified canola. *Ecological Applications, 15*, 871-881.

Morse, R. A., and Calderone, N. W. (2000). The value of honey bees as pollinators of US crops in 2000. *Bee culture, 128*, 1-15.

Nabhan, G. P., and Buchmann, S. (1997) in Nature's Services: Societal Dependence on Natural Ecosystems, ed. Daily, G. C. (Island, Washington, DC), pp. 133–150.

NASS, 2013 (http://www.agmrc.org/commodities__products/fruits/apples/commodity-apples) {Date of access: January 10th/2014}.

Novakova, E., and Hypsa, V. (2007). A new *Sodalis* lineage from bloodsucking fly *Craterina melbae* (Diptera, Hippoboscoidea) originated independently of the tsetse flies symbiont *Sodalis glossinidius*. *FEMS Microbiology Letters, 269,* 131–35.

Novakova, E., Hypsa, V., and Moran, N. A. (2009). *Arsenophonus*, an emerging clade of intracellular symbionts with a broad host dristribution. *BMC Microbiology, 9*, 143-156.

O'Neill, S. L., Giordano, R., Colbert, A. M., Karr, T. L., and Robertson, H. M. (1992). 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proceedings of the National Academy of Sciences, 89*, 2699-2702.

Park, M. G., Orr, M. C., and Danforth, B. N. (2010). The role of native bees in apple pollination. *New York Fruit Quarterly, 18*, 21-25.

Raychoudhury, R., Baldo, L., Oliveira, D. C., and Werren, J. H. (2009). Modes of acquisition of Wolbachia: horizontal transfer, hybrid introgression, and codivergence in the Nasonia species complex. *Evolution, 63*, 165-183.

Ricketts, T. H. (2004) Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology, 18*, 1262-1271.

Russell, J. A., Goldman-Huertas, B., Moreau, C. S., Baldo, L., Stahlhut, J. K., Werren, J. H., and Pierce, N. E. (2009). Specialization and geographic isolation among Wolbachia symbionts from ants and lycaenid butterflies. *Evolution, 63*, 624-640.

Ryan, S. L., and Saul, G. B. (1968). Post-fertilization effect of incompatibility factors in *Mormoniella*. *Molecular and General Genetics, 103*, 29–36.

Schilthuizen, M. O., and Stouthamer, R. (1997). Horizontal transmission of parthenogenesis–inducing microbes in *Trichogramma* wasps. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 264*, 361-366.

Semetey, O., Gatineau, F., Bressan, A., and Boudon-Padieu, E. (2007). Characterization of a gamma-3 Proteobacteria responsible for the sundrome "basses richesses" of sugar beet transmitted by *Pentastiridius* sp. (Hemiptera Cixiidae). *Phytopathology, 97*, 72-78.

Skinner, S. W. (1985). Son-killer: a third extrachromosomal factor affecting the sex ratio in the parasitoid wasp, *Nasonia (= Mormoniella) vitripennis*. *Genetics, 109*, 745–759.

Skinner, S. W. (1983). Extrachromosomal sex ratio factors in the parasitoid wasp, *Nasonia (=Mormoniella) vitripennis*. PhD dissertation, University of Utah, Salt Lake City, UT.

Smith, C. L., Weiss, B. L., Aksoy, S., and Runyen-Janecky, L. J. (2013). Characterization of the achromobactin iron acquisition operon *in Sodalis glossinidius*. *Applied and Environmental Microbiology, 79*, 2872-2881.

Steffan-Dewenter, I. (2003). Importance of habitat area and landscape context for species richness of bees and wasps in fragmented orchard meadows. *Conservation Biology, 17*, 1036-1044.

Stouthamer, R. (1997). *Wolbachia*-induced parthenogenesis. In Influential passengers: inherited microorganisms and arthropod reproduction. Edited by S. L. O'Neill, A. A. Hoffmann, and J. H. Werren. Oxford University Press, Oxford, U.K. pp. 102-124.

Stouthamer, R., Breeuwer, J. A. J., and Hurst, G. D. D. (1999). *Wolbachia pipientis*: Microbial manipulator of arthropod reproduction. *Annual Review of Microbiology 53*, 71-102.

Southwick, E. E., and Southwick Jr, L. (1992). Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States. *Journal of Economic Entomology, 85*, 621-633.

Strickler, K. (1979). Specialization and foraging efficiency of solitary bees. *Ecology, 60*, 998-1009.

Taylor, G. P., Coghlin, P. C., Floate, K. D., and Perlman, S. J. (2011). The host range of the male-killing symbiont *Arsenophonus nasoniae* in filth fly parasitioids. *Journal of Invertebrate Pathology, 106*, 371-379.

Teixeira, L., Ferreira, Á., and Ashburner, M. (2008). The bacterial symbiont *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biology, 6*, e1000002.

Thao, M. L., and Baumann, P. (2004). Evidence for multiple acquisition of *Arsenophonus* by whitefly species (Sternorrhyncha: Aleyrodidae). *Current Microbiology, 48*, 140-144.

Thomson, D. M. (2006). Detecting the effects of introduced species: a case study of competition between *Apis* and *Bombus*. *Oikos, 114*, 407-418.

Torchio, P. F. (1982a). Field experiments with *Osmia lignaria propinqua* Cresson as a pollinator in almond orchards III, 1977 studies (Hymenoptera: Megachilidae). *Journal of the Kansas Entomological Society, 55*, 101–116.

Torchio, P. F. (1982b). Field experiments with the pollinator species, *Osmia lignaria propinqua* Cresson in apple orchards II, 1976 studies (Hymenoptera: Megachilidae). *Journal of the Kansas Entomological Society, 55*, 759–778.

Torchio, P. F. (1985). Field experiments with the pollinator species, *Osmia lignaria propinqua* Cresson in apple orchards V, (1979–1980), methods of introducing bees, nesting success, seed counts, fruit yields (Hymenoptera: Megachilidae). *Journal of the Kansas Entomological Society, 58*, 448–464.

Toju, H., T. Hosokawa, R. Koga, N. Nikoh, X. Y. Meng, N. Kimura, and Fukatsu, T. (2010). "*Candidatus* Curculioniphilus buchneri," a novel clade of bacterial endocellular symbionts from weevils of the genus *Curculio*. *Applied Environmental Microbiology, 76*, 275-282.

Tylianakis, J. M., Klein, A. M., and Tscharntke, T. (2005). Spatiotemporal variation in the diversity of Hymenoptera across a tropical habitat gradient. *Ecology, 86*, 3296-3302.

United Stated Department of Agriculture: Economic Research Service (2012). http://www.ers.usda.gov/topics/crops/fruit-tree-nuts/background.aspx#.UyUaz_ldWlY {Date of access: January 12th/2014}.

vanEngelsdorp, D., Evans, J. D., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B. K., Frazier, M., Frazier, J., Cox-Foster, D., Chen, Y., Underwood, R., Tarpy, D. R., and Pettis, J. S. (2009). Colony collapse disorder: a descriptive study. *PLoS ONE 3*, e6481.

vanEngelsdorp, D., and Meixner, M. D. (2010). A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *Journal of Invertebrate Pathology, 103*, S80-S95.

Vasquez, A., Forsgren, E., Fries, I., Paxton, R. J., Flaberg, E., Szekely, L., & Olofsson, T. C. (2012). Symbionts as major modulators of insect health: lactic acid bacteria and honeybees. *PLoS One 7*, e33188.

Vavre, F., Fleury, F., Lepetit, D., Fouillet, P., and Boulétreau, M. (1999). Phylogenetic evidence for horizontal transmission of *Wolbachia* in host-parasitoid associations. *Molecular Biology and Evolution, 16*, 1711-1723.

Vavre, F., Fleury, F., Varaldi, J., Fouillet, P., and Bouletreau, M. (2000) Evidence for female mortality in *Wolbachia*-mediated cytoplasmic incompatibility in haplodiploid insects: epidemiologic and evolutionary consequences. *Evolution, 54*, 191–200.

Walker, T., Johnson, P. H., Moreira, L. A., Iturbe-Ormaetxe, I., Frentiu, F. D., McMeniman, C. J., Leong, Y. S., Dong, Y., Axford, J., Kriesner, P., Lloyd, A. L., Ritchie, S. A., O'Neill, S. L., Hoffmann, A. A. (2011). The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature, 476*, 450-453.

Walter, J., Britton, R. A., and Roos, S. (2011). Host-microbial symbiosis in the vertebrate gastrointestinal tract and the *Lactobacillus reuteri* paradigm. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 4645–4653.

Weeks, A. R., Velten, R., and Stouthamer, R. (2003). Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. *Proceedings of the Royal Society of London Series B-Biological Sciences, 270*, 1857-1865.

Weinert, L. A., Werren, J. H., Aebi, A., Stone, G. N., and Jiggins, F. M. (2009). Evolution and diversity of *Rickettsia* bacteria. *BMC Biology, 7*, 6.

Werren, J. H., Baldo, L., and Clark, M. E. (2008). *Wolbachia*: Master manipulators of invertebrate biology. *Natural Reviews of Microbiology, 6*, 741-751.

Westphal, C., Bommarco, R., Carré, G., Lamborn, E., Morison, N., Petanidou, T., Potts, S. G., Roberts, S. P. M., Szentgyorgyi, H., Tscheulin, T., Vaissiere, B. E., Woyciechowski, M., Biesmeijer, J. C., Kunin, W. E., Settele, J., and Steffan-Dewenter, I. (2008). Measuring bee diversity in different European habitats and biogeographical regions. *Ecological Monographs*, *78*, 653-671.

Winfree, R., Williams, N. M., Dushoff, J., and Kremen, C. (2007). Native bees provide insurance against ongoing honey bee losses. *Ecology Letters*, *10*, 1105-1113.

Zchori-Fein, E. I. N. A. T., and Perlman, S. J. (2004). Distribution of the bacterial symbiont *Cardinium* in arthropods. *Molecular Ecology*, *13*, 2009-2016.

Zreik, L., Bove, J. M., and Garnier, M. (1998). Phylogenetic characterization of the bacterium-like organism associated with marginal chlorosis of strawberry and proposition of a *Candidatus* taxon for the organism,'*Candidatus* Phlomobacter fragariae'. *International journal of systematic bacteriology*, *48*, 257-261.

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