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Short Communication

Surveys for maternally-inherited endosymbionts reveal novel and variable infections within solitary bee species ☆

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ABSTRACT

Maternally-inherited bacteria can affect the fitness and population dynamics of their host insects; for solitary bees, such effects have the potential to influence bee efficacy as pollinators. We screened bee species for bacterial associates using 454-pyrosequencing (4 species) and diagnostic PCR (183 specimens across 29 species). The endosymbiont *Wolbachia* was abundant, infecting 18 species, including all specimens from the family Halictidae. Among commercially-supplied orchard bees (family Megachilidae), only 2/7 species were *Wolbachia*-infected, but one species showed variable infection among specimens. Two other maternally-inherited bacteria, *Arsenophonus* and *Sodalis*, were also detected, neither of which was fixed in infection frequency. Differential endosymbiont infection could potentially compromise fitness and reproductive compatibility among commercially redistributed pollinator populations.

1. Introduction

Solitary bees are important pollinators of crops and native flora. Due to the ongoing decline of managed *Apis mellifera* populations, solitary bees act as a buffer to protect worldwide crop pollination operations (Winfree et al., 2007), and several species, particularly within the family Megachilidae, are commercially available for purchase and use in crop and orchard settings (Bosch and Kemp, 2002; Gruber et al., 2011). Given the rising economic and ecological importance of these bees, increasing attention is also being given to their biology, including their microbial associations (e.g., Gerth et al., 2011, 2013, 2015; McFrederick et al., 2012, 2013, 2014).

Solitary bees are particularly prone to infection by maternally-inherited endosymbiotic bacteria in the genus *Wolbachia* (Gerth et al., 2011, 2013, 2015), bacteria that frequently manipulate host reproduction to increase bacterial transmission (Werren et al., 2008). The preponderance of *Wolbachia* among solitary bees suggests that these bees may be susceptible to infection by other maternally-inherited endosymbionts as well, yet the frequency of infection by other endosymbionts remains largely unexplored. Gerth et al. (2015) recently screened numerous bee species using taxon specific primers for three other maternally-inherited bacteria (*Cardinium*, *Arsenophonus*, and *Rickettsia*) and found the latter two within some bee clades. It is useful, however, to complement such diagnostic approaches with more exploratory techniques that can discover unexpected members of the microbial community.

Here, we surveyed the microbial associates of several species of commercially available and wild-caught bees through 454-pyrosequencing, followed by diagnostic screening to determine frequency of infection by particular bacterial taxa. Commercial movement of solitary bees as pollinators means concurrent movement of their associated microbiota. Insect populations may be differentially infected with endosymbionts, which can affect host fitness and reproductive compatibility (e.g., Turelli and Hoffmann, 1991). For solitary bee species, such effects within commercially translocated species would have the potential to affect population dynamics and pollinator efficacy.

2. Materials and methods

We obtained specimens of seven bee species from a variety of commercial suppliers and an additional 22 species through active and passive collection methods in central Kentucky, USA, between 2011 and 2013 (Table S1). Specimens were stored in 95% ethanol at −20 °C until identification and DNA extraction. Bees were

☆ The nucleotide sequence data reported in this manuscript have been submitted to the DDBJ/EMBL/GenBank databases under accession numbers KT123206-42 and KT153630-43, with a public release date of 11 Dec, 2015, and the sequence read archive under project accession number PRJNA283198 with a public release date of 31 Aug, 2015.

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identified morphologically using the Discoverlife IDnature guides for Apoidea (http://www.discoverlife.org/20/q?search=Apoidea) and/or molecularly using COI and/or EF-1 alpha sequences. Specimens were surface sterilized in 5% bleach (60 s); followed by rinses in 95% ethanol (3×), and DI water. DNA was extracted from bee abdomens using DNeasy kits (Qiagen) following manufacturer's instructions. COI and/or EF-1 alpha gene fragments from each specimen were amplified using PCR (primers and cycling conditions in Table S2) in 10 μl reactions as described in Wulff et al. (2013). Unsuccessful extractions (in which COI did not amplify; 3/186 extractions = 1.6%) were discarded from the dataset. Amplified product for a minimum of one specimen per morphospecies per population was sequenced at the Advanced Genetic Technologies Center (University of Kentucky, Lexington, KY, USA) and/or Beckman Coulter (Danvers, MA, USA). Sequences were manually trimmed and edited in Geneious v.6.0.6 (Biomatters Ltd., Auckland, NZ), and submitted to NCBI (Table S1). We identified specimens based on closest available sequences matched in the BOLD ID database (http://barcodinglife.org/index.php/IDS_OpenIdEngine) for COI sequences and NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for EF-1 alpha sequences.

We used 454-pyrosequencing to evaluate the bacterial microbiomes of two commercial (*Osmia aglaia* and *Osmia lignaria*), and two locally captured (*Halictus ligatus* and *Lasioglossum pilosum*) bee species. For each species, DNA from 8 to 10 individuals was pooled at a DNA concentration of 20 ng/μL. Samples were multiplexed 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 using a 454 FLX instrument with Titanium chemistry (Research and Testing Laboratory, Lubbock, TX; Dowd et al., 2008). Resulting sequences underwent quality control and manual curation as described in Brady and White (2013). Final read depth ranged from 1700 to 6800 sequences per bee species. Based on pyrosequencing results, bee specimens were then individually screened for 3 bacterial endosymbiont taxa (*Wolbachia*, *Sodalis* and *Arsenophonus*) using previously published diagnostic primers (Table S2). Products were visualized on a 1% agarose gel stained with GelRed (Biotium, Hayward, CA) in comparison with both positive and negative controls. Putatively positive diagnoses were validated by Sanger sequencing whereas negative diagnoses were re-screened for confirmation; symbiont infections were confirmed when sequences matched endosymbiont taxa within the NCBI nr database at >97% using the blastn algorithm.

3. Results and discussion

Three of the four pyrosequenced bee samples were each dominated by a single bacterial taxon. In *O. aglaia*, 96% of bacterial reads came from *Sodalis* (Table 1), a genus of maternally-transmitted gram-negative bacteria whose members are facultative or obligate endosymbionts within a variety of insect hosts (Aksoy et al., 1997; Fukatsu et al., 2007). A 386bp 16S segment from this *O. aglaia* symbiont was identical to that of *Sodalis* symbionts from multiple stinkbugs and weevils (Toju et al., 2013; Hosokawa et al., 2015), and had greater than 99% similarity to many other insect-associated strains of *Sodalis*. We subsequently sequenced small fragments of genes encoding a molecular chaperonin, GroEL, and a ribosomal protein, rplB1, from *O. aglaia* (KT153640, KT153641), both of which also aligned with *Sodalis* symbionts at >99% identity. This is the first record of this genus of symbiotic bacteria within the order Hymenoptera. In *L. pilosum*, 88% of reads came from *Arsenophonus*, a genus that contains maternally-inherited arthropod-associated bacteria with a variety of functions, including reproductive manipulation (male killing; Ferree et al., 2008), insect vectored plant pathogens (Bressan et al., 2008), and obligate nutritional endosymbionts of some blood-feeding insects (Nováková et al., 2009). Our sequenced fragment (397bp of 16S) showed greater than 99% similarity to many insect-associated *Arsenophonus* strains, but intriguingly was identical to a strain isolated from a honeybee intestine (DQ837612; Babendreier et al., 2007). In *H. ligatus*, 94% of reads came from *Lactobacillus,* which is frequently associated with bees (e.g., Martinson et al., 2011; McFrederick et al., 2012), although it is likely an environmentally acquired rather than maternally inherited bacteria (McFrederick et al., 2012, 2013, 2014). Both the *L. pilosum* and *H. ligatus* samples also contained *Wolbachia* at relatively low prevalence of reads (2% of reads per sample; Table 1). In the final pyrosequenced species, *O. lignaria*, *Acidovorax*/*Diaphorobacter* reads were most prevalent, at 70% of reads. Very low prevalence reads from all three maternally-inherited bacteria (*Sodalis*, *Arsenophonus*, and *Wolbachia*) were detected in this sample (Table 1), but none could be validated through subsequent diagnostic PCR of individual specimens, suggesting that these sequences may have been

Table 1
High prevalence 454-pyrosequencing reads (and percentages) of bacterial taxa constituting >1% of total reads for at least one host species.

Bacterial taxa	Prevalence			
	H. ligatus (*N* = 10)[a]	*L. pilosum* (*N* = 8)	*O. lignaria* (*N* = 10)	*O. aglaia* (*N* = 8)
Acidovorax/*Diaphorobacter*[b]	8 (<1%)	0	1233 (70%)	10 (<1%)
Acinetobacter	0	0	26 (2%)	0
Arsenophonus[c]	1 (<1%)	5980 (88%)	63 (4%)	5 (<1%)
Enterobacteriaceae (unknown genus)	0	123 (2%)	0	90 (2%)
Enterococcus	0	0	81 (5%)	0
Hafnia	98 (2%)	0	0	0
Lactobacillus	3773 (94%)	584 (9%)	9 (1%)	5 (<1%)
Riemerella	0	0	64 (4%)	1 (<1%)
Sodalis	0	0	2 (<1%)	4035 (96%)
Staphylococcus	0	0	112 (6%)	0
Streptococcus	0	0	22 (1%)	0
Wolbachia	96 (2%)	108 (2%)	7 (<1%)	0
Xenorhabdus	0	0	0	72 (2%)
Other[d]	58 (1%)	25 (<1%)	143 (8%)	17 (<1%)
Total bacterial reads	**4033**	**6820**	**1762**	**4219**

[a] *N* = number of host specimens from which DNA was pooled.
[b] Genera indistinguishable from amplified sequence – both 100% matches.
[c] Bacterial genera in bold represent known maternally-inherited symbionts that were targeted in subsequent diagnostic screening.
[d] The taxonomic breakdown of this category is available in Table S3.