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Bacterial communities of herbivores and pollinators that have co-evolved *Cucurbita* spp.

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Data Deposition Statement: Raw sequencing data generated for this study are deposited at NCBI SRA accession SUB5883192, and processed OTUs are deposited at SUB5883184. All processing and analysis scripts are available at https://github.com/lshapiro31/insect.microbiome

Abstract

Insects, like all animals, are exposed to diverse environmental microbes throughout their life cycle. Yet, we know little about variation in the microbial communities associated with the majority of wild, unmanaged insect species. Here, we use a 16S rRNA gene metabarcoding approach to characterize temporal and geographic variation in the gut bacterial communities of herbivores (*Acalymma vittatum* and *A. trivittatum*) and pollinators (*Eucera (Peponapis) pruinosa*) that have co-evolved with the plant genus *Cucurbita* (pumpkin, squash, zucchini and gourds). Overall, we find high variability in the composition of bacterial communities in squash bees and beetles collected from different geographic locations and different time points throughout a growing season. Still, many of the most common OTUs are shared in *E. (P.) pruinosa, A. vittatum* and *A. trivittatum*. This suggests these insects may be exposed to similar environmental microbial sources while foraging on the same genus of host plants, and that similar microbial taxa may aid in digestion of *Cucurbita* plant material. The striped cucumber beetle *A. vittatum* can also transmit *Erwinia tracheiphila,* the causal agent of bacterial wilt of

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48 cucurbits. We find that few field-collected *A. vittatum* individuals have detectable *E.*
49 *tracheiphila,* and when this plant pathogen is detected, it comprises less than 1% of the gut
50 bacterial community. Together, these results are consistent with previous studies showing that
51 plant feeding insects have highly variable gut bacterial communities, and provides a first step
52 towards understanding the spatiotemporal variation in the microbial communities associated with
53 herbivores and pollinators that depend on *Cucurbita* host plants.
54
55
56 **Keywords:** *Cucurbita, Erwinia tracheiphila, Eucera, Peponapis, Acalymma,* bacterial wilt
57 disease, microbiome, pollination ecology, squash bee, striped cucumber beetle, plant pathoge

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Introduction

Herbivorous insects that consume leaf, floral, stem or root tissues are exposed to diverse communities of microbes associated with plants and/or soil. Many microbes ingested by insects likely pass transiently through the digestive tract and may not confer any impact on the host insect (Oliver et al. 2008, Hammer et al. 2017, Hammer et al. 2019). However, some microbes are able to colonize insects as hosts, and can have diverse and consequential – yet often cryptic – roles in mediating ecological interactions between plants, other insects and other microbes (Shapiro et al. 2012, Chung et al. 2013, Shapiro et al. 2013, Wang et al. 2016, Mason et al. 2018). For example, many pollinators likely transfer microbes between flowers during normal foraging that may directly affect other visiting insects, or indirectly alter traits determining floral attractiveness (Yang et al. , McArt et al. 2014, Ravoet et al. 2014, Vannette and Fukami 2016, Rering et al. 2018, Figueroa et al. 2019). Some insect pollinators and herbivores encounter plant pathogens while feeding, and can then transmit these pathogens from infected to healthy plants (Raguso and Roy 1998, Alexandrova et al. 2002, López-Villavicencio et al. 2007, Degnan et al. 2009, Shapiro et al. 2012, Shapiro et al. 2014, Mauck et al. 2018, Shapiro and Mauck 2018, Cellini et al. 2019).Yet, for almost all insect taxa, we still lack an understanding of the spatial and temporal diversity of microbes that insects are naturally exposed to, which microbes are able to persistently colonize insects as hosts vs. transiently pass through the digestive tract, and the impacts of different microbes on ecological interactions.

The pollinators and herbivores that have co-evolved with the plant genus *Cucurbita* (Cucurbitaceae) are an ideal system to investigate how a shared host plant affects microbiome composition for insect species that have different life histories and ecological functions. *Cucurbita* (zucchini, squash, pumpkin, and some yellow-flowered gourds) is comprised of 14

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81 closely related species that are native to the New World tropics and subtropics (Whitaker and
82 Bemis 1975, Metcalf and Lampman 1989, Kates et al. 2017, Castellanos-Morales et al. 2018).
83 All bee species in the genus *Eucera* and sub-genera *Peponapis* and *Xenoglossa* (Hymenoptera:
84 Anthophila: Apidae: Eucerini: *Eucera*) – commonly known as squash bees – are obligate
85 specialists of *Cucurbita* pollen (Baker and Hurd Jr 1968, Hurd et al. 1971, Hurd et al. 1974). The
86 emergence of squash bees in the summer coincides with the *Cucurbita* bloom period, and these
87 ground-nesting, solitary bees exclusively use pollen from *Cucurbita* flowers to feed their brood
88 (Hurd and Linsley 1964, Hurd and Linsley 1967, Hurd et al. 1971, Hurd et al. 1974). *Acalymma*
89 (Barber) leaf beetle herbivores (Coleoptera: Chrysomelidae: Luperini: Diabriticina) have also co-
90 evolved with *Cucurbita*, and all ~70 species of *Acalymma* are obligately dependent on *Cucurbita*
91 in all life stages. *Acalymma* larvae are rootworms, and adults feed on above-ground leaves,
92 flowers and fruits (Barber 1946, Metcalf et al. 1980, Munroe and Smith 1980, Ferguson and
93 Metcalf 1985, Ferguson et al. 1985, Tallamy and Krischik 1989, Tallamy and Gorski 1997,
94 Tallamy et al. 1997, Kistler et al. 2015).

95 The squash bee (*Eucera (P.) pruinosa*) and the striped cucumber beetle (*Acalymma*
96 *vittatum*) are the two most common *Cucurbita*-associated insects in temperate Eastern North
97 America. *E. pruinosa* has recently expanded its geographic range into this region by following
98 the human-mediated dispersal of domesticated *Cucurbita pepo* from the subtropical Eastern
99 United States (Petersen and Sidell 1996, Monaghan et al. 2006, Smith 2006, López-Uribe et al.
100 2016), and it is likely that *A. vittatum* has a similar demographic history. In temperate Eastern
101 North America, both *E. pruinosa* and *A. vittatum* exclusively rely on domesticated plants grown
102 in human managed agro-ecosystems as a food resource. Temperate Eastern North America is
103 also the only region worldwide where *A. vittatum* transmits *Erwinia tracheiphila* Smith

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104 (Enterobacteriaceae), the causal agent of bacterial wilt of cucurbits, which causes millions of
105 dollars annually in direct yield losses and indirect preventative measures (Shapiro et al. 2014,
106 Shapiro et al. 2016, Shapiro et al. 2018b). *E. tracheiphila* can persistently colonize the digestive
107 tract of beetle vectors after beetles feed on the foliage of wilting, symptomatic plants (Rand and
108 Enlows 1916, Rand and Cash 1920, Rand and Enlows 1920, Smith 1920, Shapiro et al. 2012,
109 Shapiro et al. 2014). However, dynamics of vector colonization by *E. tracheiphila* in the field,
110 and potential interactions with other microbes in the beetle's digestive tract remain almost
111 completely unknown (Fleischer et al. 1999, Shapiro et al. 2014).
112 Here, we use 16S rRNA gene barcode sequencing to describe variation in the bacterial
113 communities of the squash bee *Eucera (P.) pruinosa,* and the striped cucumber beetles
114 *Acalymma vittatum* and *A. trivittatum.* Insects were field-collected from their native range in the
115 United States Southwest and in Central Mexico, and from their introduced range in temperate
116 North America. We find high overall variation in bacterial community composition within and
117 between beetles and bees that specialize on *Cucurbita*, but substantial overlap in the most
118 common bacterial OTUs. We also find that few *Acalymma* beetles are colonized by detectable
119 *Erwinia tracheiphila*, and that OTUs that are the closest match to *E. tracheiphila* are a minor
120 component of the beetle vector's gut bacterial community. Together, these results suggest the
121 shared *Cucurbita* host plants are likely a strong factor influencing microbial community
122 composition of bees and beetles that have co-evolved with these hosts.
123
124 **Methods**
125 *The study system*

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126 Current taxonomic assignments recognize 14 closely related *Cucurbita* species (Kates et
127 al. 2017). All are fast-growing vines, and many wild species are common throughout the New
128 World tropics and subtropics (Kates et al. 2017, Castellanos-Morales et al. 2018). All *Cucurbita*
129 produce large, yellow, sweetly scented flowers that only open a single morning from sunrise
130 until midday, and then dehisce. Flowers release a blend of volatile organic compounds that act as
131 long distance host location cues for co-evolved pollinators and herbivores. *Cucurbita* also
132 produce a group of oxygenated tetracyclic tri-terpenes called 'cucurbitacins' that are among the
133 most bitter compounds known, with broad-spectrum toxicity against almost all insect and
134 mammalian herbivores (David and Vallance 1955, Fraenkel 1959, Whitaker and Bemis 1964,
135 Chambliss and Jones 1966, Shang et al. 2014, Zhou et al. 2016). For Diabroticite leaf beetle
136 herbivores (Coleoptera: Chrysomelidae: Luperini: Diabroticina), cucurbitacins are arrestants and
137 feeding stimulants. All *Acalymma* are specialists on *Cucurbita* in all life stages, while *Diabrotica*
138 and *Ceratoma* spp. only visit *Cucurbita* pharmacophagically as adults to sequester cucurbitacins
139 (Metcalf et al. 1980, Tallamy and Krischik 1989, Dinan et al. 1997, Tallamy et al. 1997, Tallamy
140 1998). *Acalymma* larvae feed underground on *Cucurbita* roots, and adults feed on leaves, flowers
141 and fruits (Barber 1946, Munroe and Smith 1980, Tallamy 1999, Eben and Espinosa 2013,
142 Shapiro and Mauck 2018).

143 While insect herbivory has driven the evolution of *Cucurbita* functional traits over their
144 millions of years of co-evolutionary history, humans have shaped the more recent evolution of
145 many *Cucurbita* species. *Cucurbita pepo* was the first plant domesticated for agriculture in the
146 Americas, and *Cucurbita moschata* was the second (Smith 1993, Smith 1997, Piperno and
147 Stothert 2003, Smith 2006). As the glaciers retreated during the Holocene around 5,000 years
148 ago, cultivated *Cucurbita* were the first crop plant introduced into temperate Eastern North

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America by humans for agriculture from a second independent domestication of *Cucurbita pepo* in the Mississippi River Valley (Petersen and Sidell 1996, Monaghan et al. 2006, Smith 2006). It is likely that movement of domesticated *Cucurbita pepo* varieties into temperate Eastern North America had profound – but still largely unknown – impacts on the biotic community that had co-evolved with *Cucurbita* for millions of years. *Eucera (P.) pruinosa* (López-Uribe et al. 2016) was the only *Cucurbita* specialist pollinator species, and *Acalymma vittatum* the only obligate herbivore species that were able to colonize the new temperate geographic areas where humans introduced *Cucurbita* cultivars (Shapiro et al. 2018b). In the temperate Eastern United States, *Acalymma vittatum* can transmit the virulent bacterial pathogen, *Erwinia tracheiphila.* This pathogen does not occur anywhere else in the world outside of this limited geographic area, and inferences from *E. tracheiphila* genomics studies suggest it was human-mediated changes to cucurbit agro-ecosystems that recently drove its emergence in this region (Shapiro et al. 2015, Shapiro et al. 2016, Andrade-Domínguez et al. 2018, Shapiro et al. 2018a, Shapiro et al. 2018b).

Insect collection:

Insects were field collected from flowers of healthy (*ie,* not infected with *Erwinia tracheiphila*) wild and cultivated *Cucurbita* spp. host plants, and all insects per location came from the same field or wild plant population (Table 1). Insects were killed in 95% ethanol and stored at -80C. Beetles were identified to species by LRS, and bees were identified to species by MMLU.

DNA extraction and sequencing:

To degrade surface DNA, ethanol-preserved insects were immersed in 10% CoveragePlus for 30sec and then rinsed in molecular grade water for 10s. After surface DNA degradation, the

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whole insect was pre-ground with a bleach treated plastic pestle, and homogenized with a bead beater at the highest settings for 1 min. DNA was extracted from ground whole insects using Zymo Microbiomics (Tustin, CA) extraction kit following manufacturer's instructions. The extracted DNA in each sample was quantified with Qubit Broad Spectrum Kit (Thermofisher), and the concentration in all samples was standardized to 10 ng/µl to be used as PCR template. Each DNA sample was amplified in triplicate using the bacterial 16S primers F515 (5'-GTGCCAGCMGCCGCGGTAA-3') and R806 (5'-GGACTACHVGGGTWTCTAAT- 3'), specific for the V4 region of 16S rRNA. In addition to the region specific for 16S rRNA hybridization, these oligonucleotides contain adapter, primer pad and linker sequences for Illumina (Supplemental Table 5). One unique reverse primer was used for each sample, which also contained a distinctive 12 nucleotide GoLay barcode (Supplemental Table 5) (Caporaso et al. 2011). Each 25 µl PCR reaction contained 0.25 µl of Q5 High Fidelity polymerase (New England Biolabs), 0.5 µl of 10 mM dNTPs, 500 nM of each primer and 5 µl of 5X Q5 reaction buffer. The PCR cycle program was: denaturation at 98°C for 30 s, then 35 cycles of 98°C for 10 s, 60°C for 30 s, and 72°C for 20 s; finally, 72 □C for 2 min. The PCR products were checked via agarose gel electrophoresis for the correct size PCR product as a visual quality control. The three triplicate PCR products per sample were then pooled, gel purified using a Gel Purification Kit (Zymo Research, Tustin CA), and quantified using Qubit Broad Range kit (Thermofisher). Equal quantities of the pooled triplicates from each sample were then combined. This final pool containing all libraries was prepared for Illumina MiSeq sequencing by diluting to 4 nM and then denatured by mixing 1:1 with 0.2 N NaOH, for a final concentration of 2 nM of DNA and 0.1 N NaOH. The pooled libraries were then mixed with 1 volume of 2 nM denatured PhiX Sequencing Control (Illumina). Three primers were used for sequencing: read 1 of 251 cycles (5'-

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TATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3'), read 2 for 251 cycles (5'-AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3') and index (5'-ATTAGAWACCCBDGTAGTCCGGCTGACTGACT-3'). The MiSeq kit V2 for 500 cycles (Illumina) was used for sequencing, following the manufacturer's instructions.

Data pre-processing and statistical analyses

All parameters for all pre-processing steps in Qiime and all statistical analyses in R are available in the qiime.txt file at https://github.com/lshapiro31/cucurbit.insect.microbiome.

Qiime 1.8 was used for intial data processing, to demultiplex raw sequencing data and join paired reads (Caporaso et al. 2010). Chimeric sequences were identified *de novo* and removed using usearch 6.1 (Edgar 2010b). PyNast (Caporaso et al. 2009) was used to align the representative 16S sequences, and Usearch (Edgar 2010b) was used to cluster unique *de novo* OTUs at 97% similarity (Edgar 2010a). Taxonomy was assigned with Uclust (Edgar 2010b) using the greengenes database as the reference (DeSantis et al. 2006, McDonald et al. 2012). The nucleotide sequences of the most prevalent and abundant OTUs were used as a query in the BLASTN (Altschul et al. 1990) web interface to assess whether the taxonomy assignments from greengenes is accurate. When BLASTN provided a more accurate taxonomic assignment, that assignment was added in a separate column to the OTUs in the taxonomy files (Supplemental Tables 1, 2, 3, 4).

All statistical analyses were carried out in the R statistical computing environment (Team 2015) following the general pipelines established in phyloseq (McMurdie and Holmes 2013). OTUs that had less than five total reads were removed. One *Acalymma* sample (#SampleID 153; Supplemental Table 4) had only 2 total reads and was excluded from all analyses due to low

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sequencing coverage. OTUs that were assigned as chloroplast or mitochondria were filtered out of the OTU table, and *Wolbachia* OTUs were filtered to a new biom table to be analyzed separately. Phyloseq was used to rarefy the number of all reads per sample to 1,000, and these rarefied sample sums were used to quantify Shannon and Simpson α-diversity indices. Eight additional beetles (#SampleID 259, 280, 271, 187, 239, 175, 247, 151; Supplemental Table 4) had fewer than 1000 reads and were removed from only the α-diversity quantification. These eight individuals were retained in all other analyses. For analyses other than α-diversity, read counts were normalized to the total number of reads obtained for each individual sample (McMurdie and Holmes 2014). To calculate ß-diversity, only the top 30 most abundant OTUs for the three sub-groups of samples were used (*Acalymma* collected from different states; *Acalymma vittatum* collected within a season; and *Eucera (P.) pruinosa*). Distance matrices were calculated using unweighted Unifrac, and a PcoA of these distances were constructed using Phyloseq and custom scripts (McMurdie and Holmes 2013). A PERMANOVA adonis test on the unweighted Unifrac distance matrix was implemented in vegan (Dixon 2003). All plots were created with phyloseq (McMurdie and Holmes 2013) and ggplot2 (Wickham 2016).

Results

Sequencing Summary

In the United States, *Eucera (P.) pruinosa* samples were collected from cultivated *Cucurbita* plants in Pennsylvania and California. *Acalymma vittatum* and *A. trivittatum* were collected from cultivated plants in New Mexico, Arizona, Alabama, Iowa, Kentucky, Massachusetts and Vermont in the United States. *Acalymma trivittatum* were also collected from wild *Cucurbita* plants in Queretero, Mexico. At each location, all insects were sampled from the same

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agricultural field, or same wild plant population. In total, bacterial communities of 59 *Acalymma* beetles and 11 *Eucera pruinosa* bee individuals were sequenced (Figure 1a and 1b, Table 1). Sequencing the V3-V4 region of the 16S rRNA gene resulted in an average of 13,696 reads per sample (when grouped at 97% similarity, and after filtering out reads belonging to OTUs assigned to chloroplast, mitochondria and *Wolbachia*). There were 1,326 unique *de novo* OTUs overall, which were classified into 17 unique phyla and 149 unique families.

Acalymma vittatum *and* A. trivittatum *microbiome composition and geographic variation*

There were a total of 1,179 unique *de novo* OTUs present in the 59 sequenced *Acalymma* leaf beetle samples, and an average of 148 OTUs in each individual beetle. There are 16 OTUs that each comprise at least 1% of the total bacterial abundance, and collectively these 16 OTUs account for 83% of the total bacterial abundance across all the *Acalymma* individuals analyzed (Supplemental Table 1). These 16 OTUs belong to Gammaproteobacteria (50%; 8 of 16), Betaproteobacteria (12.5%; 2 of 16), Lactobacillales (12.5%; 2 of 16), Bacteroidetes (12.5%; 2 of 16) and Actinobacteria (6.25%; 1 of 16).

The taxonomic distribution of the 1,163 remaining rare OTUs (that are each present as less than 1% of the overall bacterial abundance) is similar to the taxonomic distribution of the 16 most common OTUs. Most of the rare OTUs are from Proteobacteria (44%; 514 out of 1163), followed by OTUs from Firmicutes (26%; 304 out of 1163), Actinobacteria (18.5%; 215 out of 1163), Chloroflexi (3.4%; 39 out of 1163) and Bacteroidetes (2.9%; 34 out of 1163). Other rare, low abundance OTUs are assigned to Acidobacteria, Verrucomicrobia, Plantomycetes, Mollicutes and several Archeal phyla (Funaro et al. 2011, Abdul Rahman et al. 2015, Alves et al. 2016). There was moderate statistical support for differences in the α-diversity (number and

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264 evenness) of OTUs in beetles collected in the different geographic locations (Kruskal-Wallis $\chi^2 =$
265 11.9, df = 6, $P = 0.06$), but there is no clear geographic factor underlying these differences
266 (Figure 2). The bacterial community composition of beetles collected in the different states is
267 qualitatively different overall (adonis PERMANOVA on unweighted UniFrac distances of the 30
268 most abundant OTUs; df = 6, $P \leq 0.01$). Beetles collected from Iowa (USA) have the most
269 homogeneous communities, while beetles collected from Arizona (USA) and Querétaro (MX) –
270 both locations in the ancestral native range of *A. vittatum* – have the most variable composition.
271
272 *Within-season variation of* Acalymma vittatum *bacterial communities*
273 To quantify how seasonality affects the composition of beetle gut bacterial communities, adult
274 *Acalymma vittatum* were collected from cultivated *Cucurbita* plants in Cambridge,
275 Massachusetts (USA) soon after emerging from diapuase in the spring (June 7, 2016), from the
276 second generation in the middle of the growing season (July 12, 2016), and from the diapausing
277 generation towards the end of the growing season (August 14, 2016). There is high variability in
278 α-diversity in individual *A. vittatum* collected throughout a single season, and no time point had
279 significantly higher or lower α-diversity than the others (Kruskal-Wallis $\chi^2 = 0.61$, df = 2, $P =$
280 0.74) (Figure 3). However, the composition of beetle bacterial communities does significantly
281 change over the course of the growing season (adonis PERMANOVA on unweighted UniFrac
282 distances of the 30 most abundant OTUs df = 2, $P \leq 0.01$). Beetles collected soon after emerging
283 from underground winter diapause (June 7, 2016) have higher abundances of Actinomycetales
284 and Cytophagales – which are thought to predominantly be soil-dwelling species – than the other
285 two time points. Beetles collected in the middle of the season (July 12, 2016), when many
286 *Cucurbita* cultivars are flowering (and beetles are likely consuming protein-rich pollen and

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287 sugar-rich nectar (Samuelson 1994, Sasu et al. 2010a, Shapiro et al. 2012)) have the highest
288 abundance of Lactobacillales. Bacterial communities of beetles collected on August 14 are
289 dominated by OTUs assigned to Enterobacteriales and Pseudomonadales, which are likely
290 derived from foliar tissue.
291
292 *Prevalence of* Erwinia tracheiphila *in* Acalymma vittatum *and* A. trivittatum *beetles*
293 *Erwinia tracheiphila* cannot be differentiated from closely related *Erwinia* and *Pantoea* strains
294 with certainty from the sequences of V3-V4 rRNA gene fragments. To be conservative about
295 estimating *E. tracheiphila* prevalence and abundance, all OTUs that were assigned to
296 Enterobacteriaceae, and their taxonomic assignments were manually curated. Eight OTUs had at
297 least a 98% sequence similarity to *E. tracheiphila* as the first or second BLASTN (Altschul et al.
298 1990) hit (using the NCBI rRNA reference database), and the prevalence and abundance of these
299 OTUs were combined and all considered as putative *E. tracheiphila* (Supplemental File 3). Even
300 using this permissive criteria that almost certainly over-estimates *E. tracheiphila* presence, only
301 33 out of the 59 *Acalymma* beetles harbor any of these putative *E. tracheiphila* OTUs (Table 2).
302 When *E. tracheiphila* OTUs are detected in an individual beetle, they never sum to more than
303 1% of the total bacterial abundance within that insect (Table 2). Surprisingly, *E. tracheiphila*
304 OTUs are detected not only in *Acalymma* beetles collected in temperate Easterns regions where
305 this pathogen causes annual epidemics, but was also detected in a number of beetles collected
306 from Tucson, AZ, USA and Querétero, MX – areas far from where *E. tracheiphila* is known to
307 occur. This suggests there may be *Erwinia* strains or species closely related to *Erwinia*
308 *tracheiphila* that may be common, non-pathogenic commensals of wild *Cucurbita* plants.
309

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Eucera (P.) pruinosa *microbiome composition*

Overall, there is a total of 312 distinct *de novo* OTUs present in at least one of the 11 sequenced *Eucera (P.) pruinosa* samples (Table 1, Supplemental Table 2). There is an average of 93 OTUs in each individual bee collected in California, while there is an average of only 30 OTUs in each bee collected in Pennsylvania. There are 14 OTUs that comprise at least 1% of the total bacterial abundance, and these 14 OTUs collectively sum to 89.6% of the total bacterial abundance in the *Eucera* bee samples. Of the 14 common OTUs, 6 are assigned to Lactobacillales (Bacilli), 5 are assigned to Enterobacteriales and Pseudomonadales (Gammaproteobacteria), 1 is assigned to Actinobacteria and 1 to Entoplasmatales (Mollicutes). The most abundant OTU in *E. (P.) pruinosa* (*de novo* 415107) is assigned to *Acinetobacter* (Gammaproteobacteriaceae), a genus with many species commonly associated with floral nectar (Álvarez-Pérez et al. 2013, McFrederick and Rehan 2016). The Lactobacillales OTUs are likely involved in breakdown of pollen, and/or fermentation of nectar carbohydrates (McFrederick et al. 2012, McFrederick et al. 2018, Vuong and McFrederick 2019). Pollen is very high in fructose, and one of the 14 common OTUs is assigned to *Fructobacillus* (*de novo* 382787), which may also function to breakdown sugar in pollen and nectar (Endo and Salminen 2013). The same *Fructobacillus* OTU is occasionally present in *Acalymma,* but at extremely low abundance Supplemental Table 1). One of the 14 common *E. (P.) pruinosa* OTUs is assigned to *Spiroplasma* sp. nr. *apis* (Entoplasmatales) (*de novo* 410890), and is common in bees collected in both CA and PA. Some Entoplasmatales species are ancient commensals of insects (Funaro et al. 2011), while other species are emerging as pathogens of wild and managed bees (Ravoet et al. 2014). Of the 299 rare OTUs that comprise the remaining 11% of bacterial abundance, most OTUs are assigned to Gammaproteobacteria (43%; 129 out of 299), followed by Lactobacillales (37%; 111 out of

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299), Actinobacteria (6%; 19 out of 299) and Entomoplasmatales (3.7%; 11 out of 299) (Supplemental Table 2). *E. (P.) pruinosa* collected in California have marginally higher average α-diversity (Kruskal-Walls $\chi^2 = 1.75$, df = 1, $P = 0.18$) (Figure 4), and the bacterial communities from bees sampled in CA vs. PA are marginally qualitatively different (adonis PERMANOVA on unweighted UniFrac distances of the 30 most abundant OTUs df = 2, $P = 0.09$).

Comparison of microbial communities in Acalymma *and* Eucera (P.) pruinosa

Despite the high total number of *de novo* bacterial OTUs, six of the top 7 most abundant OTUs in *Acalymma* beetles are also among the 14 most abundant OTUs in *Eucera* bees (Supplemental Table 1, Supplemental Table 2, (Figure 5) (Herrera et al. 2009, Fridman et al. 2012). As expected, the taxonomic assignments of the OTUs found in *E. pruinosa* reflect microbes that are likely well-adapted to aid in digestion of the floral food sources. Surprisingly, many of these same common OTUs are found in *Acalymma*, suggesting that floral pollen and nectar may be an unappreciated nutritional source for these beetles, which are generally thought of as leaf herbivores due to the extensive foliar economic damage they inflict (Baker and Hurd Jr 1968, Samuelson 1994, Shapiro et al. 2012). A *Klebsiella* spp. OTU (*de novo* 423191) is the most abundant overall in *Acalymma* beetles, and the second most abundant in bees. Two Lactobacillales OTUs (*de novo* 432933 and *de novo* 222965), an Enterobacteriaeae (*de novo* 264045), an *Acinetobacter* (*de novo* 213309), and an Actinomycetales (*de novo* 211172) are also among the most common OTUs in both bees and beetles (Supplemental Table 4). This suggests these insects are exposed to these same OTUs through their shared floral food sources, and that these common OTUs may perform similar digestive functions related to breakdown of sugars (and perhaps proteins and fats) present in *Cucurbita* pollen and nectar.

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356

357 *Detection of* Wolbachia *in* Acalymma

358 Ten percent of *Acalymma vittatum* (6 out of 59 individuals) were infected with *Wolbachia.* This
359 is consistent with a previous study that found a 5% *Wolbachia* infection rate of field-collected *A.*
360 *vittatum* (Clark et al. 2001). All *Wolbachia*-positive beetles were collected from temperate
361 Eastern North America (Massachusetts, Kentucky and Vermont) (Supplemental Figure 1). No
362 *Acalymma* collected outside of the *E. tracheiphila* endemic area carried *Wolbachia,* and none of
363 the *E. (P.) pruinosa* were positive for *Wolbachia*. The presence of *Wolbachia* in few beetles
364 collected from within their newly expanded geographic range in temperate Eastern North
365 America may be due to a recent shift of this reproductive parasite into *A. vittatum* as a host
366 species. A better understanding of the effects of harboring *Wolbachia* on *A. vittatum*
367 reproduction and population dynamics may be useful for developing sustainable vector control
368 strategies (Clark et al. 2001, Kajtoch and Kotásková 2018).

369

370 **Discussion**

371 Here, we find that field-collected *Acalymma vittatum* and *trivittatum* beetles, and to a lesser
372 extent *Eucera (P.) pruinosa* bees are exposed to diverse bacteria during normal foraging. Squash
373 bees and beetles have co-evolved to specialize on *Cucurbita*, and are both attracted over long
374 distances to the large, yellow, highly scented flowers that secrete substantial quantities of sugary
375 nectar, and produce large amounts of protein rich pollen (Metcalf and Lampman 1991, Mena
376 Granero et al. 2005, Shapiro et al. 2012). *Cucurbita* flowers, when present, are a foraging site
377 that likely also serves as a shared source of microbial exposure for both *Acalymma* and *Eucera*
378 (*Peponapis*) (Baker and Hurd Jr 1968, Samuelson 1994, Salzman et al. 2018). Consumption of

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379 floral resources with the same nutrient and carbohydrate composition by both beetles and bees
380 also likely drives the need for microbial associates that have the same metabolic functions for
381 plant matter digestion.

382 The trend towards higher α-diversity in bees from California may be an artifact from the
383 low sample sizes of bees sequenced in this study. Alternatively, this trend may reflect that in
384 their native range, pollinators are exposed to a higher diversity of microbes while foraging on
385 multiple co-occuring species of *Cucurbita,* and on both wild and cultivated genotypes.
386 Populations of cultivated crops that are grown in homogeneous, human-managed landscapes
387 have reduced genetic diversity compared to populations of wild relatives, and may also host
388 microbial communities with lower diversity – measured as either 16S OTU richness, or total
389 physiological potential of the microbial community. The susceptibility of crop plants grown in
390 homogeneous, human managed agro-ecosystems to invasion by novel microbial pathogens is a
391 well-documented and continuing risk (McDonald and Stukenbrock 2016, Shapiro et al. 2016,
392 Shapiro et al. 2018b). How these homogeneous landscapes potentially also affect pollinator
393 health through alterations in microbial ecology, perhaps by creating landscapes with lower
394 microbial functional diversity, or increasing opportunities where wild and managed pollinators
395 can exchange pathogens, remains unknown (Colla et al. 2006, Burke et al. 2011, Fürst et al.
396 2014, López-Uribe et al. 2016, McDonald and Stukenbrock 2016, Shapiro et al. 2016, Pérez-
397 Jaramillo et al. 2017, Shapiro et al. 2018b, SA et al. 2019, Yu et al. 2019).
398 The high number of rare, low abundance OTUs in *Acalymma* herbivores is consistent with
399 accumulating evidence that the local environment – *ie*, the variable and diverse microbial
400 communities that colonize plants or the surrounding soil – are the most significant source of
401 microbial exposure for most non-social and non-sap feeding insects (Moran 2003, Kwong and

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Moran 2016, Hammer et al. 2017, McFrederick et al. 2017, Hammer et al. 2019, Hannula et al. 2019). The lower average number of OTUs per insect in bees compared to beetles may reflect that bees exclusively feed on *Cucurbita* floral resources, while *Acalymma* beetles are often found feeding on other introduced cucurbit crop plants (most notably muskmelon and cucumbers, both *Cucumis* spp.), consume both foliage and floral structures, and can occasionally be found feeding on pollen from distantly related plant species (Gould 1944). *Acalymma* are highly mobile, and move frequently between foliage and flowers of different host plants and the surrounding soil during normal foraging, feeding and oviposition. The large geographic area from which beetles were sampled may also contribute to the high number of rare OTUs. Overall, the composition and diversity patterns reflect that *Acalymma* beetles are transiently exposed to diverse bacteria that do not persistently colonize them as hosts (Hammer et al. 2017, Hammer et al. 2019).

A diminishingly small proportion of the gut bacterial community in field-collected *A. vittatum* are OTUs that could be *Erwinia tracheiphila.* The low abundance of *E. tracheiphila* OTUs in beetle vectors is consistent with laboratory experiments to quantify *E. tracheiphila* in beeltes, and field studies to quantify transmission rates. In controlled laboratory experiments, populations of *E. tracheiphila* in infective vectors or frass were too low to be detected by standard PCR (Mitchell and Hanks 2009) but can be reliably detected by more sensitive fluorescent probe-based qPCR assays (Shapiro et al. 2014). In field trials conducted at Rock Springs Experimental Station in Central Pennsylvania (USA), almost all *Cucurbita* plants were exposed to *E. tracheiphila* every morning by beetles that gather to feed and mate in flowers (Sasu et al. 2010a). Despite this daily exposure to *E. tracheiphila* deposited by vectors, less than half of the experimental plants developed an active *E. tracheiphila* infection (Sasu et al. 2009, Sasu et al. 2010b). Genotypes of *Cucurbita* that produce more flowers, and more volatiles per

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425 flower – and therefore attract more foraging beetle vectors – are also associated with a
426 significantly higher rate of *E. tracheiphila* infection in *Cucurbita pepo* (Sasu et al. 2009, Shapiro
427 et al. 2012). This suggests that individual vectors deposit few *E. tracheiphila* cells during each
428 feeding exposure, and that cumulative exposure to many infective vectors is more important than
429 exposure to a single vector (Yao 1996).

430 The composition, function and variation of microbial communities that associate with
431 most wild, unmanaged insect species is almost completely unknown. This study is the first step
432 towards understanding factors underlying variation in the composition of bacterial communities
433 that associate with the ecologically and economically important insect species that have co-
434 evolved with *Cucurbita*. These results provide an important baseline for guiding future
435 experimental studies to understand factors driving variation in bacterial community composition
436 and functions. A more comprehensive understanding of which microbial strains and communities
437 colonize *Eucera* and *Acalymma* across the diverse geographic they inhabit, and empirically
438 testing the functions of individual isolates and more complex communities for the host insects
439 will be important for developing effective vector controls and protecting pollinator health.

440

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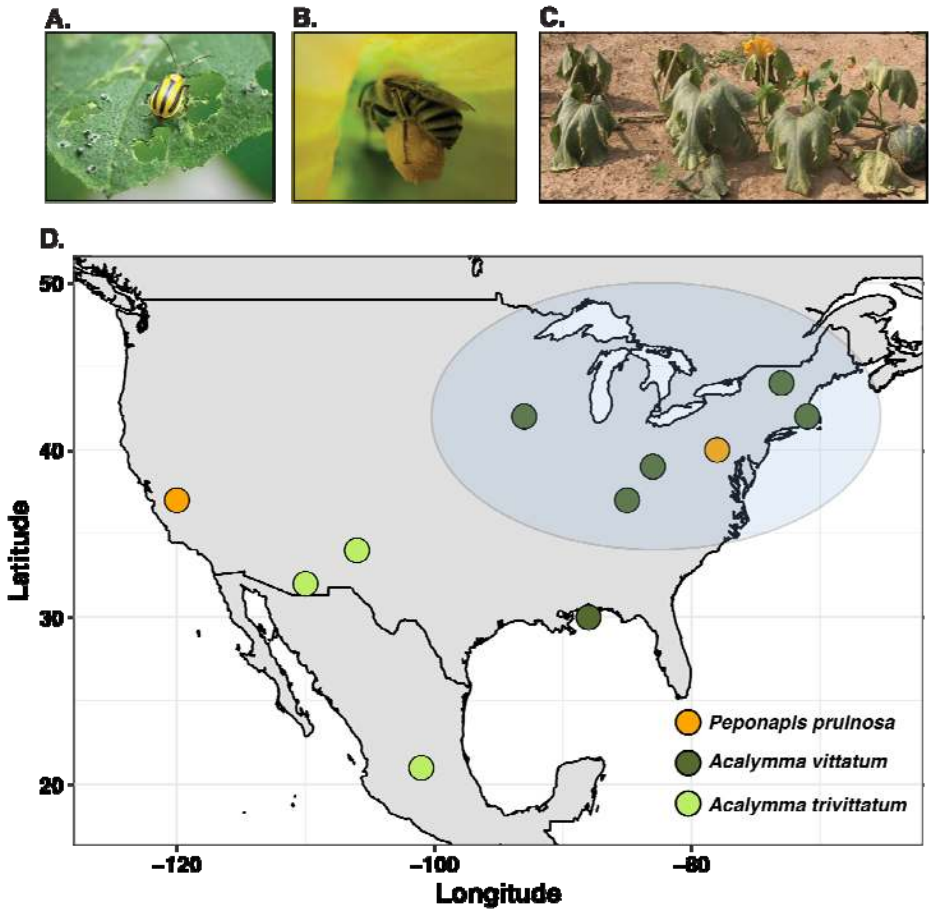
454

455 **Figures**
456



457
458 **Figure 1**: **The study system.**
459 **A)** The Eastern striped cucumber beetle, *Acalymma vittatum* resting on a *Cucurbita pepo* leaf
460 with heavy herbivory damage. This species is the most important herbivore of cucurbits in

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temperate Eastern North America, and is the predominant vector driving *Erwinia tracheiphila* transmission dynamics

B) The Eastern squash bee, *Eucera (Peponapis) pruinosa,* gathering pollen and nectar from a male *Cucurbita pepo* flower

C) A vine of a wilting, *Erwinia tracheiphila* infected wild gourd, *Cucurbita pepo* ssp. *texana.*

D) The locations where bees and beetles sequenced in this study were collected. The blue oval approximates the region where *Erwinia tracheiphila* is an annual epidemic

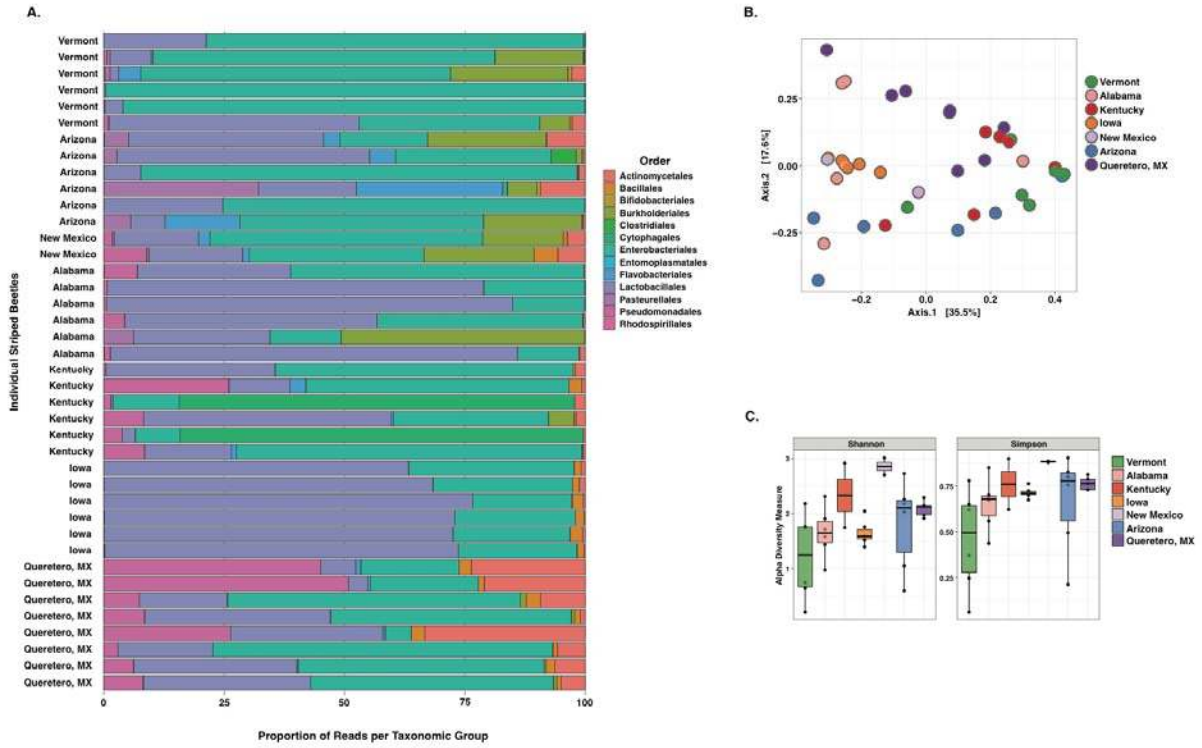

Figure 2: **Summary of bacterial community composition of *Acalymma vittatum* and *trivittatum***

A) Bacterial community composition of individual *Acalymma vittatum* and *trivittatum* beetles. Each horizontal bar is the bacterial community composition in an individual beetle. The collection location of each sample is labeled on the Y-axis. Community composition is displayed by combining OTUs within each order, and the counts in each insect were normalized to the total number of reads in that individual and displayed as a percentage of the total community.

B) Principle coordinates analysis (PCoA) of unweighted Unifrac distances, with each dot representing an individual insect. Insects are colored according to collection location.

C) Shannon and Simpson α-diversity indices for the bacterial communities of *Acalymma vittatum* and *trivittatum* collected from different geographic locations (corresponding to Table 1 and Figure 1D). The number of reads per insect was rarefied to an even depth of 1000. Black dots are individual insects, and boxplots show the median and the quartile variation.

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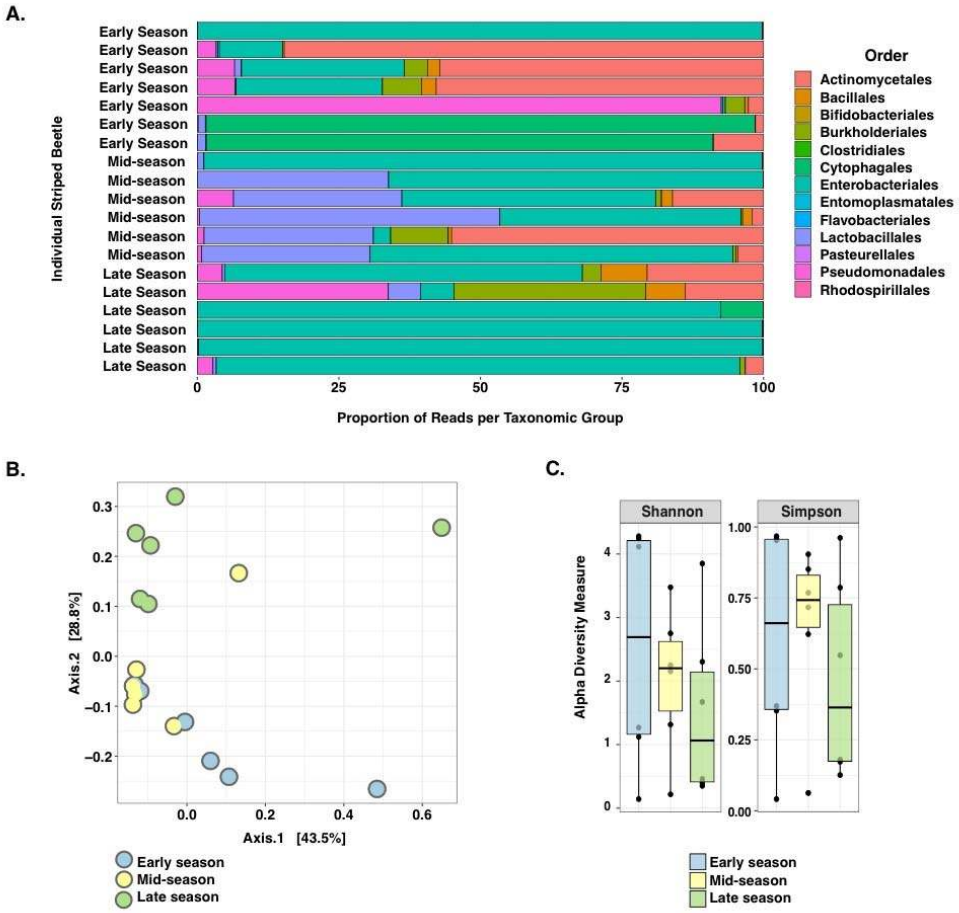

Figure 3: **Seasonal variation of microbial communities from the same population of *Acalymma vittatum* in a Massachusetts.**

A) Bacterial community composition of individual *Acalymma vittatum* beetles collected in Cambridge, Massachusetts. Beetles were collected early in the season as adults emerged from winter diapause and cucurbits had just being planted (June 7 2016); in the middle of the season when commercial summer squash are blooming (July 12 2016); and at the end of the season as the second generation was nearing diapause (August 2016). The collection group each insect belongs to is labeled on the Y-axis. Community composition is displayed by combining OTUs within each order, and the counts in each insect were normalized to the total number of reads in that individual and displayed as a percentage of the total community.

B) Principle coordinates analysis (PCoA) of unweighted Unifrac distances, with each dot representing an individual insect. Insects are colored according to date of collection.

C) Shannon and Simpson α-diversity indices for the gut bacterial communities of *Acalymma vittatum* collected at three time points in a single growing season. The number of reads per insect was rarefied to an even depth of 1000. Black dots are individual insects, and boxplots show the median and the quartile variation.

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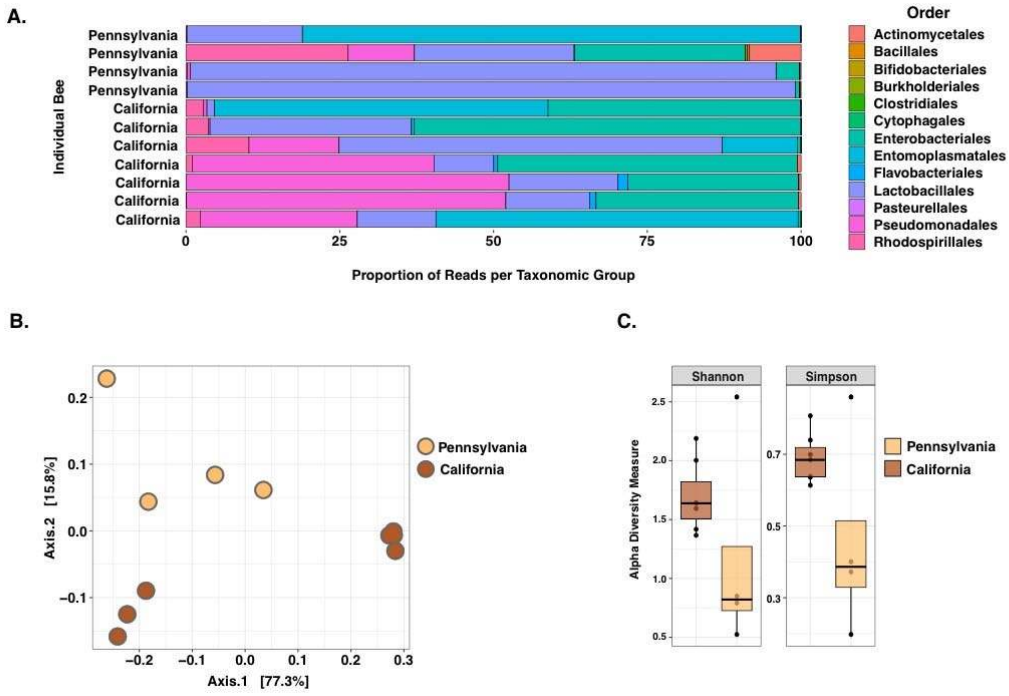

Figure 4: **Geographic variation in *Eucera (Peponapis) pruinosa* bacterial communities.** **A)** Bacterial community composition of individual *Eucera (P.) pruinosa* bees collected in California or Pennsylvania. The collection location of each sample is labeled on the Y-axis. Community composition is displayed by combining OTUs within each order, and the counts in each insect were normalized to the total number of reads in that individual and displayed as a percentage of the total community. **B)** Principle coordinates analysis (PCoA) of unweighted Unifrac distances, with each dot representing an individual insect. Insects are colored according to collection location. **C)** Shannon and Simpson α-diversity indices for the gut bacterial communities of *Eucera (P.) pruinosa* collected in California vs. Pennsylvania. The number of reads per insect was rarefied to an even depth of 1000. Black dots are individual insects, and boxplots show the median and the quartile variation.

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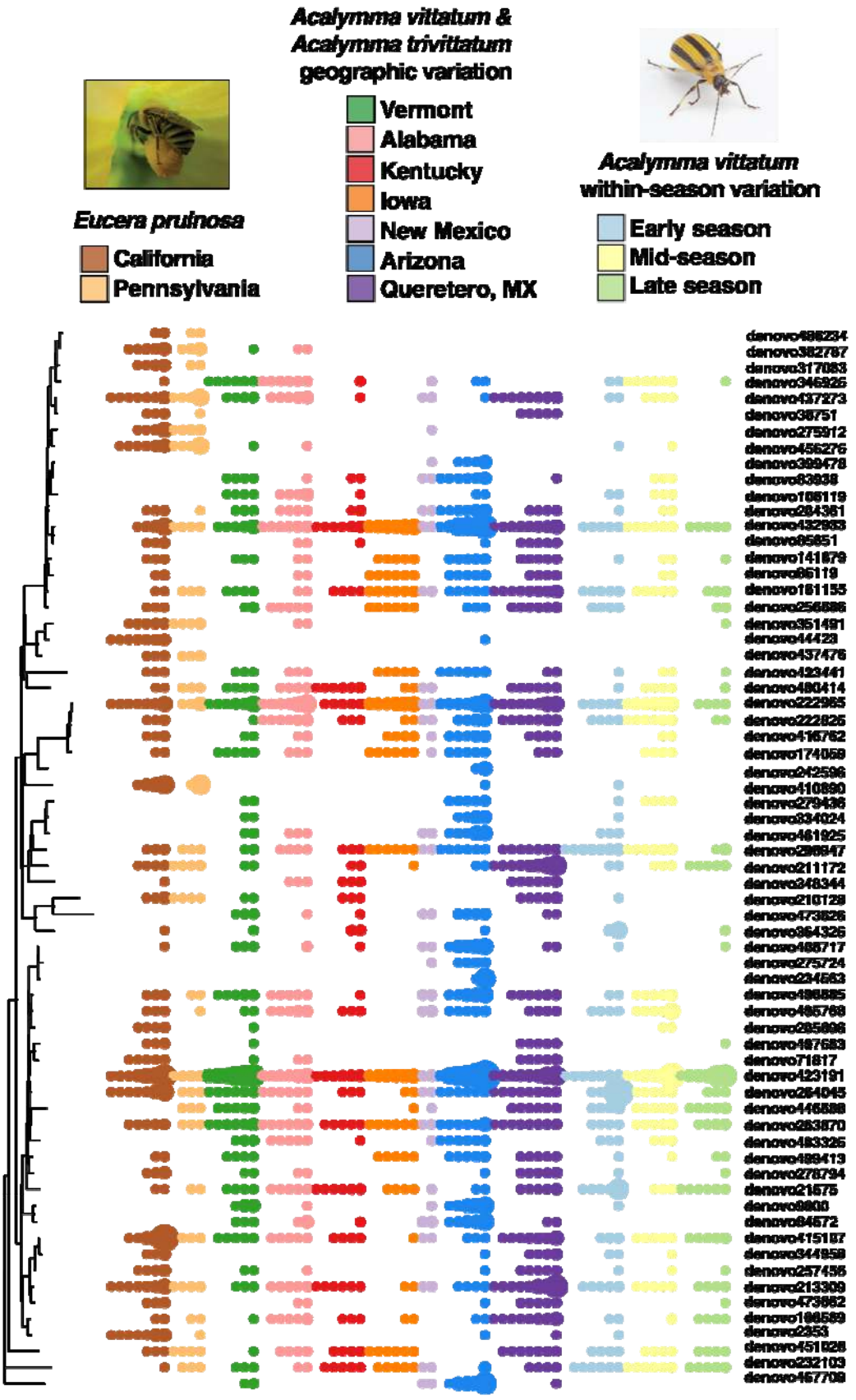

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Figure 5: Prevalence and abundance of the most common bacterial OTUs in *Acalymma* beetles and *Eucera* (*Peponapis*) bees.
The tree shows the phylogenetic distance relationships of the 50 most abundant OTUs in all *Acalymma* beetles and the 40 most abundant OTUs in the *Eucera (P.) pruinosa* bees. Each tip of the tree is an individual OTU, and corresponds to taxonomic assignments provided in Supplemental Table 4 Each colored dot represents the occurrence of that OTU in an individual insect, and the size of the dot corresponds to the relative abundance of that OTU in that individual.

Supplemental Figure 1: *Wolbachia* occurrence in *Acalymma* beetles.

Table 1: Collection location, date, and host plant for all insects sequenced in this study

Insect Species	Collection Location	Host Plant	Number of Insects Sequenced	Collection Date	Lattitude	Longitude
Acalymma vittatum	Christian Herter Community Garden, Watertown, Massachusetts	Cultivars of *Cucurbita* spp. in a diverse community garden	6	June 7, 2016	42.366338	-71.134525
Acalymma vittatum	Christian Herter Community Garden, Watertown, Massachusetts	Cultivars of *Cucurbita* spp. in a diverse community garden	7	July 12, 2016	42.366338	-71.134525
Acalymma vittatum	Christian Herter Community Garden, Watertown, Massachusetts	Cultivars of *Cucurbita* spp. in a diverse community garden	6	August 14, 2016	42.366338	-71.134525
Acalymma vittatum	University of Vermont Horticultural Farm, Burlington, Vermont	*Cucurbita pepo* (Zucchini)	6	August 8, 2015	44.430052	-73.204011
Acalymma vittatum	Iowa State University Horticultural Research Station, Ames Iowa	Cultivated *Cucurbita* spp. and *Cucumis* spp. cultivars	6	August 18, 2016	42.107812	-93.589034
Acalymma vittatum	Gulf Coast Research and Extension Center, Mobile, Alabama	Cultivars of *Cucurbita* spp.	6	June 3, 2015	30.547286	-87.88255
Acalymma vittatum	University of Kentucky Horticultural Research Farm, Lexington, Kentucky	Acorn squash plot (*Cucurbita pepo*)	6	August 9, 2016	37.976154	-84.533393
Acalymma trivittatum	New Mexico	Wild roadside *Cucurbita foetidissima*	2	July 2, 2015	32.5225	-108.730833
Acalymma trivittatum	University of Arizona Pima Outreach Center, Tucson, Arizona	Cultivars of *Cucurbita* spp. and *Cucumis sativus* in demonstration garden beds	6	July 7, 2015	32.282077	-110.94240
Acalymma trivittatum	Quertaro, Mexico	Wild *Cucurbita foetidissima* on roadside	8	July 28, 2016	20.6889	-100.326
Peponapis pruinosa	California	Flowers of cultivated *Cucurbita*	7	August 2, 2010	38.7678	-122.0379

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Peponapis pruinosa	Pennsylvania	Flowers of cultivated *Cucurbita*	4	July 29, 2011	39.9461	-77.3018

528

Table 2: Percentage of the total bacterial community in each individual insect that is *Erwinia tracheiphila*

Individual Sample ID	Location	*Erwinia tracheiphila* geographic zone	Total Number of Bacterial Reads (top 20 OTUs)	*Total Number of *Reads in* OTUs near *Erwinia tracheiphila*	Percentage of total reads putatively *Erwinia tracheiphila*
6	Massachusetts (early)	Yes	1544	0	0
203	Massachusetts (early)	Yes	387	0	0
215	Massachusetts (early)	Yes	13763	1	0.0073
239	Massachusetts (early)	Yes	623	0	0
251	Massachusetts (early)	Yes	7488	0	0
263	Massachusetts (early)	Yes	43316	18	0.0416
275	Massachusetts (early)	Yes	247	0	0
106	Massachusetts (mid-season)	Yes	10217	0	0
118	Massachusetts (mid-season)	Yes	2972	4	0.1346
155	Massachusetts (mid-season)	Yes	2990	2	0.0669
167	Massachusetts (mid-season)	Yes	1448	1	0.0691
179	Massachusetts (mid-season)	Yes	6280	1	0.0159
191	Massachusetts (mid-season)	Yes	28553	4	0.0140
90	Massachusetts (late-season)	Yes	732	0	0
105	Massachusetts (late-season)	Yes	1160	5	0.4310
117	Massachusetts (late-season)	Yes	19665	59	0.3000
129	Massachusetts (late-season)	Yes	14516	59	0.4064
141	Massachusetts (late-season)	Yes	1734	0	0
165	Massachusetts (late-season)	Yes	2765	13	0.4702
101	Vermont	Yes	5760	2	0.0347
138	Vermont	Yes	22983	14	0.0609
150	Vermont	Yes	7344	58	0.7898
162	Vermont	Yes	6830	0	0
174	Vermont	Yes	12054	27	0.2240
186	Vermont	Yes	17541	6	0.0342
260	Alabama	No	1041	0	0
248	Alabama	No	2638	0	0
236	Alabama	No	2518	0	0
224	Alabama	No	4531	0	0
212	Alabama	No	13223	2	0.0151
200	Alabama	No	4651	7	0.1505
268	Kentucky	Yes	1080	1	0.0926
280	Kentucky	Yes	258	0	0
283	Kentucky	Yes	1630	0	0
271	Kentucky	Yes	455	0	0
259	Kentucky	Yes	138	0	0
247	Kentucky	Yes	641	0	0
190	Iowa	Yes	9058	2	0.0221
272	Iowa	Yes	10720	0	0
166	Iowa	Yes	3849	0	0
154	Iowa	Yes	2544	0	0

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142	Iowa	Yes	6035	0	0
130	Iowa	Yes	4014	1	0.0249
257	New_Mexico	No	2525	1	0.0396
269	New_Mexico	No	1856	0	0
216	Arizona	No	27221	1	0.0037
204	Arizona	No	36866	19	0.0515
40	Arizona	No	56257	0	0
28	Arizona	No	48542	5	0.0103
16	Arizona	No	51246	67	0.1307
4	Arizona	No	57625	10	0.0174
187	Querétero MX	No	557	0	0
78	Querétero MX	No	14615	16	0.1095
175	Querétero MX	No	644	2	0.3106
163	Querétero MX	No	14801	15	0.1013
151	Querétero MX	No	719	0	0
139	Querétero MX	No	1469	1	0.0681
127	Querétero MX	No	8398	58	0.6906
66	Querétero MX	No	52178	483	0.9257

*The number of reads in all individual putative *Erwinia tracheiphila* OTUs were summed

Supplemental Table 1: Taxonomic assignments of OTUs, the number of individuals in which that OTU occurs, and the percentage of each OTU in the cumulative community in *Acalymma vittatum* and *trivittatum* beetles.

Supplemental Table 2: Taxonomic assignments of OTUs, the number of individuals in which that OTU occurs, and the percentage of each OTU in the cumulative community in *Eucera (P.) pruinosa* squash bees.

Supplemental Table 3: Manual curation of OTUs assigned to Enterobacteriaceae to assess which are most likely to be *Erwinia tracheiphila.*

Supplemental Table 4: Manual curation of taxonomic assignments of the dominant OTUs in *Acalymma* and *Eucera (Peponapis).*

Supplemental Table 5: Primers, barcodes, and individual #SampleIDs for the insects analyzed in this study.

References

Abdul Rahman, N., D. H. Parks, D. L. Willner, A. L. Engelbrektson, S. K. Goffredi, F. Warnecke, R. H. Scheffrahn, and P. Hugenholtz. 2015. A molecular survey of Australian and North American termite genera indicates that vertical inheritance is the primary force shaping termite gut microbiomes. Microbiome 3: 5.

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552 **Alexandrova, M., B. Cimini, C. Bazzi, E. Carpana, S. Massi, and A. G. Sabatini. 2002.** The role of
553 honeybees in spreading *Erwinia amylovora*. Acta Horticulturae 590: 55-60.
554 **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990.** Basic local alignment
555 search tool. Journal of Molecular Biology 215: 403-410.
556 **Álvarez-Pérez, S., B. Lievens, H. Jacquemyn, and C. M. Herrera. 2013.** *Acinetobacter nectaris*
557 sp. *nov*. and *Acinetobacter boissieri* sp. *nov*., isolated from floral nectar of wild
558 Mediterranean insect-pollinated plants. International Journal of Systematic and
559 Evolutionary Microbiology 63: 1532-1539.
560 **Alves, M., A. Pereira, P. Matos, J. Henriques, C. Vicente, T. Aikawa, K. Hasegawa, F.**
561 **Nascimento, M. Mota, A. Correia, and I. Henriques. 2016.** Bacterial community
562 associated to the pine wilt disease insect vectors *Monochamus galloprovincialis* and
563 *Monochamus alternatus*. Scientific Reports 6: 23908.
564 **Andrade-Domínguez, A., R. Kolter, and L. R. Shapiro. 2018.** Complete genome sequence of
565 EtG, the first phage sequenced from *Erwinia tracheiphila*. genomeA 6.
566 **Baker, H. G., and P. D. Hurd Jr. 1968.** Intrafloral ecology. Annual Review of Entomology 13: 385-
567 414.
568 **Barber, H. 1946.** *Diabrotica* and two new genera (Coleoptera, Chrysomelidae). Proceedings of
569 the Entomological Society of Washington 49: 151-161.
570 **Burke, C., P. Steinberg, D. Rusch, S. Kjelleberg, and T. Thomas. 2011.** Bacterial community
571 assembly based on functional genes rather than species. Proceedings of the National
572 Academy of Sciences 108: 14288-14293.
573 **Caporaso, J. G., K. Bittinger, F. D. Bushman, T. Z. DeSantis, G. L. Andersen, and R. Knight.**
574 **2009.** PyNAST: a flexible tool for aligning sequences to a template alignment.
575 Bioinformatics 26: 266-267.
576 **Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N.**
577 **Fierer, and R. Knight. 2011.** Global patterns of 16S rRNA diversity at a depth of millions
578 of sequences per sample. Proceedings of the National Academy of Sciences 108: 4516-
579 4522.
580 **Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N.**
581 **Fierer, A. G. Pena, J. K. Goodrich, and J. I. Gordon. 2010.** QIIME allows analysis of high-
582 throughput community sequencing data. Nature Methods 7: 335-336.
583 **Castellanos-Morales, G., L. M. Paredes-Torres, N. Gámez, H. S. Hernández-Rosales, G.**
584 **Sánchez-de la Vega, J. Barrera-Redondo, E. Aguirre-Planter, A. Vázquez-Lobo, S.**
585 **Montes-Hernández, and R. Lira-Saade. 2018.** Historical biogeography and phylogeny of
586 *Cucurbita*: Insights from ancestral area reconstruction and niche evolution. Molecular
587 Phylogenetics and Evolution 128: 38-54.
588 **Cellini, A., V. Giacomuzzi, I. Donati, B. Farneti, M. T. Rodriguez-Estrada, S. Savioli, S. Angeli,**
589 **and F. Spinelli. 2019.** Pathogen-induced changes in floral scent may increase honeybee-
590 mediated dispersal of *Erwinia amylovora*. The ISME Journal 13: 847.
591 **Chambliss, O. L., and C. M. Jones. 1966.** Cucurbitacins: specific insect attractants in
592 Cucurbitaceae. Science 153: 1392-1393.
593 **Chung, S. H., C. Rosa, K. Hoover, D. S. Luthe, and G. W. Felton. 2013.** Colorado potato beetle
594 manipulates plant defenses in local and systemic leaves. Plant Signaling & Behavior 8:
595 e27592.

bioRxiv preprint doi: https://doi.org/10.1101/691378; this version posted July 3, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Clark, T. L., L. J. Meinke, S. R. Skoda, and J. E. Foster. 2001. Occurrence of *Wolbachia* in selected Diabroticite (Coleoptera : Chrysomelidae) beetles. Annals of the Entomological Society of America 94: 877-885.

Colla, S. R., M. C. Otterstatter, R. J. Gegear, and J. D. Thomson. 2006. Plight of the bumble bee: pathogen spillover from commercial to wild populations. Biological conservation 129: 461-467.

David, A., and D. Vallance. 1955. Bitter principles of Cucurbitaceae. Journal of Pharmacy and Pharmacology 7: 295-296.

Degnan, P. H., Y. Yu, N. Sisneros, R. A. Wing, and N. A. Moran. 2009. *Hamiltonella defensa*, genome evolution of protective bacterial endosymbiont from pathogenic ancestors. Proceedings of the National Academy of Sciences 106: 9063-9068.

DeSantis, T. Z., P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu, and G. L. Andersen. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology 72: 5069-5072.

Dinan, L., P. Whiting, J.-P. Girault, R. Lafont, S. T. Dhadallia, E. D. Cress, B. Mugat, C. Antoniewski, and J.-A. Lepesant. 1997. Cucurbitacins are insect steroid hormone antagonists acting at the ecdysteroid receptor. Biochemical journal 327: 643-650.

Dixon, P. 2003. VEGAN, a package of R functions for community ecology. Journal of Vegetation Science 14: 927-930.

Eben, A., and A. Espinosa. 2013. Tempo and mode of evolutionary radiation in Diabroticina beetles (genera *Acalymma, Cerotoma*, and *Diabrotica*). Zookeys 332: 207-231.

Edgar, R. 2010a. Usearch. Lawrence Berkeley National Lab.(LBNL), Berkeley, CA (United States).

Edgar, R. C. 2010b. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26: 2460-2461.

Endo, A., and S. Salminen. 2013. Honeybees and beehives are rich sources for fructophilic lactic acid bacteria. Systematic and Applied Microbiology 36: 444-448.

Ferguson, J. E., and R. L. Metcalf. 1985. Cucurbitacins. Journal of Chemical Ecology 11: 311-318.

Ferguson, J. E., R. L. Metcalf, and D. C. Fischer. 1985. Disposition and fate of cucurbitacin B in five species of diabroticites., pp. 1307-1321, J Chem Ecol.

Figueroa, L. L., M. Blinder, C. Grincavitch, A. Jelinek, E. K. Mann, L. A. Merva, L. E. Metz, A. Y. Zhao, R. E. Irwin, and S. H. McArt. 2019. Bee pathogen transmission dynamics: deposition, persistence and acquisition on flowers. Proceedings of the Royal Society B 286: 20190603.

Fleischer, S. J., D. de Mackiewicz, F. E. Gildow, and F. L. Lukezic. 1999. Serological estimates of the seasonal dynamics of *Erwinia tracheiphila* in *Acalymma vittata* (Coleoptera: Chrysomelidae). Environmental Entomology 28: 470-476.

Fraenkel, G. S. 1959. The raison d'etre of secondary plant substances Science 129: 1466-1470.

Fridman, S., I. Izhaki, Y. Gerchman, and M. Halpern. 2012. Bacterial communities in floral nectar. Environmental Microbiology Reports 4: 97-104.

Funaro, C. F., D. J. C. Kronauer, C. S. Moreau, B. Goldman-Huertas, N. E. Pierce, and J. A. Russell. 2011. Army ants harbor a host-specific clade of Entomoplasmatales bacteria. Applied and Environmental Microbiology 77: 346-350.

bioRxiv preprint doi: https://doi.org/10.1101/691378; this version posted July 3, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Fürst, M., D. P. McMahon, J. Osborne, R. Paxton, and M. Brown. 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature 506: 364.

Gould, G. E. 1944. The biology and control of the striped cucumber beetle. Bulletin. Purdue University Experimental Station.

Hammer, T. J., J. G. Sanders, and N. Fierer. 2019. Not all animals need a microbiome. FEMS microbiology letters.

Hammer, T. J., D. H. Janzen, W. Hallwachs, S. P. Jaffe, and N. Fierer. 2017. Caterpillars lack a resident gut microbiome. Proceedings of the National Academy of Sciences 114: 9641-9646.

Hannula, S. E., F. Zhu, R. Heinen, and T. M. Bezemer. 2019. Foliar-feeding insects acquire microbiomes from the soil rather than the host plant. Nature Communications 10: 1254.

Herrera, C. M., C. de Vega, A. Canto, and M. I. Pozo. 2009. Yeasts in floral nectar: a quantitative survey. Annals of Botany 103: 1415-1423.

Hurd, P. D., and E. G. Linsley. 1964. The squash and gourd Bees - genera *Peponapis* (Robertson) and *Xenoglossa* (Smith) inhabiting America north of Mexico (Hymenoptera: Apoidea), University of Calif.

Hurd, P. D., and E. G. Linsley. 1967. South American squash and gourd bees of the genus Peponapis (Hymenoptera: Apoidea). Annals of the Entomological Society of America 60: 647-661.

Hurd, P. D., EG Linsley, and T. Whitaker. 1971. Squash and gourd bees (*Peponapis, Xenoglossa*) and the origin of the cultivated *Cucurbita*. Evolution 25: 218.

Hurd, P. D., E. G. Linsley, and A. E. Michelbacher. 1974. Ecology of the squash and gourd bee, *Peponapis pruinosa*, on cultivated cucurbits in California (Hymenoptera: Apoidea), Smithsonian Institution Press.

Kajtoch, Ł., and N. Kotásková. 2018. Current state of knowledge on *Wolbachia* infection among Coleoptera: a systematic review. PeerJ 6: e4471.

Kates, H. R., P. S. Soltis, and D. E. Soltis. 2017. Evolutionary and domestication history of *Cucurbita* (pumpkin and squash) species inferred from 44 nuclear loci. Molecular Phylogenetics and Evolution 111: 98-109.

Kistler, L., L. A. Newsom, T. M. Ryan, A. C. Clarke, B. D. Smith, and G. H. Perry. 2015. Gourds and squashes (*Cucurbita* spp.) adapted to megafaunal extinction and ecological anachronism through domestication. Proceedings of the National Academy of Sciences 112: 15107-15112.

Kwong, W. K., and N. A. Moran. 2016. Gut microbial communities of social bees. Nature Reviews Microbiology 14: 374.

López-Uribe, M. M., J. H. Cane, R. L. Minckley, and B. N. Danforth. 2016. Crop domestication facilitated rapid geographical expansion of a specialist pollinator, the squash bee *Peponapis pruinosa*. Proceedings of the Royal Society B: Biological Sciences 283: 20160443.

López-Villavicencio, M., O. Jonot, A. Coantic, M. E. Hood, J. Enjalbert, and T. Giraud. 2007. Multiple infections by the anther smut pathogen are frequent and involve related strains. PLoS Pathogens 3: e176.

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Mason, C. J., A. G. Jones, and G. W. Felton. 2018. Co-option of microbial associates by insects and their impact on plant–folivore interactions. Plant, Cell & Environment 42: 1078-1086.

Mauck, K. E., Q. Chesnais, and L. R. Shapiro. 2018. Evolutionary determinants of host and vector manipulation by plant viruses. Advances in Virus Research 101: 189-250.

McArt, S. H., H. Koch, R. E. Irwin, and L. S. Adler. 2014. Arranging the bouquet of disease: floral traits and the transmission of plant and animal pathogens. Ecology Letters 17: 624-636.

McDonald, B. A., and E. H. Stukenbrock. 2016. Rapid emergence of pathogens in agro-ecosystems: global threats to agricultural sustainability and food security. Philosophical transactions - Royal Society. Biological sciences 371: 20160026.

McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight, and P. Hugenholtz. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME Journal 6: 610.

McFrederick, Q. S., and S. M. Rehan. 2016. Characterization of pollen and bacterial community composition in brood provisions of a small carpenter bee. Molecular Ecology 25: 2302-2311.

McFrederick, Q. S., H. Q. Vuong, and J. A. Rothman. 2018. *Lactobacillus micheneri* sp. nov., *Lactobacillus timberlakei* sp. nov. and *Lactobacillus quenuiae* sp. nov., lactic acid bacteria isolated from wild bees and flowers. International Journal of Systematic and Evolutionary Microbiology 68: 1879-1884.

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

McFrederick, Q. S., J. M. Thomas, J. L. Neff, H. Q. Vuong, K. A. Russell, A. R. Hale, and U. G. Mueller. 2017. Flowers and wild megachilid bees share microbes. Microbial ecology 73: 188-200.

McMurdie, P., and S. Holmes. 2014. Waste not, want not: why rarefying microbiome data is inadmissible PLoS Computational Biology 10: e1003531.

McMurdie, P. J., and S. Holmes. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8: e61217.

Mena Granero, A., F. J. Egea Gonzalez, J. M. Guerra Sanz, and J. L. Martínez Vidal. 2005. Analysis of biogenic volatile organic compounds in zucchini flowers: identification of scent sources. Journal of Chemical Ecology 31: 2309-2322.

Metcalf, R., and R. Lampman. 1989. The chemical ecology of Diabroticites and Cucurbitaceae. Experientia 45: 240-247.

Metcalf, R. L., and R. L. Lampman. 1991. Evolution of diabroticite rootworm beetle (Chrysomelidae) receptors for *Cucurbita* blossom volatiles. Proceedings of the National Academy of Sciences 88: 1869-1872.

Metcalf, R. L., R. A. Metcalf, and A. M. Rhodes. 1980. Cucurbitacins as kairomones for diabroticite beetles. Proceedings of the National Academy of Sciences 77: 3769-3772.

Mitchell, R. F., and L. M. Hanks. 2009. Insect frass as a pathway for transmission of bacterial wilt of cucurbits. Environmental Entomology 38: 395.

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Monaghan, G. W., W. A. Lovis, and K. C. Egan-Bruhy. 2006. Earliest *Cucurbita* from the Great Lakes, Northern USA. Quaternary Research 65: 216-222.

Moran, N. A. 2003. Tracing the evolution of gene loss in obligate bacterial symbionts. Current Opinion in Microbiology 6: 512-518.

Munroe, D. D., and R. F. Smith. 1980. A revision of the systematics of *Acalymma* sensu stricto Barber (Coleoptera: Chrysomelidae) from North America including Mexico Memoirs of the Entomological Society of Canada 112: 1-92.

Oliver, K. M., J. Campos, N. A. Moran, and M. S. Hunter. 2008. Population dynamics of defensive symbionts in aphids. Proceedings of the Royal Society B: Biological Sciences 275: 293-299.

Pérez-Jaramillo, J. E., V. J. Carrión, M. Bosse, L. F. Ferrão, M. de Hollander, A. A. Garcia, C. A. Ramírez, R. Mendes, and J. M. Raaijmakers. 2017. Linking rhizosphere microbiome composition of wild and domesticated *Phaseolus vulgaris* to genotypic and root phenotypic traits. The ISME Journal 11: 2244.

Petersen, J. B., and N. A. Sidell. 1996. Mid-Holocene evidence of *Cucurbita* sp. from central Maine. American Antiquity 61: 685-698.

Piperno, D. R., and K. E. Stothert. 2003. Phytolith evidence for early Holocene *Cucurbita* domestication in southwest Ecuador. Science 299: 1054-1057.

Raguso, R. A., and B. A. Roy. 1998. 'Floral' scent production by *Puccinia* rust fungi that mimic flowers. Molecular Ecology 7: 1127-1136.

Rand, F. V., and E. M. A. Enlows. 1916. Transmission and control of bacterial wilt of cucurbits. Journal of Agricultural Research 6: 7-434.

Rand, F. V., and E. M. A. Enlows. 1920. Bacterial wilt of cucurbits, US Dept. of Agriculture.

Rand, F. V., and L. C. Cash. 1920. Some insect relations of *Bacillus tracheiphilus* Erw. Sm. Phytopathology 10: 133-140.

Ravoet, J., L. De Smet, I. Meeus, G. Smagghe, T. Wenseleers, and D. C. de Graaf. 2014. Widespread occurrence of honey bee pathogens in solitary bees. Journal of Invertebrate Pathology 122: 55-58.

Rering, C. C., J. J. Beck, G. W. Hall, M. M. McCartney, and R. L. Vannette. 2018. Nectar-inhabiting microorganisms influence nectar volatile composition and attractiveness to a generalist pollinator. New Phytologist 220: 750-759.

SA, A., B. PA, B. HF, and B. AK. 2019. RNA virus spillover from managed honeybees (Apis mellifera) to wild bumblebees (Bombus spp.). PLoS One 14: e0217822.

Salzman, S., M. Whitaker, and N. E. Pierce. 2018. Cycad-feeding insects share a core gut microbiome. Biological Journal of the Linnean Society 123: 728-738.

Samuelson, G. A. 1994. Pollen consumption and digestion by leaf beetles, pp. 179-183, Novel aspects of the biology of Chrysomelidae. Springer.

Sasu, M., I. Seidl-Adams, K. Wall, J. Winsor, and A. Stephenson. 2010a. Floral transmission of *Erwinia tracheiphila* by cucumber beetles in a wild *Cucurbita pepo*. Environmental Entomology 39: 140-148.

Sasu, M. A., M. J. Ferrari, and A. G. Stephenson. 2010b. Interrelationships among a virus-resistance transgene, herbivory, and a bacterial disease in a wild *Cucurbita*. International Journal of Plant Sciences 171: 1048-1058.

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Sasu, M. A., M. J. Ferrari, D. Du, J. A. Winsor, and A. G. Stephenson. 2009. Indirect costs of a nontarget pathogen mitigate the direct benefits of a virus-resistant transgene in wild *Cucurbita*. Proceedings of the National Academy of Sciences 106: 19067-19071.

Shang, Y., Y. Ma, Y. Zhou, H. Zhang, L. Duan, H. Chen, J. Zeng, Q. Zhou, S. Wang, and W. Gu. 2014. Biosynthesis, regulation, and domestication of bitterness in cucumber. Science 346: 1084-1088.

Shapiro, L., C. M. De Moraes, A. G. Stephenson, and M. C. Mescher. 2012. Pathogen effects on vegetative and floral odours mediate vector attraction and host exposure in a complex pathosystem. Ecology Letters 15: 1430-1438.

Shapiro, L., I. Seidl-Adams, C. De Moraes, A. Stephenson, and M. Mescher. 2014. Dynamics of short-and long-term association between a bacterial plant pathogen and its arthropod vector. Scientific Reports 4.

Shapiro, L. R., and K. E. Mauck. 2018. Chemically-mediated Interactions among cucurbits, insects and microbes, pp. 55-90. In J. Tabata (ed.), Chemical Ecology of Insects. CRC Press.

Shapiro, L. R., A. Andrade, E. D. Scully, J. Rocha, J. N. Paulson, and R. Kolter. 2018a. Draft genome sequence of an *Erwinia tracheiphila* isolate from an infected muskmelon (*Cucumis melo*). Microbiology Resource Announcements 7: e01058-01018.

Shapiro, L. R., L. Salvaudon, K. E. Mauck, H. Pulido, C. M. De Moraes, A. G. Stephenson, and M. C. Mescher. 2013. Disease interactions in a shared host plant: effects of pre-existing viral infection on cucurbit plant defense responses and resistance to bacterial wilt disease. PLoS One 8: e77393.

Shapiro, L. R., J. N. Paulson, B. J. Arnold, E. D. Scully, O. Zhaxybayeva, N. Pierce, J. Rocha, V. Klepac-Ceraj, K. Holton, and R. Kolter. 2018b. An introduced crop plant is driving diversification of the virulent bacterial pathogen *Erwinia tracheiphila*. mBio 9: e01307-01318.

Shapiro, L. R., E. D. Scully, T. J. Straub, J. Park, A. G. Stephenson, G. A. Beattie, M. L. Gleason, R. Kolter, M. C. Coelho, C. M. D. Moraes, M. C. Mescher, and O. Zhaxybayeva. 2016. Horizontal gene acquisitions, mobile element proliferation, and genome decay in the host-restricted plant pathogen *Erwinia tracheiphila*. Genome Biology and Evolution 8: 649-664.

Shapiro, L. R., E. D. Scully, D. Roberts, T. J. Straub, S. M. Geib, J. Park, A. G. Stephenson, E. S. Rojas, Q. Liu, G. Beattie, M. Gleason, C. M. D. Moraes, M. C. Mescher, S. J. Fleischer, R. Kolter, N. Pierce, and O. Zhaxybayeva. 2015. Draft genome sequence of *Erwinia tracheiphila*, an economically important bacterial pathogen of cucurbits. genomeA 3: e00482-00415.

Smith, B. D. 1993. Rivers of change: essays on early agriculture in eastern North America. JSTOR.

Smith, B. D. 1997. The initial domestication of *Cucurbita pepo* in the Americas 10,000 years ago. Science 276: 932-934.

Smith, B. D. 2006. Eastern North America as an independent center of plant domestication. Proceedings of the National Academy of Sciences 103: 12223-12228.

Smith, E. F. 1920. An introduction to bacterial diseases of plants, W.B. Saunders Company, Philadelphia.

bioRxiv preprint doi: https://doi.org/10.1101/691378; this version posted July 3, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Tallamy, D. W. 1998. Sequestered cucurbitacins and pathogenicity of Metarhizium anisopliae (Moniliales: Moniliaceae) on spotted cucumber beetle eggs and larvae (Coleoptera: Chrysomelidae). Environmental Entomology 27: 366.

Tallamy, D. W. 1999. An alternate route to insect pharmacophagy: the loose receptor hypothesis. Journal of Chemical Ecology 25: 1987.

Tallamy, D. W., and V. A. Krischik. 1989. Variation and function of cucurbitacins in *Cucurbita*: an examination of current hypotheses. The American Naturalist 133: 766-786.

Tallamy, D. W., and P. M. Gorski. 1997. Long-and short-term effect of cucurbitacin consumption on *Acalymma vittatum* (Coleoptera: Chrysomelidae) fitness. Environmental Entomology 26: 672-677.

Tallamy, D. W., J. Stull, N. P. Ehresman, P. M. Gorski, and C. E. Mason. 1997. Cucurbitacins as feeding and oviposition deterrents to insects. Environmental Entomology 26: 678-683.

Team, R. C. 2015. R: A language and environment for statistical computing. R Foundation of Statistical Computing Vienna, Austria.

Vannette, R. L., and T. Fukami. 2016. Nectar microbes can reduce secondary metabolites in nectar and alter effects on nectar consumption by pollinators. Ecology 97: 1410-1419.

Vuong, H. Q., and Q. S. McFrederick. 2019. Comparative genomics of wild bee and flower isolated *Lactobacillus* reveals potential adaptation to the bee host. Genome Biology and Evolution *Accepted Manuscript*.

Wang, J., S. H. Chung, M. Peiffer, C. Rosa, K. Hoover, R. Zeng, and G. W. Felton. 2016. Herbivore oral secreted bacteria trigger distinct defense responses in preferred and non-preferred host plants. Journal of Chemical Ecology 42: 463-474.

Whitaker, T. W., and W. P. Bemis. 1964. Evolution in the genus Cucurbita. Evolution 18: 553-559.

Whitaker, T. W., and W. Bemis. 1975. Origin and evolution of the cultivated *Cucurbita*. Bulletin of the Torrey Botanical Club: 362-368.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis, Springer.

Yang, M., G.-C. Deng, Y.-B. Gong, and S.-Q. Huang. Nectar yeasts enhance the interaction between *Clematis akebioides* and its bumblebee pollinator. Plant Biology 0.

Yao, C., Zehnder, G., Bauske, E., Kloepper, J. 1996. Relationship between cucumber beetle (Coleoptera: Chrysomelidae) density and incidence of bacterial wilt of cucurbits. Journal of Economic Entomology 89: 510-514.

Yu, X., M. F. Polz, and E. J. Alm. 2019. Interactions in self-assembled microbial communities saturate with diversity. The ISME journal: 1.

Zhou, Y., Y. Ma, J. Zeng, L. Duan, X. Xue, H. Wang, T. Lin, Z. Liu, K. Zeng, and Y. Zhong. 2016. Convergence and divergence of bitterness biosynthesis and regulation in Cucurbitaceae. Nature Plants 2: 16183.