

# Metagenomic analysis of host plant-associated microbiomes in the cotton fleahopper, *Pseudatomoscelis seriatus* (Miridae)



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## Abstract

Herbivorous insects can form mutualistic associations with bacterial symbionts that provide ecological advantages and may promote host plant-associated genetic differentiation among populations. The cotton fleahopper, *Pseudatomoscelis seriatus* (Miridae), exhibits genetic differentiation among populations in Texas, USA associated with two host plants: cotton and horsemint. We investigated the potential role of microbial diversity in host plant-associated differentiation by comparing bacterial microbiomes among populations using 16S rDNA pyrosequencing. We detected both geographic and host-plant associated variation in bacterial community diversity and composition. This preliminary study indicates that symbiotic associations of insects might play an important role underlying genetic structure in herbivorous populations.

## Background

- ❖ Cotton fleahopper, *Pseudatomoscelis seriatus* (Miridae), is an important early season cotton pest attacking developing flowers (squares).
- ❖ Barman *et al.* (2012) recently demonstrated the presence of genetically-divergent cotton fleahopper populations feeding on either cotton or horsemint, an alternative wild host, in Texas, USA (see Fig. 1).
- ❖ Herbivorous insects can establish mutualistic associations with bacterial symbionts that provide ecological advantages, such as the ability to use otherwise inadequate host plants.
- ❖ Such mutualistic associations can lead to the formation of genetically-divergent host plant-associated populations of insects.
- ❖ We investigated the potential role of bacterial symbionts in cotton fleahopper genetic divergence by conducting a metagenomic analysis of microbiome variation across host plant-associated populations using bacterial 16S rDNA pyrosequencing.



Figure 1. The cotton fleahopper and two host plants, cotton and horsemint, upon which host plant-associated genetic differentiation exists in Texas, USA (Barman *et al.* 2012).

## Key questions

1. Do cotton fleahopper bacterial communities vary by HOST PLANT?
2. Do cotton fleahopper bacterial communities vary by LOCATION?

## Methods

- ❖ Sampled 10 individuals from both cotton and horsemint at 5 locations across Texas (Fig. 2).
- ❖ Whole genomic DNA from individual insects was isolated using the Qiagen DNeasy® kit.
- ❖ Pooled DNA from 10 insects per plant/site.
- ❖ PCR amplification of the bacterial 16S rDNA gene and subsequent 454 pyrosequencing were performed by the Research and Testing Laboratory (Lubbock, TX).
- ❖ Analyses conducted using QIIME (Caporaso *et al.* 2010) and R (R Development Core Team).

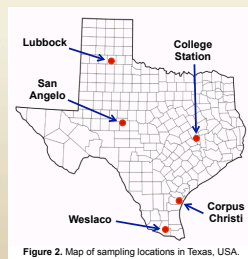


Figure 2. Map of sampling locations in Texas, USA.

## Results

- ❖ We recovered a total of 392,297 bacterial sequences representing 766 Operational Taxonomic Units (OTUs).
- ❖ Rarefaction curves did not reach asymptote, indicating more sequencing is needed to fully capture cotton fleahopper bacterial community species richness (Fig. 3).

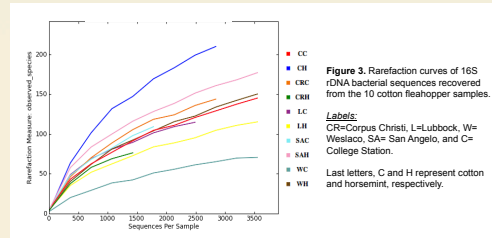


Figure 3. Rarefaction curves of 16S rDNA bacterial sequences recovered from the 10 cotton fleahopper samples. Labels: CR=Corpus Christi, L=Lubbock, W=Weslaco, SA=San Angelo, and C=College Station. Last letters, C and H represent cotton and horsemint, respectively.

- ❖ Overall, the species diversity of fleahopper bacterial communities was higher among insects from cotton compared to horsemint (Fig. 4).

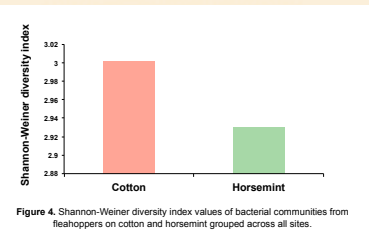


Figure 4. Shannon-Weiner diversity index values of bacterial communities from fleahoppers on cotton and horsemint grouped across all sites.

- ❖ A hierarchical cluster analysis based on abundances of the most commonly encountered taxa failed to reveal any clear pattern of similarity between microbiomes depending upon either host plant or geographic location (Fig. 5).

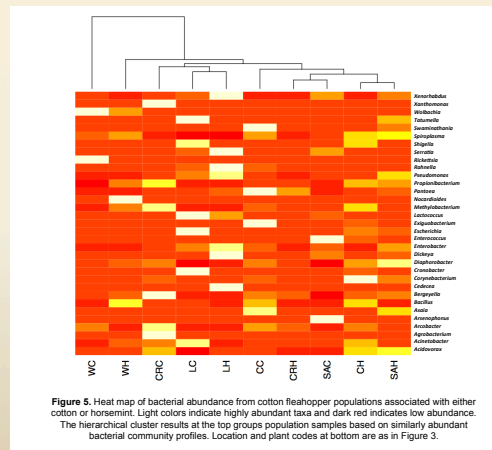


Figure 5. Heat map of bacterial abundance from cotton fleahopper populations associated with either cotton or horsemint. Light colors indicate highly abundant taxa and dark red indicates low abundance. The hierarchical cluster results at the top groups population samples based on similarly abundant bacterial community profiles. Location and plant codes at bottom are as in Figure 3.

- ❖ Although community composition could not be fully explained by either host plant or location, fleahoppers associated with different plants do harbor unique bacteria at specific locations (Table 1). For example, *Wolbachia*, a maternally inherited bacterial symbiont was found only in insects collected at Weslaco, TX (Table 1).

Table 1. Frequency of some of the most commonly occurring bacterial genera sampled from cotton fleahoppers associated with cotton (C) and horsemint (H) at five locations in Texas.

	College Station		Corpus Christi		Weslaco		Lubbock		San Angelo	
	C	H	C	H	C	H	C	H	C	H
<i>Wolbachia</i> sp.	0	0	0	0	12052	3886	0	0	0	0
<i>Xanthomonas</i> sp.	2	3	2700	0	0	0	0	0	0	0
<i>Cedacia neteri</i>	3	2	2	0	1	1	774	0	5	5
<i>Nacardioides</i> sp.	0	3	0	0	0	116	0	0	1	0
<i>Agrobacterium</i> sp.	0	0	176	0	0	0	0	0	0	0

- ❖ Phylogenetic analysis based on our 16S rDNA sequences revealed different *Wolbachia* strains in the cotton and horsemint-associated fleahoppers.
- ❖ *Wolbachia* found in cotton-associated fleahoppers was most closely related to that found in the rice weevil, *Sitophilus oryzae*, whereas the *Wolbachia* in horsemint-associated fleahoppers was more closely related to that in the brown planthopper, *Nilaparvata lugens* (Fig. 6).

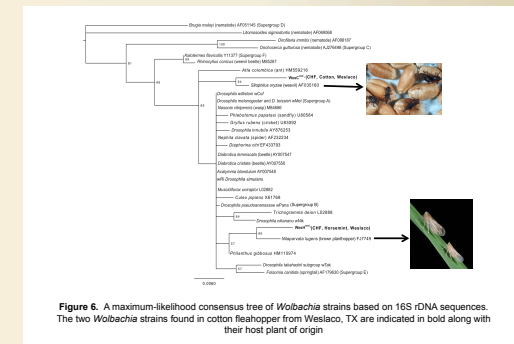


Figure 6. A maximum-likelihood consensus tree of *Wolbachia* strains based on 16S rDNA sequences. The two *Wolbachia* strains found in cotton fleahopper from Weslaco, TX are indicated in bold along with their host plant of origin.

## Conclusions and future work

- ❖ The cotton fleahopper hosts different bacterial communities on cotton and horsemint, but community compositions vary across sites.
- ❖ Different bacterial communities could provide site-specific ecological advantages to fleahoppers experiencing different environmental conditions at our sampling locations.
- ❖ We are currently investigating how environmental conditions affect bacterial communities of fleahoppers on cotton across the cotton belt of U.S.
- ❖ We are also investigating the relationship between microbiomes and host plant use in other sucking insects.

## Acknowledgements

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## References

- Barman, A.K., Parajulee, M.N., Sansone, C.G., Suh, C.P.C., and Medina, R.F. (2012). Geographic pattern of host-associated differentiation in the cotton fleahopper, *Pseudatomoscelis seriatus*. *Entomologia Experimentalis et Applicata* 143, 31-41.
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