



# Environmental and host factors affect bacterial community structure in the mosquito gut

Sofia Bertoloni Meli<sup>1</sup>, Kerri L. Coon<sup>2</sup>, Michael R. Strand<sup>2,3</sup>

<sup>1</sup>Indiana University, Bloomington, IN

<sup>2</sup>Department of Entomology, University of Georgia, Athens, GA

<sup>3</sup>Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA



## Introduction

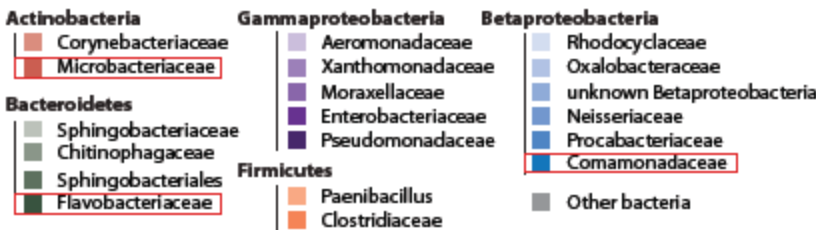
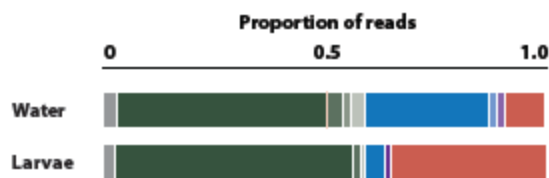
- Insect species host many microbial symbionts.
- Mosquitoes acquire a gut microbiota by ingesting bacteria in their larval aquatic environment.
- These bacteria are necessary for larvae to develop to adulthood (Coon et al. 2014).
- This larval bacterial community persists into adulthood and is important for maintaining mosquito health by aiding in nutrient acquisition and reproduction (Minard et al. 2013).
- By investigating the bacterial communities in three species of mosquitoes, we assessed how environment x species interactions affect community composition.
- We had two main objectives:

1. Characterize the gut bacterial communities that develop in *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* when reared separately.

2. Characterize the gut bacterial communities of these species when reared together.

## Background

Bacteria in the genera *Chryseobacterium*, *Microbacterium*, and *Comamonas* are dominant members of the *A. aegypti* gut community



Bacterial community in conventionally-reared *A. aegypti* larvae and their aquatic environment in the laboratory. Data were generated by high-throughput sequencing of 16S rRNA. Phylum and family names are provided. Each bar indicates the proportion of reads that matched the given bacterial family. Only categories >2% are shown. Shown boxed in red are three dominant families from which bacterial strains were selected to perform experiments with artificially structured communities. From Flavobacteriaceae, *Chryseobacterium*; from Microbacteriaceae, *Microbacterium*; from Comamonadaceae, *Comamonas*. Figure adapted from Coon et al. (2014).

## Methods

Produce bacteria-free (axenic) mosquito larvae

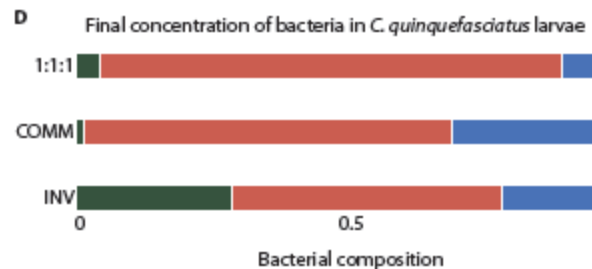
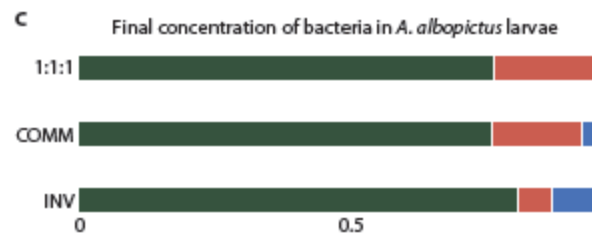
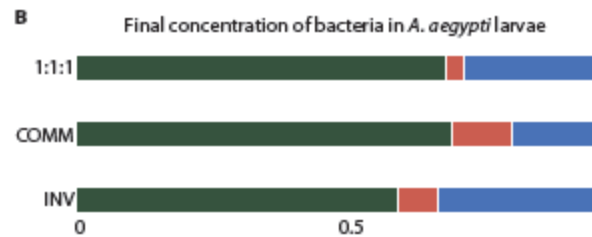
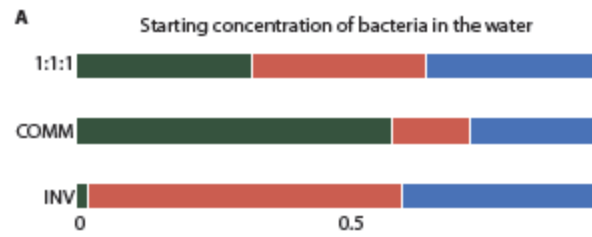
Add axenic larvae to dish containing sterile water and diet

Inoculate with bacterial isolates of interest

Characterize relative abundance of each bacterial isolate in fourth instar larvae

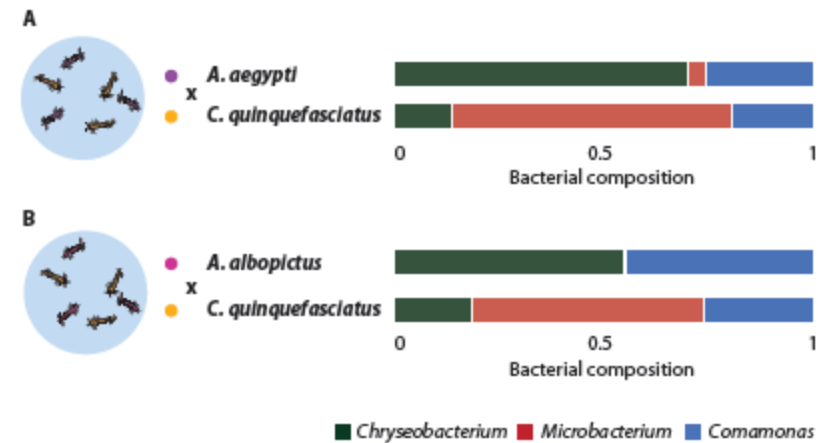
## Results

1. The initial composition of bacteria in the aquatic environment does not affect the final bacterial community in the larval gut



Relative abundance of three dominant community members in water and larvae measured by quantitative PCR (qPCR). Initial bacterial concentrations in the water were determined using a hemocytometer. Mosquito larvae were reared in the presence of three different ratios of community members: a 1:1:1 ratio, a ratio simulating that found in lab-reared *A. aegypti* (COMM), and the inverse (INV) (A). Graphs B, C, and D show the relative abundance of each bacterial isolate after the larvae reached the fourth instar. For each mosquito species, the relative abundance of community members in the water had no effect on the final community structure in the gut ( $p > 0.05$ ). However, the final community in *C. quinquefasciatus* differed from both *A. aegypti* and *A. albopictus* ( $p < 0.0001$ ).

2. Cohabitation with another species of mosquito does not affect the gut bacterial community



Relative abundance of three dominant community members in fourth instar larvae measured by qPCR. Each dish was inoculated with a 1:1:1 mixture of the three bacterial isolates of interest. In experiment A, *A. aegypti* and *C. quinquefasciatus* were reared together and in experiment B, *A. albopictus* and *C. quinquefasciatus* were reared together. The final community in *C. quinquefasciatus* differed from both *A. aegypti* and *A. albopictus* even when reared in the same environment ( $p < 0.0001$ ).

## Conclusions

- Community structure in larvae is robust to changes in the environment.
- Closely related mosquito species have more similar gut communities.
- This indicates that both the environment and the larval host play an important role in the development of the gut community.
- Future studies will focus on identifying physiological factors underlying host determination of gut bacterial communities.

## References

1. Coon KL, Vogel KJ, Brown MR, Strand MR. 2014. Mosquitoes rely on their gut microbiota for development. *Molecular Ecology* 23(11): 2727-2739.
2. Minard G, Mavingui P, Moro CV. 2013. Diversity and function of bacterial microbiota in the mosquito holobiont. *Parasites & Vectors* 6(1): 146-157.

## Acknowledgements

We thank Kip Lacy for preliminary results on *A. aegypti*. This work was sponsored by the NSF Population Biology of Infectious Diseases REU program at the University of Georgia. This material is based upon work supported by the National Science Foundation (Graduate Research Fellowship 038550-04) (KLC) and the National Institutes of Health (R01 A1033108/A106892)(MRS).

