

Using the mosquito gut microbiome
to teach phylogeny reconstruction,
species concepts, and symbioses

Background

- Mosquitoes are important vectors of disease for many vertebrates
- Females feed on vertebrate blood in order to lay eggs
- In doing so, they acquire and then transmit diseases like malaria, Dengue fever, West Nile Virus, etc.

Background

- Blood is an unusual meal
- Many blood sucking insects have a unique microbiota to aid in digesting that meal and to provide necessary nutrients
- E.g., *Wigglesworthia* sp in tsetse

Background

- Mosquitoes, as a group, are the most deadly animals on earth.
- *Anopheles* sp., e.g., transmit between 300 and 500 million cases of malaria every year.
- Understanding their microbiota may reveal ways to control them or manipulate their ability to vector disease, especially **paratransgenesis**.
- Paratransgenesis is the ability to alter the phenotype of an organism by altering its microbiota.

Thinking about the microbiota and symbioses (specific aims)

- To what extent do individual mosquitoes of the same species have the same gut microbiome?
- To what extent do *Anopheles* from different regions share any OTUs in the gut microbiome (combining published data sets)?
- Is there a “core” mosquito microbiome?
- Do these blood-feeding insects share any gut microbiota with other blood feeding species (e.g., sandflies, fleas, kissing bugs, tsetse, leeches, etc)?
- Is it possible to predict the role of particular gut microbiome OTUs?
- Does the microbiome of mosquitoes change with life stage and/or seasonally?

Learning goals

- Discuss the importance of insect vectors of disease for human health. Understand the general life cycle of mosquito vectors.
- Identify organisms at different levels of symbiosis (obligate, facultative, commensual)
- Utilize the correct species concepts for appropriate taxa (e.g., bacteria vs mosquitoes).
- Perform the appropriate method(s) of phylogeny reconstruction (and understand how they work)
- Evaluate microbiome data to detect patterns and suggest paratransgenesis candidate species
- Detect co-evolution

Core concepts and biological literacy

- Evolution (speciation, adaptation, co-evolution)
- Systems (symbiosis)

Core competencies and practices

- Ability to apply the process of science (hypothesis generation and testing)
- Ability to use quantitative reasoning (statistical tests to test hypotheses, phylogeny reconstruction)
- Ability to use modeling and simulation (data sampling algorithms in qiime, understanding that trees are hypotheses)
- Ability to tap into interdisciplinarity (ability to utilize computer programs and program languages, especially R)

Core competencies and practices, part 2

- Ability to communicate and collaborate with other disciplines (making sense of the mosquito microbiome: microbiology, phylogenetics, entomology)
- Ability to understand the relationship of science and society (why mosquitoes are important for human health, why controlling them is essential for human health and the costs of not doing so)

GCAT-SEEK requirements

- Use Illumina MiSeq and 16S V4 pcr data generated during GCAT-seek workshop
- Use other similar data from other species' microbiomes
- No new wet-lab resources required.
Computer resources required (laptop and mainframe, depending on level of analysis required)

Computer program requirements

- Lots of qiime!
- Comparisons of otus from different individuals
- Comparisons of otus from different species of similar taxa (mosquitoes)
- Comparisons of otus from organisms with similar diets, but divergent taxa
- Principle component analysis
- Decisions regarding otu cutoffs
- Phylogenetic reconstruction (this can be web-based)
- Phylogeny viewers (anything that displays newick)

Appropriate classes for this module

- Evolution (jr/sr level lecture)
- Invertebrate Biology (jr/sr level lecture)
- Parasitology or Pathogenic Micro (jr/sr lecture)
- Environmental microbiology (jr/sr lecture)

Assessments

- A problem set on phylogeny reconstruction comparing parsimony and distance. Reconstructing trees by hand on a small data set (5 sequences of 6 nucleotides).
- A problem set on tree topology. What is a tree, rooted vs unrooted trees, tree space, etc. These are done by hand.
- Tree building using sequences from the 16S data set. Probably a subset of the entire data set. Are particular taxa common? MEGA would be appropriate here and it is free.
- Rarefaction analysis to analyze community species richness using the MiSeq data set. How rich is the microbiota in mosquitoes? Compare to humans, to termites, etc.
- PCoA for detecting paratransgenesis candidates using the MiSeq data set and other published data. What are the common universal species for *Anopheles* mosquitoes? For other blood sucking insects?

Lecture topics for class

- Species concepts (biological, morphological, phylogenetic)
- Symbiosis
- The importance of the microbiome on the phenotype of eukaryotes
- Vector biology
- Approaches to phylogeny reconstruction (distance, parsimony, likelihood)
- Technology of metagenomics and nextgen sequencing

Discussion topics for class

- What is a species?
- How do we reconstruct evolutionary history?
- What is the microbiome?
- What are the basic components of the phenotype?
- Can we change the phenotype of an organism by changing its microbiome?

Time line of module

- This is probably a two to three-week module covering phylogeny reconstruction, some vector biology, species concepts, and some quantitative analysis of microbiological communities. This could be spread out throughout a semester, depending on the syllabus and the course
- One week (three lectures) on phylogeny reconstruction
- One lecture on vector biology
- Two lectures on species concepts
- Two lectures on symbioses
- Two to three lectures on next gen sequencing and analysis of data

References

- Caporaso, JG et al. 2010. **QIIME allows analysis of high-throughput community sequencing data.** Nature Methods 7, 335–336. (The software package to perform metagenome analysis. Download from <http://qiime.org/>).
- Ridley, M. 1996. Evolution, 2nd ed. Wiley-Blackwell. (A good reference for phylogeny reconstruction and the organization of phylogenetic trees. Contains exercises for building trees by hand).
- Tamura K. et al. 2011. **MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods.** Mol. Biol Evol. 28, 2731-2739. (Free software for sequence alignment and phylogenetic analysis. <http://megasoftware.net/>)