THE IDENTIFICATION AND CHARACTERIZATION OF BACTERIAL ENDOSYMBIONTS IN THE PLANTHOPPER FAMILY, ISSIDAE (STERNORRHYNCHA: FULGOROIDEA)

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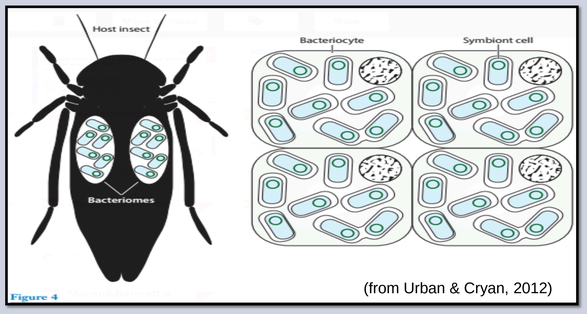
ABSTRACT

Many insects live on nutrient-poor diets; to compensate, they have evolved obligate associations with microorganisms that are transmitted directly between generations. The bacterial associates of some Hemiptera insects have been identified and understood, but several remain unknown. Our objective is to identify the diversity of bacterial endosymbionts in Issidae using modern microscopy and molecular methods, and compare their identities to known symbionts from related insects. To do so, we will extract DNA from insect samples, amplify bacterial symbiont 16S genes, sub-clone PCR products, sequence the products, and determine their phylogenetic relationships to other insect symbionts and free-living bacteria. Our study will contribute to a broader understanding of the diversity and evolution of symbiotic associations in insects.



(from Urban & Cryan, 2012)

INTRODUCTION

Many insects that feed on nutrient-poor diets contain symbiotic microorganisms. In plant-sap-feeding insects these associates are housed within a specialized organ in their abdomen (the bacteriome), and are typically endosymbiotic bacteria. For example, the association of aphids is with the Gammaproteobacteria, *Buchnera aphidicola*. Most of these insects could not have existed without this association: genomic and experimental studies on these symbionts indicate that they produce the nutrients missing in the insects' diets, including essential amino acids and vitamins (Moran & Bennett 2014). Thus, symbiosis can open up nutritionally unbalanced diets as new ecological niches for hosts, leading to evolutionary diversification and ecological expansion. Symbiosis may also accelerate speciation rates, when maternally inherited, obligate symbionts thrust lineages into a peculiar, irreversible co-evolutionary relationship (Bennett & Moran 2015).

A comprehensive understanding of the co-evolutionary dynamics of insect-symbiont relationships can be accomplished only when knowledge of many associations is obtained. However, several plant-sap-feeding Hemiptera remain incompletely explored. For example, recent molecular studies have failed to identify the presence of symbionts in certain Fulgoroidea (planthoppers) (Urban & Cryan 2012), despite that early microscopy studies indicated their presence (Muller 1940a,b). In this study, we aim to identify and characterize the bacterial endosymbionts of Issidae planthoppers. We will determine whether their endosymbionts are typical of other planthoppers, or whether they might represent novel associations.

HYPOTHESIS

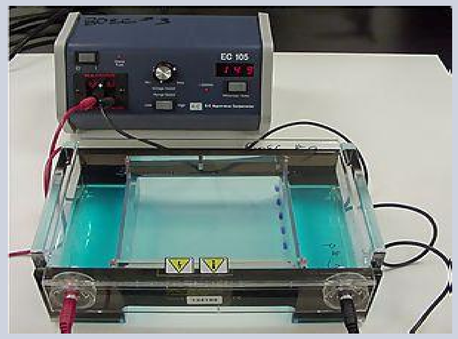
Two symbionts, *Sulcia* and *Vidania*, will be detected in the samples of Issidae using our experimental techniques.

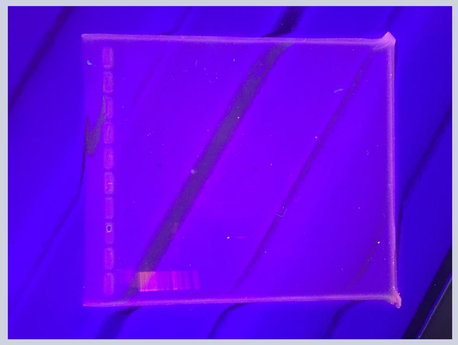
METHODS

The first step is to obtain samples of the target family, Issidae, from field collections or alcohol samples in the von Dohlen Lab.


Thionia bullata (photo by Clyde Sorensen, North Carolina State University)

The next step is to dissect out the bacteriome and amplify the bacterial symbiont genes with polymerase chain reaction (PCR) and general eubacterial primers using a thermocycler.

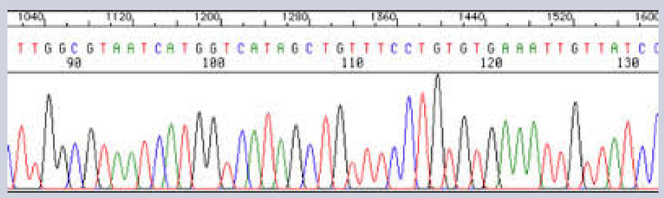




Using gel electrophoresis, the DNA amplified from the PCR is checked for expected product size. From the migration of bands, we can accurately determine the length of the DNA segments by running them on an agarose gel alongside a DNA ladder which has known lengths of DNA.

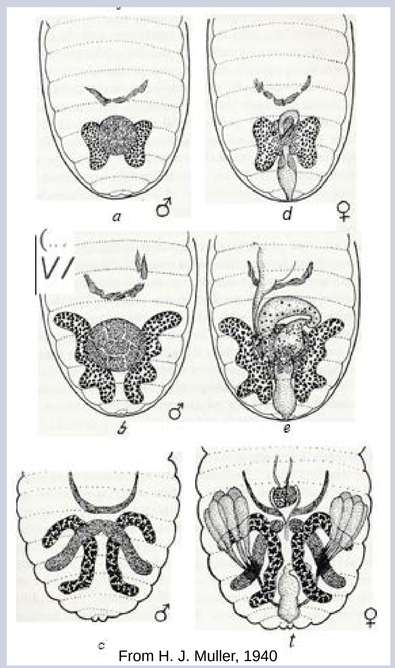
We follow that with sub-cloning the PCR products to separate the different bacterial species.

Using the products from our sub-cloning, we send it off to sequencing.



Sequences are assembled into full-length 16S and compared against GenBank sequences using BLAST to determine closest similarity to known bacteria.

Finally, we assemble a general bacterial 16S alignment including new sequences, to determine their phylogenetic relationships to other insect symbionts and free-living bacteria.



From H. J. Muller, 1940

Postembryonic development of the symbiotic organs: (a, b, c) in the male; (d, e, j) in the female.

Expected Results

We expect to identify several unique 16S sequences, which should represent the projected diversity of the bacterial endosymbionts in Issidae. These symbionts, *Sulcia* and *Vidania,* were not detected in a recent study (Urban & Cryan, 2012), but are expected to be present because these are the typical associates in other planthopper families.

We will be able to determine whether these symbionts are closely related to those in insects of related planthopper families, or whether they represent novel associations.

Future Goals

Once we have identified the bacterial endosymbionts of Issidae samples, we will learn cryosectioning techniques and *in situ* hybridization methods. We will use these methods to localize sequences amplified from putative symbionts to the bacteriome. This will confirm that the sequences we amplified actually came from the symbionts residing in the bacteriome.

ACKNOWLEDGMENTS

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