Evolution of B Vitamin Synthesis Genes in the Bacterial Endosymbiont *Arsenophonus*, found in the aphid *Obtusicauda coweni*

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Introduction

Aphids and the Sagebrush Steppe Ecosystem

Aphids are small phloem sucking insects in the Hemiptera family. They live on a variety of plants and have a restricted nutrient intake due to their plant phloem diet. *Obtusicauda coweni* feed exclusively on the native and widespread *Artemisia tridentata* (Big Sage) of the intermountain west. *A. tridentata* provide crucial habitat for endangered species and other insects. These insects are home to bacterial endosymbionts that can be obligate (primary) or facultative (secondary). Depending on the role of the endosymbiont inside insects, insect physiology and host plant physiology can change in response to the endosymbiont. Our research investigates how endosymbionts impact the evolution of their aphid host *O. coweni* and sagebrush *A. tridentata.*

Obtusicauda (Black Aphids) on *Artemisia*
Peter Bryant www.bugguide.net

Artemisia tridentata (Big Sage)
www.usu.edu

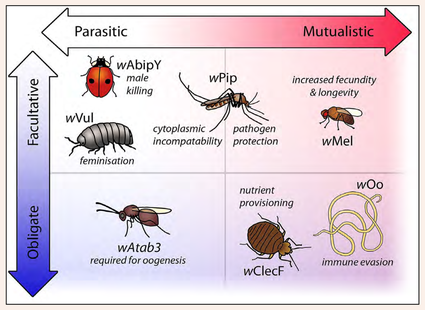


Figure 1. Diagram of phenotypes resulting from *Wolbachia* infection in various insects. Alessandra Christina Gill Alistair C. Darby Benjamin L. Makepeace (2014)

Endosymbiont Survey

Our lab performed a molecular genetic survey of the endosymbionts in a local *O. coweni* population. *Arsenophonus* was the most prevalent endosymbiont, followed by *Wolbachia* and *Rickettsia*. *Arsenophonus* is a gammaproteobacterium known to be a male-killer in aphids. In other insects, *Arsenophonus* is thought to produce B vitamins for the host. **This research investigates the ability of *Arsenophonus* to produce B vitamins for *O. coweni.***

Endosymbionts

Bacterial endosymbionts live inside the host or host cell, most commonly in insects. They have a wide range of relationships to their insect host, including parasitism, mutualism, and commensalism. Mutualistic endosymbionts provide nutrients for the host, and/or protect the host against parasites and heat shock. Parasitic endosymbionts can manipulate the host reproduction to cause male killing, cytoplasmic incompatibility (CI), and parthenogenesis, all of which result in increased infection rates of the endosymbiont.

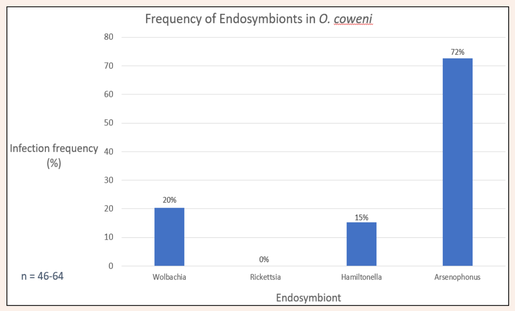
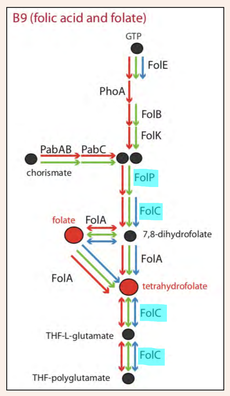
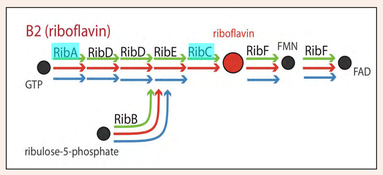


Figure 2. Bar chart depicting the infection frequency of the endosymbionts *Wolbachia*, *Rickettsia*, *Hamiltonella*, and *Arsenophonus* in local aphids (*Obtusicauda coweni*) collected from Sagebrush (*Artemesia tridentata*) using PCR.

B Vitamin Synthesis

B vitamins such as Folate and Biotin are a class of water-soluble vitamins necessary for cellular function such as fatty acid synthesis and respiration. Endosymbionts produce many nutrients, including B vitamins, for their diet-restricted hosts. Each B vitamin synthesis pathway is unique, involving different genes. Endosymbionts can make part of or produce the whole vitamin. Within the insect host, several endosymbionts can collaborate to produce one complete B vitamin.





Supplementary Figure S3. B vitamin and related cofactor biosynthetic pathways based on draft genomes of *Arsenophonus*, *Sodalis* and *Bartonella*. Genes of interest are highlighted in blue. Nováková et al. 2015.

Methods

- DNA extraction of *Obtusicauda coweni* aphids with Qiagen DNeasy®Blood and Tissue Kit (1 population in Caldwell, ID)
- Polymerase Chain Reactions (PCR) using endosymbiont specific primers:
  - RibASG and RibAN primers for RibA gene in *Arsenophonus*
  - RibCSG, and RibCN primers for Rib C gene in *Arsenophonus*
  - BioASG and BioAN primers for Bio A gene in *Arsenophonus*
  - BioDSG and BioDN primers for Bio D gene in *Arsenophonus*
  - FolPSG and FolPN primers for FolP gene in *Arsenophonus*
  - FolCSG and FolCN primers for FolC gene in *Arsenophonus*
- Target gene regions and length ranged from 493 to 780 bp.
- Gel electrophoresis
- Standard sequencing by GENEWIZ
- Sequenced samples were analyzed using Geneious Prime® 2021.2.2.
- BLAST on NCBI to confirm target genes.

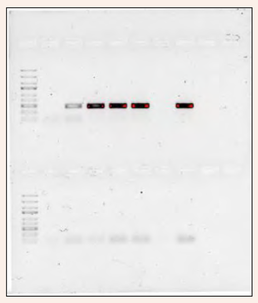


Figure 3. A SYBR Safe stained 1% agarose gel showing DNA fragments produced by PCR amplification. Top row is RibA gene using RibASG primers. Bottom row is RibC gene using RibCN primers. The negative control was sterile distilled water used in place of template DNA.

Results

Primers for the Riboflavin pathway (RibASG), Folate pathway, (FolPSG), and Biotin pathway (BioASG) produced strong bands with *Arsenophonus* positive DNA samples but not in *Arsenophonus* negative DNA samples. The top NCBI BLAST hit for all 3 genes is *Arsenophonus* from *Aphis craccivora*, verifying amplification of the correct target genes.

Primers for B vitamins in Tsetse flies (Nováková et al. 2015) did not produce any bands. All bands were from primers for B vitamins in Whiteflies Santos-Garcia et al. 2018). Primers RibCSG and FolCSG did not produce bands. Primer BioDSG displayed non-specificity (multiple bands) and was not sequenced.

Riboflavin

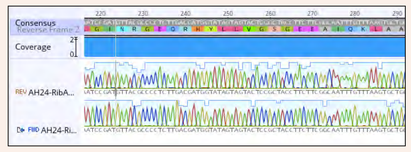


Figure 4. Chromatogram from an aligned RibASG PCR product. Reverse Frame 2 features no stop codons, indicating functional gene.

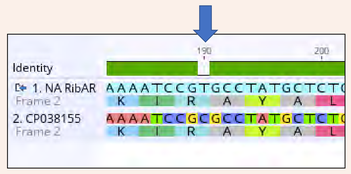


Figure 5. RibASG PCR product sequence compared to Arsenophonus in *Aphis craccivora.* (CP038155), showing silent mutation at 190 bp (arrow). RibASG PCR product had 2 silent mutations and 1 missense mutation.

Folate

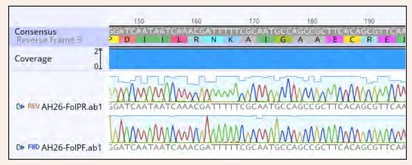


Figure 6. Chromatogram from an aligned FolPSG PCR product. Reverse Frame 3 features no stop codons, indicating functional gene.

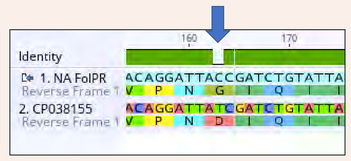


Figure 7. FolPSG PCR product sequence compared to Arsenophonus in *Aphis craccivora.* (CP038155), showing missense mutation at 163 bp (arrow). FolPSG PCR product had 3 missense mutations.

Biotin

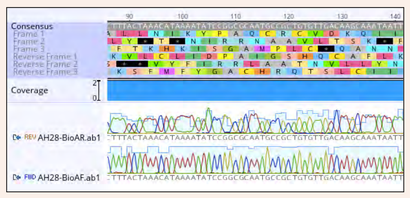


Figure 8. Chromatogram from an aligned BioASG PCR product. No open reading frame was found because of stop codons (black star) indicating nonfunctional gene.

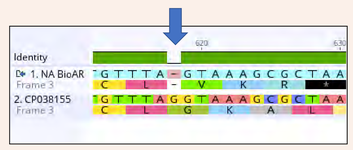


Figure 9. BioASG PCR product sequence compared to Arsenophonus in *Aphis craccivora.* (CP038155), showing deletion at 618 bp (arrow). This deletion was found in 8 aphid samples. BioASG PCR product had 4 silent mutations and 2 missense mutations.

Discussion and Conclusion

Arsenophonus in *O. coweni* aphids possess a functional RibA gene in the Riboflavin synthesis pathway and a functional FolP gene in the Folate synthesis pathway. These genes contained no stop codons in the middle of the gene, however there were missense mutations at different positions that changed the amino acids sequence in the protein. *Arsenophonus* does not appear to have a functional BioA gene in the Biotin synthesis pathway. The BioA gene had a base pair deletion observed in 8 samples and no usable reading frame, rendering the protein nonfunctional. **Overall, *Arsenophonus* contributes to the production of Riboflavin and Folate for its aphid host *O. coweni.* In contrast, Biotin must be acquired from other sources, most likely a different endosymbiont, such as *Wolbachia* or *Hamiltonella.***

Future Studies

1. With this sequence data, we can construct RNA primers and test our samples for the expression of these genes. RNA expression levels would allow us to measure how much of these B vitamins *Arsenophonus* is producing for *O. coweni.*

2. *Arsenophonus* has more genes in the Riboflavin and Folate synthesis pathways that could be tested. Testing for all genes will provide further metabolic information on the role of Arsenophonus in *O. coweni.* FolE from Folate synthesis and RibB, and RibF from Riboflavin synthesis will be our genes of interest.

3. Endosymbionts can spread vertically or horizontally, and both methods have different implications for the host insect and host plant. DNA extraction of plant tissue from sagebrush and a PCR test for *Arsenophonus* will establish the mode of transmission for *Arsenophonus* in *O. coweni.*

4. Florescence In Situ Hybridization (FISH) will allow us to visualize the location and concentration of *Arsenophonus* in the various organs of *O. coweni.* If *Arsenophonus* appears to be obligate in FISH, it means *Arsenophonus* provides essential B vitamins to the insect host.

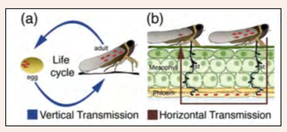


Figure 10. A: representation of vertical transmission of endosymbiont in insect.
B: representation of horizontal transmission.
C: Location of endosymbionts based on relationship to insect and plant. Bressan 2014.

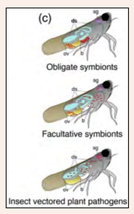
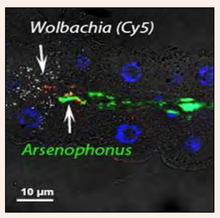


Figure 11. Florescence In Situ Hybridization shows Wolbachia and Arsenophonus inside a louse fly (Nováková et al. 2015).



References

1. Nováková E, Husník F, Šochová E, Hypša V. 2015. *Arsenophonus* and *Sodalis* symbionts in louse flies: an analogy to the *Wigglesworthia* and *Sodalis* system in tsetse flies. *Appl Environ Microbiol* 81:6189–6199.
2. Riegler M, O'Neill SL. (2007). Evolutionary dynamics of insect symbiont associations. *Trends in Ecology & Evolution*, 22(12), 625–627.
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(2018). To B or not to B: comparative genomics suggests *Arsenophonus* as a source of B vitamins in whiteflies. *Front. Microbiol*., 9 p. 2254.

Acknowledgements

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