Presence of Wolbachia in sandfly populations and correlation with pesticide resistance

I. Introduction

Problem: Leishmaniasis is a serious skin disease, affecting 12 million people worldwide, caused by a protozoan called Leishmania and vectored by sand flies. Currently there is no vaccine for humans to prevent the disease. Vector control via insecticides is the primary method for battling the disease, however, many species of sand flies are developing resistance to insecticides currently in use (Kassen and Osman 2007).

Solution: Sand flies can harbor the bacterium Wolbachia, a microorganism which blurs the line between symbiont and parasite. On the one hand, it skews sex ratios in mosquito populations, but on the other hand, it confers certain benefits, specifically, increased insecticide resistance. (Charlat et al. 2003) An estimated 65% of insect species are infected with Wolbachia (Hilgenboecker et al. 2008). Biological control utilizing Wolbachia is emerging as a potential method to stem the spread of flyborne diseases. (Berticato et al. 2002) In closely related flies, such as mosquitoes, insecticide resistance is developed when Wolbachia infection rates increase. The range of impacts of Wolbachia on their hosts may offer a alternative control pathway for sand fly populations where insecticide resistance has developed. A better understanding of Wolbachia can offer insight into how to maintain insecticide effectiveness. Before Wolbachia can be exploited for this purpose, however, basic information about infection rates and titers within populations must be gathered.

II. Methods

Testing Sand fly samples:
For three years, David Denlinger, a graduate student at USU in the Bernhardt laboratory, has been raising colonies of Lutzomyia longipalpis and Phlebotomus papatasi sand flies, to assess susceptibility to insecticides (Denlinger et al. 2015). He has consented to donate live sand flies for screening of Wolbachia. He also has extracted DNA from nine other species that can be tested.

Detecting Wolbachia:
To screen the DNA for Wolbachia, PCR was used to amplify the wsp gene specific to Wolbachia genomes. Using sand flies from the Bernhardt lab and perform DNA extractions according to our own protocols.

Analysis of the DNA:
The specimens were frozen and then crushed for extraction. The extracted DNA was then PCR-amplified for the wsp gene. The product was evaluated on an agarose gel with a DNA ladder to determine successful amplification. Positive PCR products were purified and submitted for sequencing to verify the presence of the wsp gene and determine the particular strain of the Wolbachia present. Because some specimens have already been determined to carry Wolbachia, they have been used as a positive control throughout the screening process. A negative control has been incorporated in order to ensure that contamination doesn’t occur.

Testing insecticide resistance:
The final step will be to use quantitative PCR to amplify the wsp gene. This method will allow me to quantify the numbers of Wolbachia templates in each sample. I can then correlate the levels of Wolbachia infections in individual sand flies to levels of insecticide resistance in the populations from which they were derived.

III. Results

Eleven species have been tested so far. DNA from a total of 76 specimens has been extracted and tested for the wsp gene via PCR. Of those, only 12 from two species have resulted positive for the wsp gene. Detection of Wolbachia infection was greatest by far in Phlebotomus papatasi. The number tested for each species is shown in Table 1. The “Code” column corresponds with the data shown in Figure 2.

IV. Conclusions

Now that Wolbachia has been successfully detected in sand flies via PCR, the next step is to gain secondary confirmation via DNA sequencing. The biggest challenges so far have been to perfect the PCR protocol and perform a DNA extraction that produces high quality results. The PCR protocol has been improved thereby facilitating detection of Wolbachia, however, DNA extractions continue to be a challenge. I will continue working on this project throughout the summer to test a greater number of sand flies using fresh DNA extractions, and begin testing with quantitative PCR. My goal is to publish a report on this project in a scientific journal and share the information with other scientists. This study could provide a foundation for future research into the possibility of implementing Wolbachia as a method for controlling sand flies, thus limiting the spread of a potentially fatal disease.