Infection Rate of Two Parasites Found in *Leprus Wheeleri* Captured in The Northern Chihuahuan Desert

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Abstract

During the summer of 2023, seventy-nine *Leprus wheeleri* (Thomas) grasshoppers were captured in a 5-hectare section of the Northern Chihuahua Desert. Grasshoppers are important to the ecosystem for several reasons. First, they add a significant amount of biomass to a region. Secondly, they enrich the soil by facilitating the breakdown of organic nutrients. And third, they stimulate plant growth. However, they can also be a detriment to agricultural crops when their population numbers exceed the carrying capacity of the region. There are usually checks and balances in nature. Several parasites infect *Leprus wheeleri* populations. Among them are nematodes, mites, and bacteria. Our study examines the percentage of *Leprus wheeleri* captured that were infected with the mite *Eutrombidium locustarum* (Walsh, 1866) and an alpha proteo-bacterium *Wolbachia pipientis* (Hertig, 1936). *Eutrombidium locustarum* is a microparasite and *Wolbachia pipientis* is an endosymbiont, that is sometimes mutualistic with its hosts. *Wolbachia* was identified by targeting the 16S rDNA gene of the small unit of the bacterial ribosome. Insect DNA was identified by targeting the Cytochrome C Oxidase gene. DNA was extracted from the grasshoppers through a 24-step process, then amplified by PCR, and finally identified by running an electrophoresis gel. *Wolbachia* DNA is visible at 438 base pairs (bp), and insect DNA is visible at 709 bp. *Eutrombidium locustarum* was identified by examination under a dissecting microscope. The mites were located on the wings, head, neck, and legs. The mites were also tested for *Wolbachia*. *Eutrombidium locustarum* DNA is also visible at 709 bp.

Keywords: *Leprus wheeleri, Eutrombidium locustarum, Wolbachia pipientis,* Chihuahua Desert.

1. Introduction

The Chihuahua Desert is the largest desert in North America, and home to an array of arid dwelling flora and fauna (National Park Service, 2018). A significant portion of the New Mexico Chihuahua Desert is located in Luna County. The area receives approximately 23.37 centimeters of precipitation per year. The average high temperature is 35.5°C and the average low is 18.3°C (NOAA, 2021). The flora mainly consists of a high percentage of creosote bushes, *Larrea*

tridentate, and a few mesquite trees, *Prosopis glandulosa*, native grasses and several species of the genus *Opuntia,* prickly pear cacti (Dobson, 2012). Orthopterans, grasshoppers, katydids, and crickets, are an important part of the ecosystem (Richman, Lightfoot, Sutherland & Ferguson, 1993). Grasshoppers are an essential part of arid ecosystems. They stimulate plant growth, participate in nutrient recycling, and play an important role in food webs (Capinera, Scott & Walker, 2004). Nutrient recycling is accomplished by grasshoppers breaking down cellulose in to smaller pieces facilitating degradation by soil florae and fauna. They stimulate plant growth by combining acids from their crop and midgut with auxin, a plant hormone (Latchininsky, Sword, Sergeev, Cigliano & Lecoq, 2011; Street & McGuire, 1990). However, when populations grow exponentially, they are devastating enemies of agriculturists. Grasshopper are hosts for several types of parasites which help to keep the population in check. Bacteria, nematodes and mites are among the most prevalent. The nematodes and mites can also be infected with *Wolbachia*. It has been suggested that these parasites could be used as an agent of control for grasshopper populations (Street & McGuire, 1990).

Leprus wheeleri is one of the most common grasshoppers in the Chihuahua Desert. They emerge annually in the spring, and feed mostly on the creosote bushes. *Leprus wheeleri* inhabits the central and northern regions of the desert. Most populations of *Leprus wheeleri* have blue wings but, in Luna County the grasshoppers have a yellow-wing dimorphism. Possible reasons for the yellow-wing dimorphism in *Leprus wheeleri* are thermoregulation and homochromy. In a desert it is logical that absorption of less energy would be beneficial. But, the yellow-wing coloration blends with the yellow-tan desert soil color allowing the grasshoppers protection from predators (Valverde & Schielzeth, 2015).

Wolbachia pipientis is a rickettsia alpha-proteobacteria, that infects arthropods and filarial nematodes. It is known as a major parasite/ mutualist that can alter the reproduction of its hosts. *Wolbachia* causes cytoplasmic incompatibility (CI), male killing, parthenogenesis, and feminization (Werren, 1996). Cytoplasmic incompatibility is the most common method of *Wolbachia's* reproductive manipulation (Dobson, Fox, & Jiggins, 2002; LePage et al., 2017). There are two types of CI, unidirectional and bidirectional. In unidirectional, if the male is infected, but the female is not there will be no offspring. In bidirectional, if the male and female are infected with different strains of *Wolbachia,* there will be no offspring (Stouhamer, Russel, Vavre, & Nunney, 2010). *Wolbachia* facilitates the production of female offspring because it is mainly transferred vertically from the mother (Stouthamer, 2001; Serbus, Casper-Lindley, Landmann, & Sullivan, 2008)). However, horizontal transfer does happen from predator to prey (Werren & Bartos, 2001). Parthenogenesis has only been found in haplodiploid organisms. With insects, males are produced from fertilized eggs and are diploid. Females are the opposite. It is different with arachnids. *Eutrombidium locustarum* mites are arachnids which produce males from unfertilized eggs, then the female mates with her offspring to produce diploid females (Belovsky, Branson, Chase, Barker, & Hammond, 1996). In feminization, a male can be turned into a fully functioning female. Several populations of insects have been completely feminized by *Wolbachia* (Asgharian,

Chang, Mazzoglio, & Negri, 2014; Kageyama, Nishimura, Koshizaki, & Ishikawa, 2002). The effects of *Wolbachia* infestation are complex. If mosquitoes are infected with *Wolbachia,* they cannot transmit diseases such as West Nile Virus, Dengue Fever, (Frentiu, Robinson, Young, McGraw, & O'Neill, 2010) Malaria, and many more. Currently Vietnam, Australia, Saudi Arabia and Columbia are purposely infecting populations of mosquitoes to successfully combat diseases in their countries (Cook & McGraw, 2010; Iturve-Ormaetxe, Walker, & O'Neill, 2011; Slatko, Luck, Dobson, & Foster, 2014). *Wolbachia* has also been known improve the immune systems of its hosts allowing them to resist RNA viruses (Eleftherianos, Atri, Accetta, & Castillo, 2013; Pimentel, Cesar, Martins, & Cogni, 2021) and insecticides (Liu & Guo, 2019). But, increased *Wolbachia* infected populations decrease the number of males which in-turn decreases the variation in a population. Variation in populations is needed in a changing environment (Uyeda & Mcglothun, 2024).

Eutrombidium locustarum is a micro-parasite of grasshoppers that ingest the insect's hemolymph to gain nutrients. *Eutrombidium locustarum* can be infected with *Wolbachia* both by vertical and horizontal transmission (Werren & Bartos, 2001). The mites can be infected via the hemolymph because *Wolbachia* has been found in the intercellular fluid of insects (Fallon, 2021). It can also be infected vertically from mother to offspring (Werren, 1996). The mites can be found on the wings, head, and legs of the grasshopper. *Wolbachia* infection can affect the mites several ways. First, the mite loses some of its tolerance to heat (Zhu, Song, Zhang, Hoffmann, & Hong, 2021) there is decreased egg hatchability, plus the sex-ratio is distorted (Wybouw, Mortier, & Bonte, 2022). However, *Wolbachia* protects the mites by boosting its immune system (Eleftherianos, Atri, Accetta, & Castillo, 2013; Pimentel, Cesar, Martins, & Cogni, 2021;Ros & Breeuwer, 2009).

2. Methods

Collection Methods: The specimens were collected from a 5-hectare area (~32.17^oN, 107.63^oW) at an elevation of ~1400 m from the bajada on the north side of the mountains. Weekly sweeps of the area were made using insect nets. The specimens were placed in 50 mL centrifuge tubes and frozen until DNA extraction. The grasshoppers were identified using A Manual of the Grasshoppers of New Mexico by Richman, Lightfoot, Sutherland, and Ferguson (1993). The mites were identified using Mites and Nematode Parasites of Grasshoppers by Belovsky, Branson, Chase, Barker and Hammond (1996).

DNA Extraction Methods: Two millimeters (mm) were removed from the specimen's posterior abdomen. The abdominal segment was then placed in a 1.5 milliliters (mL) microfuge tube with 200 microliters (µL) of lysis buffer. The abdominal segment was macerated for 1 minute. Eight-hundred µL of lysis buffer was added to the microfuge tube then vortexed. The tube was placed in a 95°C water bath for 5 minutes. After heating, the tube was opened briefly to release pressure then centrifuged for 5 minutes at 10,000 rpm. Another microfuge tube was obtained and 400 µL of the supernatant and put into the new tube. Forty µL of 5.0 M NaCl was added and placed on ice for 5 minutes. Tubes were placed in the centrifuge at 10,000 rpm's for 5 minutes.. Another clean

microfuge tube was obtained and 300µL of supernatant was transferred. Four-hundred microliters of cold isopropanol was added and then centrifuged at 10,000 rpm for 5 minutes. The supernatant was carefully poured out and the mouth of tube was tapped lightly to remove most of the liquid. The pellet was air dried for 10 minutes. Two-hundred µL of TE/RNase was added. The pellet was disturbed by pipetting and then tube was centrifuged at 10,000 rpm for 1 minute. The DNA was frozen until PCR amplification. PCR amplification was done with a Bio-Rad thermocycler t100; 20 uL of primer, 5 uL of DNA, and 10 uL of Master Mix were used. The primer for 16S rDNA was used to identify *W. pipientis*, and primer for the Cytochrome C oxidase gene was used to identify insect DNA. PCR cycles included 95 degrees for 2 minutes, 30 cycles of: 94 degrees for 30 seconds, 55 degrees for 45 seconds, 72 degrees for 1 minute, then 72 degrees for 10 minutes, and finally left at 4 degrees for the rest of the allotted time. One point two percent agarose electrophoresis gels were run at 150V for 30 minutes. SYBR safe green loading dye was used with lithium bromide buffer. An EDVOTEK TruBlu2 DNA illuminator was used to view the DNA. *Wolbachia pipientis* DNA is identified at 438 kilo-basepairs (kbp) and insect DNA is identified at 708 kbp. With *Eutrombidium locustarum,* DNA Extraction the same procedure was used for the mites, however, but we decreased the compounds by 75%. Also, the entire organism was macerated due to the small size of the mites.

3. Results

Seventy-nine *Leprus wheeler*i, grasshoppers 33 males and 46 females, were captured in the northern region of the Chihuahua Desert at the base of the Florida Mountains. The specimens were tested both for *Wolbachia pipientis* and *Eutrombidium locustarum* mites. The mites were also tested for *Wolbachia pipientis*. Thirty-three mites were collected from the grasshoppers, 15 from the legs, 15 from the necks, 2 from the wings, and 1 from the head. Six of the mites were infected with *Wolbachia.* Three leg mites, 2 neck mites, and 1 wing mite were infected.

Table 1. Results of *Leprus wheeleri* and *Eutrombidium locustarum* infected with *Wolbachia pipientis*

Leprus wheeleri Specimens	Number of Specimens	Number Infected with *Wolbachia*	Percentage Infected with *Wolbachia*	*Eutrombidium locustarum* Specimens	Number of Specimens	Number Infected with *Wolbachia*	Percentage Infected with Wolbachia
Male	33	17	51.5%	Leg Mites	15	3	20%
				Neck Mites	15	2	13%
Female	46	20	43.4%	Wing Mites	2	1	50%
				Head Mites	1	0	0%
Total	79	37	46.8%	Total Mites	33	6	18%

Figures 1 and 2 are electrophoresis gels showing the DNA from *L. wheeleri* (Figure 1), *E. locustarum* (Figure 2), *Wolbachia* (Figures 1 and 2), and a second bacterial DNA. Figure 1 shows *L. wheeleri* specimens 38-45 going left to right. Only specimen 41 was not infected with *Wolbachia*. Figure 2 shows *E. locustarum* 37a, 37b, 50b, 50g, 26a, 46a, and 46b. The mite numbers correspond to the grasshopper they were found on. Only 46a and 46b were infected with *Wolbachia.*

Figure 1 and 2: Going from left to right; Figure 1 is a photograph of *L. wheeleri* electrophoresis showing *Wolbachia* infection. Figure 2 is a photograph of *E. locustarum* electrophoresis showing *Wolbachia* infection.
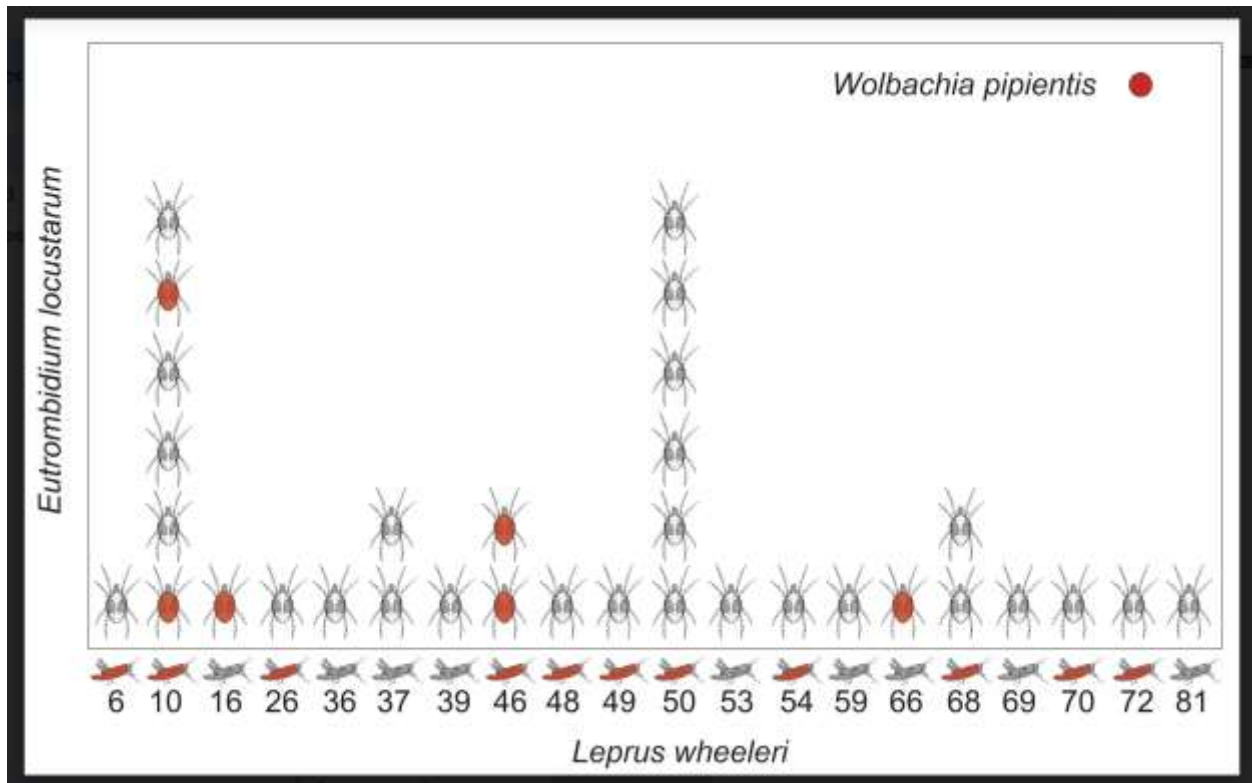

Figure 3. A pictograph showing which *L. wheeleri* grasshoppers (1) were infected with mites, (2) red denotes *Wolbachia* infection both in the mites and grasshoppers.

4. Discussion

This was an interesting study, and it gave evidence for both vertical and horizontal transfer of Wolbachia. As per table 1, 37 grasshoppers and 6 mites were infected with Wolbachia. In the grasshopper population, there were only an eight-point difference between the percentage rates of Wolbachia infection between genders. This was not enough difference to be significant. The mites were so small, their diameter being less than 1 μm, even with a dissecting microscope we could not determine their genders. The pictograph (Figure 3) shows the grasshoppers that were infected with mites. Red denotes Wolbachia infestation. Some of them were also infected with Wolbachia. Grasshoppers 16 and 66 were not infected with Wolbachia, however, mite 16a and mite 66a were indicating vertical transfer. Grasshoppers 10 and 50 were both infected with Wolbachia, and both were infected with 6 mites. With grasshopper 50, none of the mites were infected with Wolbachia. But, grasshopper 10 had 2 mites infected with Wolbachia showing a possible horizontal transfer. The mite Wolbachia infection rate, 18%, was much lower than the grasshopper Wolbachia infection rate of 46.8%. A second bacterial infection was visible on our electrophoresis gels. We believe the second bacterium was Cardinium. Co-infections have been documented (Wasala, et al., 2019), Our primer for the bacterial gene is the 16S rDNA gene, which has to have a similar nucleotide sequence between bacterial species. More research is needed addressing the relationship between mite and grasshopper infection rates. This study will be expanded during the next few years to (1) evaluate the mite infection rate on grasshoppers, (2) explore the dual infection rate of the bacteria Wolbachia and Cardinium, and (3) expand the study to different species of grasshopper dwelling in the Luna County section of the Chihuahua Desert.

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