2020

A Tangled Web: The Dynamics of Endosymbiotic Infections in a Linyphiid Spider

Laura Cecilia Rosenwald
University of Kentucky, lcro227@uky.edu
Author ORCID Identifier:
https://orcid.org/0000-0001-7846-644X
Digital Object Identifier: https://doi.org/10.13023/etd.2020.424

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Laura Cecilia Rosenwald, Student

Dr. Jennifer A. White, Major Professor

Dr. Kenneth F. Haynes, Director of Graduate Studies

A Tangled Web: The Dynamics of Endosymbiotic Infections in a Linyphiid Spider

---

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
at the University of Kentucky

By
Laura Cecilia Rosenwald
Lexington, Kentucky
Director: Dr. Jennifer A. White, Professor of Entomology
Lexington, Kentucky
2020

https://orcid.org/0000-0001-7846-644X

ABSTRACT OF THESIS

A Tangled Web: The Dynamics of Endosymbiotic Infections in a Linyphiid Spider

Many arthropods are infected with bacterial endosymbionts that manipulate host reproduction, but few bacterial taxa have been shown to cause such manipulations. *Mermessus fradeorum* (Linyphiidae) is a sheet-weaving spider that displays both feminization and cytoplasmic incompatibility (CI). To correlate reproductive manipulations to endosymbionts, I surveyed the bacterial community of *M. fradeorum* using high throughput sequencing and found that individuals can be infected with up to five different strains of bacteria from the genera *Wolbachia*, *Rickettsia,* and *Rickettsiella*. *Rickettsiella* was found in all 23 tested spider matrilines. I used antibiotic curing to generate uninfected matrilines that I reciprocally crossed with individuals infected with *Rickettsiella*. Only 13% of eggs hatched when uninfected females were mated with *Rickettsiella*-infected males, while at least 83% of eggs hatched in the other cross types. This is the first documentation of *Rickettsiella*, or any Gammaproteobacteria, causing CI. I then characterized symbiotypes of *M. fradeorum* in central Kentucky to determine the variation in endosymbiotic community composition among host populations. Overall, regional populations of *M. fradeorum* share endosymbiont strains, but the frequency of infection varied among populations. These results suggest that endosymbiotic infections of *M. fradeorum* are dynamic and that populations are composed of a mixture of symbiont-induced phenotypes.

KEYWORDS: Araneae, cytoplasmic incompatibility, endosymbiont, reproductive manipulation, co-infection, symbiotype

Laura Cecilia Rosenwald

(Name of Student)

10/14/2020

Date

A TANGLED WEB: THE DYNAMICS OF ENDOSYMBIOTIC INFECTIONS IN A LINYPHIID SPIDER

By
Laura Cecilia Rosenwald

Dr. Jennifer A. White
Director of Thesis

Dr. Kenneth F. Haynes
Director of Graduate Studies

10/14/2020
Date

ACKNOWLEDGMENTS

Every house needs a good foundation to build on, and the same is true for scientists. I would like to thank both Dr. Gina Wimp and Dr. Martha Weiss from my undergraduate career for fostering my love of research and gently guiding me to answer my scientific inquiries in a valid and repeatable fashion. I also would like to thank Dr. John Obrycki and Dr. Stephen Dobson for their time and guidance while serving on my committee. Lastly, an extra special thanks to Dr. Jen White. I am eternally grateful for your patience, endosymbiont knowledge, friendship, and the various adventures that always seem to find ourselves in.

I thank everyone in the Entomology department at the University of Kentucky for their friendship, time, assistance, and teachings. I also am extremely appreciative and extend thanks to all of the growers who graciously allowed me to traipse through their alfalfa fields in search of spiders. Special thanks for moral support and the occasional trivia knowledge to: Caitlin Stamper, Kacie Athey, Scott Bessin, Allyssa Kilanowski, Nathan Mercer, and Amanda Dunaway, and Josiah Ritchey. Thanks are also due to all of the musical artists and podcasts that have kept me company over many hours of writing and spider care. An added thank you to everyone in my life who has patiently listened to my excited chatter about endosymbionts in spiders.

And to my parents: endless thanks still do not seem to be enough. Your love and support mean the absolute world to me. Thank you for encouraging me in everything I do from horses, to beer, to bugs. Cheers.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. ENDOSYMBIOTIC *RICKETTSIELLA* CAUSES CYTOPLASMIC INCOMPATIBILITY IN A SPIDER HOST

Chapter contents published in: Rosenwald, L.C., Sitvarin, M.I., White, J.A. 2020. Endosymbiotic *Rickettsiella* causes cytoplasmic incompatibility in a spider host. Proceedings of the Royal Society B. 287 (20201107).

1.1 Introduction

Maternally transmitted endosymbiotic bacteria are common in arthropods, and are estimated to infect a majority of arthropod species (Duron et al. 2008a, Weinert et al. 2015, Zhang and Yun 2018, White et al. 2020). These inherited endosymbionts frequently manipulate host reproduction to promote symbiont transmission and spread in the host population (Engelstädter and Telschow 2009). The most prevalent and well-studied symbiont-driven manipulation is cytoplasmic incompatibility (CI) (O'Neill et al. 1992, Hunter et al. 2003, Werren et al. 2008, Engelstädter and Telschow 2009). CI is a conditional sterility phenotype in which crosses between infected males and uninfected females result in offspring mortality, sabotaging the production of uninfected progeny. This phenotype functionally increases the proportion of infected females in the host population over time (Engelstädter and Telschow 2009). CI influences host population dynamics and gene flow, acting both as a gene drive mechanism that spreads traits through a population (Sinkins and Gould 2006), and as a reproductive barrier that can enforce reproductive isolation and contribute toward speciation (Bordenstein et al. 2001, Gebiola et al. 2016). In application, CI has been used to locally repress pest populations as a form of sterile insect technique (Mains et al. 2016), and also as a gene drive/defensive mechanism to reduce disease transmission by mosquitoes (Walker et al. 2011, Pan et al. 2012a).

To date, few bacterial taxa have been shown to cause CI. The most widespread and well-investigated are members of the genus *Wolbachia* (Phylum Proteobacteria, Class Alphaproteobacteria, Order Rickettsiales), which infects many arthropod species (Duron et al. 2008b, Werren et al. 2008, Zug and Hammerstein 2012, Sazama et al. 2019). Recently, another unnamed strain in the Rickettsiales has also been suggested to induce CI (Takano et al. 2017). Finally, members of the more distantly related *Cardinium* genus (Phylum Bacteroidetes) also induce CI, although using a mechanism that appears to have evolved independently from that of *Wolbachia* (Penz et al. 2012). No other bacterial taxa have been documented to cause CI to date.

In the present study, I provide evidence that CI can be caused by a strain of *Rickettsiella* (Phylum Proteobacteria, Class Gammaproteobacteria, Order Legionellales), a bacterium that is not closely related to either *Wolbachia* or *Cardinium* (Duron et al. 2016). I began by characterizing the microbiome of *Mermessus fradeorum* (Araneae: Linyphiidae), an agricultural spider that had been previously shown to be infected by multiple *Wolbachia* and *Rickettsia* (Phylum Proteobacteria, Class Alphaproteobacteria, Order Rickettsiales) symbionts, and to exhibit CI (Curry et al. 2015). Upon discovery of an unexpected *Rickettsiella* symbiont that was pervasive throughout the sampled population, I proceeded to experimentally document that *Rickettsiella* causes CI in *M. fradeorum*. This is the first demonstration of CI by a Gammaproteobacteria, which suggests that the taxonomic distribution of bacteria that cause CI may be wider than previously appreciated, and could have important implications for the population genetics, biodiversity, and evolution of arthropod hosts.

1.2 Materials and methods

1.2.1 Study system

Mermessus fradeorum were initially collected from alfalfa at the University of Kentucky's Spindletop Research Farm (38.127, −84.508). Individual spiders were placed into deli cups (6 cm diam.) with hydrated plaster in the bottom for humidity control, and maintained them in the laboratory at 22°C 24 h dark conditions. Spiders collected as juveniles were fed symbiont-free collembola (*Sinella curviseta*) until maturity. Adult spiders were fed fruit flies (*Drosophila melanogaster*) at 2–3 day intervals; these flies were infected with a distinct strain of *Wolbachia* (MLST ST1) (Baldo et al. 2006) that was never detected in the microbiome survey or diagnostic assays. I tested whether infected prey could result in false-positive infection diagnoses in *M. fradeorum* using *Wolbachia*-infected *Drosophila melanogaster*. Thirty spiders (10 males, 20 females) from *Wolbachia*-free matrilines were deprived of food for a week before the trial began. Spiders were randomly assigned to one of five treatments: unfed (n=6) or fed a single *D. melanogaster* with DNA extracted one hour (n=6), one day (n=6), three days (n=6), or five days (n=6) after feeding. All DNA extractions were completed using DNEasy Blood and Tissue kits (Qiagen, Germantown, MD). The six unfed spiders and six positive fruit fly controls were extracted at the same time as the one hour post-feeding treatment. Presence or absence of *Wolbachia* was determined by diagnostic PCR (wsp primers) followed by gel electrophoresis of resultant products (Baldo et al. 2006). I found that uninfected *M. fradeorum* did not test positive for *Wolbachia*, even an hour after feeding on *Wolbachia*-infected flies (Figure 1.1). I initiated matrilines from individual field-

collected gravid females, or by mating field-collected females to males within the laboratory.

1.2.2 Microbiome survey

To evaluate endosymbiont diversity in the population of *M. fradeorum*, I characterized the microbiome of 23 matrilines that were initiated between August and October 2016. For each matriline, I evaluated the field-collected female specimen when possible; if the field-collected female died of natural causes (n = 8), I instead randomly selected an adult female F1 offspring. I withheld food from each spider for at least 5 days before preserving the specimen in 95% ethanol. I surface-sterilized each specimen with a series of washes with 0.5% bleach and PCR water. I then extracted total DNA from each individual specimen using DNEasy Blood and Tissue kits (Qiagen, Germantown, MD) according to the manufacturer's instructions. Using PCR with dual-indexed 515F/806R primers (Kozich et al. 2013), I amplified the V4 region of bacterial 16S from each specimen and visualized an aliquot of the resulting product on a 1% agarose gel stained with GelRed (Biotium). After verifying the presence of a strong band of the expected product size, I included a 1 µl aliquot of the corresponding product in a multiplexed library for sequencing. This library also included samples from aphids and parasitoid wasps that served as controls (see below). After purification using GenCatch PCR Cleanup kit (Epoch Life Sciences, Missouri City, TX) I sequenced the library on an Illumina Miseq 2500 instrument at the University of Kentucky's core sequencing facility.

Sequences were demultiplexed, trimmed, and quality filtered within BaseSpace (Illumina) then imported into qiime2 (v.2017.11) (Caporaso et al. 2010) using a manifest. Additional quality control was implemented using deblur (Bokulich et al.

2013), implemented in qiime2 using default parameters and a trim length of 251 bases. To determine the taxonomic placement of each operational taxonomic unit (OTU), a naive Bayes classifier trained on the V4 region of the Greengenes 13_8 99% OTUs reference database was used (DeSantis et al. 2006).

The most prevalent OTU in the library, a strain of *Rickettsiella*, constituted 46% of total reads and was associated with every component specimen in the multiplexed sample, albeit at low prevalence for non-*M. fradeorum* samples. I inferred that index swapping may have occurred between samples that shared either forward or reverse indices, resulting in reads that were misallocated to the wrong sample (Larsson et al. 2018). To validate that *Rickettsiella* was genuinely present in all *M. fradeorum* samples, I used *Rickettsiella*-specific primers to diagnostically test for the presence of *Rickettsiella* in the 23 matriline samples, as well as the eight controls (four *Aphis craccivora* (aphid) specimens and four *Habrobracon hebetor* (parasitoid wasp) specimens). Diagnostic primers and protocols followed Duron et al. (2016); samples that tested negative for *Rickettsiella* were rescreened at least two more times, and additionally tested for extraction quality via diagnostic PCR of a segment of the arthropod cytochrome oxidase subunit 1 (COI) gene (White et al. 2020).

To assess the vertical transmission of *Rickettsiella*, I included specimens from several generations in the microbiome dataset. In total, 148 mother-offspring pairs from the 23 matrilines (representing 83 *Rickettsiella*-infected mothers) were initially included in the dataset. As a quality control measure for the final dataset, I retained only pairs in which both mother and offspring yielded more than 5000 reads (119 pairs, representing 73 different mothers, sometimes with multiple offspring). To be scored positive,

the *Rickettsiella* read number for a given microbiome needed to exceed the upper 95% confidence boundary of *Rickettsiella* reads found in the eight control samples (145 reads).

1.2.3 *Rickettsiella* cytoplasmic incompatibility assay

To assess the ability of *Rickettsiella* to cause CI, I first created a population of uninfected spiders using antibiotics. Spiderlings originating from seven infected matrilines were treated with a combination of tetracycline (0.1%) and ampicillin (0.1%) by a fine mist spray daily until subadulthood (Vanthournout and Swaegers 2011, Curry et al. 2015). Upon maturity, treated spiders were mated to one another, and a subset of those offspring were diagnostically tested for endosymbionts (*Wolbachia*, *Rickettsia*, *Rickettsiella*) (Curry et al. 2015, White et al. 2020). Sibships that appeared negative for all endosymbionts were used to initiate the putatively uninfected population for experimental assays, with the understanding that not all spiders within this group would be truly cleared of infection. Antibiotic treatment often destabilizes symbiont transmission to offspring, resulting in inconsistent infection status among sibships and/or low titer infections that rebound in subsequent generations (Koga et al. 2007, White et al. 2009). It is common practice to treat hosts with antibiotics for several generations to ensure symbiont clearance, but due to the labor-intensive rearing of cannibalistic spiders, here I chose instead to diagnostically test all parental spiders to properly classify mating cross types, as described below. Spiders used in experiments were 1, 2, or 3 generations removed from antibiotic treatment.

To determine if *Rickettsiella* was causing CI in *M. fradeorum*, I reciprocally mated uninfected spiders with spiders that were infected with only *Rickettsiella*. I

randomly assigned virgin spiders to mate with a partner that I expected to be either *Rickettsiella*-infected or uninfected, ensuring that siblings and cousins were not paired with one another. Males and females were paired for 8 h, and were observed at approximately 15 min intervals for the first 2 h and periodically thereafter. If the pair was not observed to mate, they were excluded from the trial. Females were then allowed up to six weeks to lay three egg masses; females that failed to lay three egg masses within this time period (n = 2) were excluded from the sample set. Egg masses were checked daily for spiderlings. Once spiderlings were observed, I allowed 24 h for emergence, then dissected the egg mass to quantify unhatched eggs. Egg masses hatched within a mean ± s.e. of 14.1 ± 0.3 days. If no spiderlings were observed within 20 days, the entire egg mass was scored as unhatched, and dissected to count the number of unhatched eggs. Throughout the experiment, I found a small number of spiderlings (n = 30) that hatched but died prior to exiting the egg mass. These unemerged spiderlings were excluded from the dataset, as they accounted for only 2% of all eggs.

The infection status of all parents in the experiment was validated using diagnostic PCR for *Wolbachia*, *Rickettsia,* and *Rickettsiella* (Curry et al. 2015, Duron et al. 2016, White et al. 2020). The final classification of all matings was based on these diagnostic results, rather than initial expectations based on a sibling or parental infection status. Matings were reclassified if *Rickettsiella* presence/absence in either parent deviated from expectation, and were excluded altogether if either parent tested positive for a symbiont other than *Rickettsiella* (Table 1.1). The final distribution of mating cross types across four trial dates included 18 pairings in which both female and male were infected with only *Rickettsiella* (+/+), 14 in which the female was *Rickettsiella*-infected

and the male was uninfected (+/−), 12 in which the female was uninfected and the male was *Rickettsiella*-infected (−/+), and eight in which both female and male were uninfected (−/−).

I compared total eggs laid among treatments using ANOVA (JMP) after verifying that model assumptions of normality and homoscedasticity were not violated. I compared egg hatch among treatments using logistic regression (Arc v.1.06) with Williams' correction (Williams 1982) to account for moderate overdispersion. To verify that generations removed from antibiotic treatment (1, 2, or 3 generations) did not influence the experimental outcome, I considered a separate model that incorporated this factor, but found no significant effect (Wald = −0.93, $p = 0.35$). Generation from antibiotic treatment was, therefore, not included in the final model. I additionally tested whether the diagnostic reclassification and exclusion criteria affected my conclusions by running the statistical tests on alternative datasets in which I (i) completely ignored the diagnostic results and had no reclassification, (ii) reclassified according to *Rickettsiella* infection but ignored the presence of other symbionts (no exclusions), or (iii) excluded all matings that did not fit with their initial expectations (exclusions instead of reclassifications) (Table 1.1). All of these variant analyses yielded statistically significant results that concur with the analysis of the original dataset presented below.

1.3 Results

1.3.1 Microbiome survey

The most notable feature on the *M. fradeorum* microbiome was the pervasiveness of a single strain of *Rickettsiella*, present in all 23 matrilines (Figure 1.2). Mean ± s.e

reads per sample was 22054 ± 2739, of which 16172 ± 2109 (80 ± 6%) were *Rickettsiella*. In most specimens (20/23), *Rickettsiella* constituted the majority of reads, often representing greater than 95% of reads (Figure 1.2, Table 1.2). Proportional representation of *Rickettsiella* was lower in samples that were also infected with *Rickettsia* (seven samples) and/or up to three strains of *Wolbachia* (eight samples), but still constituted half the reads returned for this subset of samples (50 ± 9%). By contrast, aphid and parasitoid wasp control samples that were included in the same sequencing run had few reads of this *Rickettsiella* strain (115 ± 15 reads/sample, constituting 0.3 ± 0.05% of reads/sample; Table 1.3). Diagnostic validation confirmed the presence of *Rickettsiella* from all 23 matriline samples, and the absence of *Rickettsiella* from the aphids and parasitoid wasps. I observed 100% vertical transmission efficiency in the unmanipulated mother-offspring pairs in the dataset (Table 1.4). Across 119 pairs, all offspring of *Rickettsiella*-infected mothers were themselves infected with *Rickettsiella.*

1.3.2 Reproductive manipulation assay: cytoplasmic incompatibility

When I reciprocally mated spiders bearing only *Rickettsiella* with those from matrilines that had been cured of all facultative symbionts, I found a large difference in the number of hatchlings produced. Total egg number was consistent and not significantly different across treatments (ANOVA: $F_{3,48} = 0.56$, $p = 0.644$; Figure 1.3, Table 1.5), but the odds that these eggs hatched was significantly lower for uninfected females mated to *Rickettsiella*-infected males, relative to all three other treatments, which did not differ significantly from one another ($\chi^2 = 119$, d.f. = 3, $p < 0.001$). Only 13.2 ± 3.8% of eggs hatched in the incompatible cross between uninfected females and

Rickettsiella-infected males, compared to 99.7 ± 0.2% of eggs when both parents were infected, 91.3 ± 4.6% from *Rickettsiella*-infected females mated with uninfected males, and 83.9 ± 12.2% when both parents were uninfected.

1.4 Discussion

I found that the spider *M. fradeorum* is infected by up to five strains of bacterial symbiont, including a strain of *Rickettsiella* that was present in 100% of the field collected matrilines. I then generated uninfected matrilines, and used crossing experiments to document that this *Rickettsiella* strain induced CI in the spider host. Previous examples of CI-causing bacteria have been restricted to *Wolbachia* and closely allied bacteria in the Alphaproteobacteria, and *Cardinium* in the Bacteroidetes (O'Neill et al. 1992, Hunter et al. 2003, Gotoh et al. 2007, Takano et al. 2017). This is the first demonstration of a Gammaproteobacteria, *Rickettsiella*, causing CI.

Previous studies with this spider species diagnostically documented three endosymbiotic bacterial strains (two *Wolbachia* and one *Rickettsia*) associated with bacterially induced reproductive manipulations, but could not attribute causality to individual bacterial strains (Curry et al. 2015). The present microbiome study confirmed the presence of these same three symbiont strains at similar field frequencies as the previous work, and additionally revealed a third strain of *Wolbachia* along with the ubiquitous *Rickettsiella*. I was able to screen archived specimens from the previous study (Curry et al. 2015) and found that the CI-inducing matriline from that study was in fact co-infected with *Rickettsiella* and one strain of *Wolbachia*. It is, therefore, likely that the CI observed in this previous study was at least partially attributable to *Rickettsiella*, although both co-infecting symbionts may have contributed to the CI phenotype (Zhu et

al. 2012). Focus on taxa that are well-established as reproductive manipulators, such as *Wolbachia*, may lead researchers to overlook co-infecting bacteria that cause or contribute to manipulated host reproductive phenotypes, as co-infections are common in arthropods (Duron et al. 2008a, Zhang and Yun 2018).

The mechanism by which *Rickettsiella* induces CI is unknown. In *Wolbachia*, CI is induced by phage-encoded operons in which one gene modifies the sperm of infected males and the other rescues the modification in the cytoplasm of the egg of infected females (Beckmann et al. 2017, Le Page et al. 2017). In *Cardinium*, the mechanism to induce CI appears to be independently evolved from *Wolbachia*, and is not phage associated (Penz et al. 2012). No previously described *Rickettsiella* strains have been documented to cause CI, but CI-induction has been hypothesized based on genetic analysis of a *Rickettsiella* strain in fleas (Gillespie et al. 2018). Strains of *Rickettsiella* infect a wide range of arthropods (Cordaux et al. 2007, Duron et al. 2016), sometimes as a pathogen (Cordaux et al. 2007), sometimes as a mutualist (Tsuchida et al. 2010), and sometimes as a potential reproductive manipulator (Kurtti et al. 2002). Speculatively, it is possible that horizontal gene transfer among co-infecting symbionts may be responsible for *Rickettsiella*'s ability to induce CI (Ochman et al. 2000, Ishmael et al. 2009), particularly given the high frequency of symbiotic co-infection in spiders in general (Goodacre et al. 2006, Duron et al. 2008a, Zhang and Yun 2018, White et al. 2020) and *M. fradeorum* in particular.

These data also reinforce the perception that spiders can harbor exceptionally diverse communities of co-infecting symbionts, rivalling the most symbiont-rich inherited communities among insects (Ferrari et al. 2011, Russell et al. 2013, White et al.

2020). The discovery of an unexpected reproductive manipulator within such a community highlights the hazard of focusing only on well-characterized manipulative taxa (e.g. *Wolbachia*), when reproductive anomalies are observed in a host population. These data emphasize the need for careful characterization of the entire inherited microbiome before attributing reproductive phenotypes to particular bacterial strains.

Reproductive manipulation by endosymbionts, through mechanisms such as CI, can influence arthropod behavioral ecology (Angelella et al. 2018), population dynamics (Jaenike 2009), and evolutionary trajectory (Cordaux et al. 2011). As the known roster of symbionts capable of undertaking these manipulators increases, the potential for understanding and potentially exploiting their effects on the biology of their hosts increases as well. CI is currently being used as part of a symbiont-based strategy to decrease disease transmission in mosquitoes (Mains et al. 2016); newly discovered CI-inducing symbionts, such as described in the present work, have similar potential in a wider range of arthropod hosts. In particular, *Wolbachia* is rare or absent in ticks (Class Arachnida, Order Acari), but strains of *Rickettsiella* are routine (Kurtti et al. 2002, Tijsse-Klasen et al. 2011, Duron et al. 2016, 2017). It would be worthwhile to evaluate the function of *Rickettsiella* and other members of tick microbiomes, similarly to what I have done with *Rickettsiella* in the spider *M. fradeorum*, as the potential opportunities for symbiont-based pest control and vector management are substantial.

Table 1.1 Parental infections from *Rickettsiella* cytoplasmic compatibility assay.

	Rickettsiella *infection*		*# generations from antibiotics*			*intended* Rickettsiella *infection*		
Mating Pair	*Females*	*Males*	*Females*	*Males*	*Reclassified?*	*Females*	*Males*	*Excluded?*
1	+	+	NA	NA	No			No
2	+	+	NA	NA	No			No
3	+	+	NA	1	Yes	+	-	No
4	+	+	NA	NA	No			No
5	+	+	NA	NA	No			No
6	+	+	NA	NA	No			No
7	+	+	NA	NA	No			No
8	+	+	1	NA	Yes	-	+	No
9	+	+	NA	1	Yes	+	-	No
10	+	+	1	1	Yes	-	-	No
11	+	+	NA	2	Yes	+	-	No
12	+	+	NA	NA	No			No
13	+	+	NA	2	Yes	+	-	No
14	+	+	1	2	Yes	-	-	No
15	+	+	NA	NA	No			No
16	+	+	NA	1	Yes	+	-	No
17	+	+	2	NA	Yes	-	+	No
18	+	+	2	2	Yes	-	-	No
19	+	-	NA	NA	No			No
20	+	-	NA	1	No			No
21	+	-	NA	NA	No			No
22	+	-	1	1	Yes	-	-	No
23	+	-	NA	1	No			No
24	+	-	NA	1	No			No
25	+	-	NA	1	No			No
26	+	-	NA	2	No			No
27	+	-	NA	2	No			No
28	+	-	NA	1	No			No
29	+	-	NA	1	No			No
30	+	-	NA	2	No			No
31	+	-	1	1	Yes	-	-	No
32	+	-	1	3	Yes	-	-	No
33	-	+	1	NA	No			No
34	-	+	1	NA	No			No
35	-	+	1	NA	No			No
36	-	+	1	NA	No			No

Table 1.1 (continued) Parental infections from *Rickettsiella* cytoplasmic compatibility assay.

	Rickettsiella *infection*		*# generations from antibiotics*			*intended* Rickettsiella *infection*		
Mating Pair	*Females*	*Males*	*Females*	*Males*	*Reclassified?*	*Females*	*Males*	*Excluded?*
37	-	+	1	1	Yes	-	-	No
38	-	+	2	2	Yes	-	-	No
39	-	+	2	NA	No			No
40	-	+	2	NA	No			No
41	-	+	3	NA	No			No
42	-	+	2	NA	No			No
43	-	+	2	NA	No			No
44	-	+	3	NA	No			No
45	-	-	1	NA	No			No
46	-	-	1	1	No			No
47	-	-	1	1	No			No
48	-	-	1	1	No			No
49	-	-	2	1	No			No
50	-	-	2	1	No			No
51	-	-	2	2	No			No
52	-	-	2	2	No			No
53	-	-	1	1	No			Yes[1]
54	+	+	1	NA	Yes	-	+	Yes[2]
55	+	-	1	1	Yes	-	-	Yes[2]
56	-	+	1	NA	No			Yes[3]
57	-	-	1	1	No			Yes[3]
58	-	-	1	1	No			Yes[3]
59	-	-	1	1	No			Yes[4]
60	-	-	2	2	No			Yes[3]
61	-	+	2	NA	No			Yes[3]

[1]Male tested positive for *Wolbachia*
[2]Female tested positive for *Wolbachia*
[3]Female tested positive for *Rickettsia*
[4]Male tested positive for *Rickettsia*

Table 1.2 Read results per matriline from high throughput 16S microbiome sequencing.

OTU	Total reads across all matriline samples	Microbiome read distributions from *Mermessus fradeorum* matriline representatives. Specimens with just letters were field collected females. Specimens with a number are adult female F1 offspring.							
		A3	AM	AC	AK	I2	U2	AD1	X
Rickettsiella	371946	4907	9432	15779	15170	2485	25025	14970	5512
Rickettsia	41722	7259	2	11474	4579	5382	3504	8223	1027
Wolbachia 1	26446	9120	3265	7771	5978	0	0	0	7
Wolbachia 2	15560	854	120	3041	1123	2217	2935	4868	278
Wolbachia 3	11412	3053	1204	3851	3106	0	11	0	14
Stenotropomonas	1910	1195	18	59	23	0	0	46	13
Pseudomonas 1	23149	23127	0	0	0	0	0	0	0
Pseudomonas 2	2639	0	0	0	0	0	0	0	0
Pseudomonas 3	476	0	0	0	0	0	0	0	0
Methylobacterium	1062	8	10	91	45	0	13	53	25
Mycobacterium	1042	165	0	0	0	4	0	12	0
Enterobacteriaceae 1	866	858	0	0	2	0	0	0	0
Enterobacteriaceae 2	1032	1005	0	2	9	0	0	0	0
Norcardiaceae	957	0	0	0	0	0	332	0	30
Other	5583	619	38	277	152	36	669	289	36
	Total	42345	30187	53552	14120	10124	32489	28462	6942

Table 1.2 (continued) Read results per matriline from high throughput 16S microbiome sequencing.

OTU	Total reads across all matriline samples	Microbiome read distributions from *Mermessus fradeorum* matriline representatives. Specimens with just letters were field collected females. Specimens with a number are adult female F1 offspring.								
		AF	AH	AI	AJ	AA	AL	AN	AP	G2
Rickettsiella	371946	27655	11669	21164	18224	26736	11584	11706	48476	8090
Rickettsia	41722	10	45	10	24	13	15	10	13	13
Wolbachia 1	26446	3	97	28	10	6	3	2	0	14
Wolbachia 2	15560	12	9	10	9	10	2	0	3	5
Wolbachia 3	11412	10	38	12	31	1	3	0	0	30
Stenotropomonas	1910	21	26	13	254	22	25	0	107	29
Pseudomonas 1	23149	0	0	0	0	2	0	0	0	2
Pseudomonas 2	2639	0	0	0	2636	0	0	0	0	0
Pseudomonas 3	476	0	0	0	0	0	0	0	4	0
Methylobacterium	1062	74	84	72	130	72	124	18	21	9
Mycobacterium	1042	0	17	0	85	35	0	0	0	7
Enterobacteriaceae 1	866	4	0	0	2	0	0	0	0	0
Enterobacteriaceae 2	1032	0	0	3	0	0	0	0	0	0
Norcardiaceae	957	0	0	3	470	0	0	0	0	0
Other	5583	271	369	591	592	210	255	28	47	82
	Total	28060	12354	21906	22467	27114	12024	11764	48680	8281

Table 1.2 (continued) Read results per matriline from high throughput 16S microbiome sequencing.

OTU	Total reads across all matriline samples	Microbiome read distributions from *Mermessus fradeorum* matriline representatives. Specimens with just letters were field collected females. Specimens with a number are adult female F1 offspring.					
		M4	S	V	Y1	Z5	W1
Rickettsiella	371946	15607	8399	17905	27943	14262	9246
Rickettsia	41722	18	5	9	52	28	7
Wolbachia 1	26446	5	9	16	38	43	31
Wolbachia 2	15560	5	7	1	26	25	0
Wolbachia 3	11412	17	3	3	5	12	8
Stenotropomonas	1910	15	7	0	15	12	10
Pseudomonas 1	23149	18	0	0	0	0	0
Pseudomonas 2	2639	0	3	0	0	0	0
Pseudomonas 3	476	0	0	472	0	0	0
Methylobacterium	1062	8	11	89	15	79	11
Mycobacterium	1042	0	0	0	0	717	0
Enterobacteriaceae 1	866	0	0	0	0	0	0
Enterobacteriaceae 2	1032	0	0	0	13	0	0
Norcardiaceae	957	0	0	0	122	0	0
Other	5583	186	27	59	431	231	88
	Total	15879	8471	18554	28660	15409	9401

Table 1.3 Total reads from aphid (*Aphis craccivora*) and wasp controls (*Habrobracon hebetor)* from high throughput 16S sequencing.

OTU	Total Control Reads	Aphid Controls – *Aphis craccivora*				Wasp Controls – *Habrobracon hebetor*			
		Aphid1	Aphid2	Aphid3	Aphid4	Wasp1	Wasp2	Wasp3	Wasp4
Rickettsiella	918	158	180	71	109	115	137	94	54
Rickettsia	196	49	44	29	9	30	21	6	8
Wolbachia 1	197	69	69	37	22	0	0	0	0
Wolbachia 2	115	35	10	4	5	12	22	4	23
Wolbachia 3	62	9	39	6	8	0	0	0	0
Stenotropomonas	18	0	12	0	0	1	0	0	5
Pseudomonas 1	13	4	5	0	0	2	2	0	0
Pseudomonas 2	0	0	0	0	0	0	0	0	0
Pseudomonas 3	0	0	0	0	0	0	0	0	0
Methylobacterium	38	7	10	4	4	5	4	0	4
Mycobacterium	0	0	0	0	0	0	0	0	0
Enterobacteriaceae 1	17	0	6	11	0	0	0	0	0
Enterobacteriaceae 2	0	0	0	0	0	0	0	0	0
Norcardiaceae	0	0	0	0	0	0	0	0	0
Other	298148	58416	26499	40423	49409	35143	39147	29019	20092
Total	**299722**	58747	26874	40585	49566	35308	39333	29123	20186

Table 1.4 High throughput 16S microbiome reads between mother and offspring to assess vertical transmission of endosymbionts.

	identity			*Rickettsiella* reads		Total reads		proportion *Rickettsiella*	
pair	mother	offspring	off. sex	mother	offspring	mother	offspring	mother	offspring
1	A3	A3F2-4	f	4907	8667	53552	31750	0.092	0.273
2	A3F2-4	A3F3-4	f	8667	8386	31750	59405	0.273	0.141
3	A3F3-4	A3F4-9	m	8386	5387	59405	32027	0.141	0.168
4	A4	A4F2-1	f	5242	19184	22918	107562	0.229	0.178
5	A4	A4F2-4	f	5242	6226	22918	50573	0.229	0.123
6	A4	A4F2-6	f	5242	6189	22918	38717	0.229	0.160
7	A4F2-1	A4F3-5	f	19184	6206	107562	55269	0.178	0.112
8	A4F2-1	A4F3-6	f	19184	5656	107562	43643	0.178	0.130
9	A4F3-6	A4F4-5	f	5656	3536	43643	22630	0.130	0.156
10	A4F3-5	A4F4-8	f	6206	8815	55269	60151	0.112	0.147
11	A5	A5F2-2	f	6750	15992	19800	83553	0.341	0.191
12	A5	A5F2-5	f	6750	2861	19800	29667	0.341	0.096
13	A5	A5F2-6	f	6750	2504	19800	30472	0.341	0.082
14	A5	A5F2-7	f	6750	3016	19800	25673	0.341	0.117
15	A5F2-2	A5F3-4	f	15992	4574	83553	45245	0.191	0.101
16	A5F3-4	A5F4-1	f	4574	3644	45245	14748	0.101	0.247
17	A6	A6F2-1	m	10257	8287	37097	40606	0.276	0.204
18	A6F3-7	A6F4-1	f	12218	8414	43931	66954	0.278	0.126
19	AA	AA5	f	26736	16877	27114	17327	0.986	0.974
20	AA	AA6	f	26736	12174	27114	16793	0.986	0.725
21	AA6	AA6F2-2	f	12174	11466	16793	11567	0.725	0.991
22	AA6F2-2	AA6F3-9	f	11466	15036	11567	15517	0.991	0.969
23	AC	AC8	f	15779	16124	42345	16741	0.373	0.963
24	AC8	AC8F2-2	m	16124	4031	16741	5146	0.963	0.783
25	AC8	AC8F2-5	f	16124	14619	16741	14949	0.963	0.978
26	AC8F2-5	AC8F3-5	m	14619	5780	14949	6293	0.978	0.918
27	AC8F2-5	AC8F3-6	f	14619	9619	14949	10123	0.978	0.950
28	AD1	AD1F2-10	f	14970	8197	28462	27076	0.526	0.303
29	AD1	AD1F2-11	f	14970	8887	28462	43024	0.526	0.207
30	AD1	AD1F2-7	m	14970	7521	28462	28814	0.526	0.261
31	AD1F2-10	AD1F3-1	m	8197	5435	27076	6993	0.303	0.777
32	AD1F2-11	AD1F3-8	f?	8887	10963	43024	14907	0.207	0.735
33	AF	AF1	f	27655	11448	28060	11831	0.986	0.968
34	AF1	AF1F2-1	f	11448	60063	11831	60164	0.968	0.998
35	AF1	AF1F2-10	m	11448	12729	11831	13165	0.968	0.967

Table 1.4 (continued) High throughput 16S microbiome reads between mother and offspring to assess vertical transmission of endosymbionts.

	identity			*Rickettsiella* reads		Total reads		Proportion *Rickettsiella*	
pair	mother	offspring	off. sex	mother	offspring	mother	offspring	mother	offspring
36	AF1F2-1	AF1F3-4	m	60063	48330	60164	48575	0.998	0.995
37	AF1F2-1	AF1F3-5	f	60063	42044	60164	42394	0.998	0.992
38	AF	AF2	m	27655	5681	28060	6230	0.986	0.912
39	AF	AF3	m	27655	22382	28060	22970	0.986	0.974
40	AF	AF4	m	27655	10159	28060	10233	0.986	0.993
41	AF	AF6	m	27655	17639	28060	17705	0.986	0.996
42	AF	AF9	m	27655	24393	28060	24901	0.986	0.980
43	AH	AH6	f	11669	26609	12354	27122	0.945	0.981
44	AH6	AH6F2-10	f	26609	22369	27122	23091	0.981	0.969
45	AH6	AH6F2-9	m	26609	12258	27122	12459	0.981	0.984
46	AH6F2-10	AH6F3-5	f	22369	9418	23091	10207	0.969	0.923
47	AI	AI5	f	21164	7924	21906	7983	0.966	0.993
48	AI5	AI5F2-8	m	7924	6926	7983	7366	0.993	0.940
49	AI5	AI5F2-9	f	7924	22741	7983	23199	0.993	0.980
50	AJ	AJ3	f	18224	6130	22467	6280	0.811	0.976
51	AJ	AJ4	f	18224	7311	22467	7397	0.811	0.988
52	AJ3	AJ3F2-9	m	6130	5196	6280	5625	0.976	0.924
53	AK	AK10	f	15170	4199	30187	24918	0.503	0.169
54	AK10	AK10F2-4	f	4199	6685	24918	37252	0.169	0.179
55	AK10F2-4	AK10F3-1	f	6685	5161	37252	50070	0.179	0.103
56	AK10F3-1	AK10F4-1	m	5161	3020	50070	22423	0.103	0.135
57	AK10F3-1	AK10F4-2	f	5161	4533	50070	26744	0.103	0.169
58	AM	AM1	f	9432	9165	14120	31433	0.668	0.292
59	AM	AM2	f	9432	4249	14120	41622	0.668	0.102
60	AM2	AM2F2-5	f	4249	8521	41622	40060	0.102	0.213
61	AM2F2-5	AM2F3-1	f	8521	5163	40060	44446	0.213	0.116
62	AM2F3-1	AM2F4-1	m	5163	6112	44446	14794	0.116	0.413
63	AN	AN1	f	11706	20083	11764	20267	0.995	0.991
64	AN1	AN1F2-10	f	20083	10053	20267	11274	0.991	0.892
65	AN1	AN1F2-9	m	20083	23806	20267	24195	0.991	0.984
66	AN	AN3	f	11706	12255	11764	12414	0.995	0.987
67	AP	AP1	m	48476	28441	48680	28463	0.996	0.999
68	AP	AP2	f	48476	27166	48680	27201	0.996	0.999
69	G2	G2F2-5	f	8090	12710	8281	13331	0.977	0.953
70	G2	G2F2-7	f	8090	16911	8281	17057	0.977	0.991

Table 1.4 (continued) High throughput 16S microbiome reads between mother and offspring to assess vertical transmission of endosymbionts.

	identity			*Rickettsiella* reads		Total reads		Proportion *Rickettsiella*	
pair	mother	offspring	off. sex	mother	offspring	mother	offspring	mother	offspring
71	I2	I2F2-11	m	2485	6276	10124	18913	0.245	0.332
72	I2	I2F2-13	f	2485	4628	10124	14744	0.245	0.314
73	I2	I2F2-5	f	2485	13323	10124	50117	0.245	0.266
74	I2	I2F2-6	m	2485	7743	10124	36172	0.245	0.214
75	I2	I2F2-9	m	2485	8347	10124	38243	0.245	0.218
76	I2F2-5	I2F3-7	f	13323	9016	50117	38618	0.266	0.233
77	M2	M2F2-1	f	16479	10792	16974	11580	0.971	0.932
78	M2F2-1	M2F3-4	f	10792	11603	11580	12017	0.932	0.966
79	M2F3-4	M2F4-1	f	11603	20597	12017	21280	0.966	0.968
80	M4	M4F2-10	f	15607	8444	15879	13362	0.983	0.632
81	M4	M4F2-4	f	15607	12785	15879	14556	0.983	0.878
82	M4	M4F2-5	f	15607	32903	15879	33343	0.983	0.987
83	M4	M4F2-8	f	15607	35368	15879	54026	0.983	0.655
84	M4	M4F2-9	f	15607	23136	15879	23680	0.983	0.977
85	M4F2-5	M4F3-8	f	32903	4088	33343	5614	0.987	0.728
86	M5	M5F2-1	f	19511	14340	19633	14576	0.994	0.984
87	M5	M5F2-8	f	19511	12317	19633	22119	0.994	0.557
88	M5F2-1	M5F3-11	f	14340	28007	14576	28590	0.984	0.980
89	M5F2-1	M5F3-12	f	14340	22905	14576	23262	0.984	0.985
90	M5F3-11	M5F4-3	f	28007	7934	28590	8469	0.980	0.937
91	S	S4	f	8399	19273	8471	19789	0.992	0.974
92	S	S7	f	8399	4297	8471	8721	0.992	0.493
93	S7	S7F2-6	m	4297	12015	8721	12264	0.493	0.980
94	S7	S7F2-7	f	4297	7790	8721	8033	0.493	0.970
95	S7F2-7	S7F3-6	f	7790	8879	8033	11569	0.970	0.767
96	U2	U2F2-1	m	25025	4007	32489	9533	0.770	0.420
97	U5	U5F2-1	f	6897	9789	20500	28022	0.336	0.349
98	U5F2-1	U5F3-3	f	9789	7508	28022	32073	0.349	0.234
99	W1	W1F2-1	f	9246	8608	9401	11287	0.984	0.763
100	W1F2-1	W1F3-1	f	8608	10552	11287	10684	0.763	0.988
101	W4	W4F2-3	f	24199	10532	25637	11081	0.944	0.950
102	W4	W4F2-4	m	24199	9869	25637	29277	0.944	0.337

Table 1.4 (continued) High throughput 16S microbiome reads between mother and offspring to assess vertical transmission of endosymbionts.

	identity			*Rickettsiella* reads		Total reads		Proportion *Rickettsiella*	
pair	mother	offspring	off. sex	mother	offspring	mother	offspring	mother	offspring
103	X	X2	f	5512	10939	6942	14364	0.794	0.762
104	X2	X2F2-1	f	10939	7325	14364	8674	0.762	0.844
105	X2	X2F2-5	f	10939	5095	14364	11126	0.762	0.458
106	X2	X2F2-7	f	10939	3901	14364	12256	0.762	0.318
107	X2	X2F2-8	f	10939	5785	14364	22874	0.762	0.253
108	X2F2-1	X2F3-3	f	7325	12328	8674	41498	0.844	0.297
109	Y5	Y5F2-3	f	26707	11319	26928	14306	0.992	0.791
110	Y5	Y5F2-5	f	26707	26241	26928	26503	0.992	0.990
111	Y5	Y5F2-6	f	26707	16244	26928	16866	0.992	0.963
112	Y5	Y5F2-7	f	26707	8066	26928	8353	0.992	0.966
113	Y5F2-3	Y5F3-4	f	11319	11078	14306	11652	0.791	0.951
114	Y5F3-4	Y5F4-1	f	11078	5283	11652	5589	0.951	0.945
115	Z4F2-1	Z4F3-9	f	9057	16433	9170	16900	0.988	0.972
116	Z4F3-9	Z4F4-1	m	16433	4524	16900	5117	0.972	0.884
117	Z5	Z5F2-2	f	14262	10488	15409	10645	0.926	0.985
118	Z5F2-2	Z5F3-8	f	10488	18059	10645	18746	0.985	0.963
119	Z5F3-8	Z5F4-1	f	18059	6156	18746	6616	0.963	0.930

Figure 1.1 Gel visualization of PCR products from *Mermessus fradeorum* gut symbiont detection assay. *Drosophila melanogaster* prey were infected with *Wolbachia* and readily detected by diagnostic *Wolbachia* PCR. However, when the flies were fed to spiders, the *Wolbachia* was undetectable, even in spiders that had been fed a fly only one hour before. The positive control for *Wolbachia* diagnostic were previously extracted flies (n = 3).

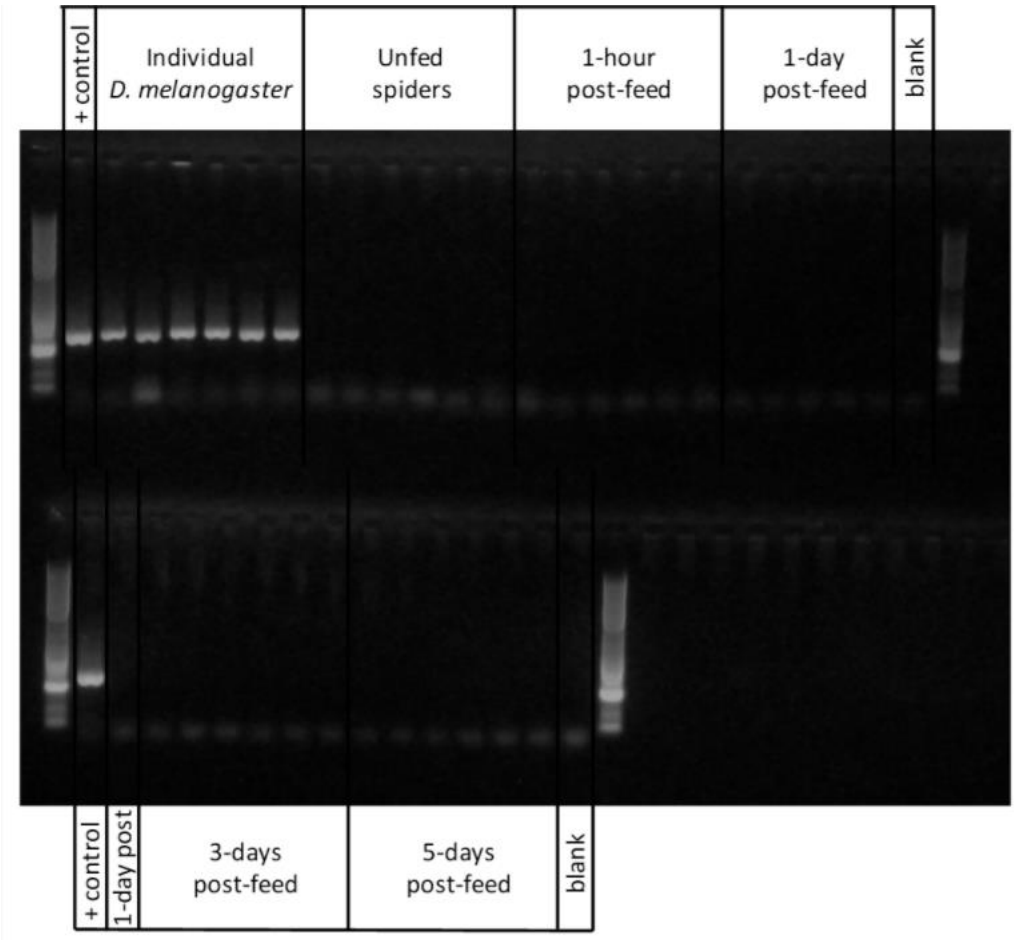


Figure 1.2 Proportional composition of 16S bacterial reads from 23 adult female *Mermessus fradeorum* spiders. All spiders were either field collected or adult offspring of different field-collected mothers. Each shade represents a different bacterial strain; different strains from the same genus are designated by different numbers in the legend. Bacteria known to be maternally transmitted are brightly colored (yellow, blue, red), other strains are shades of grey and brown.

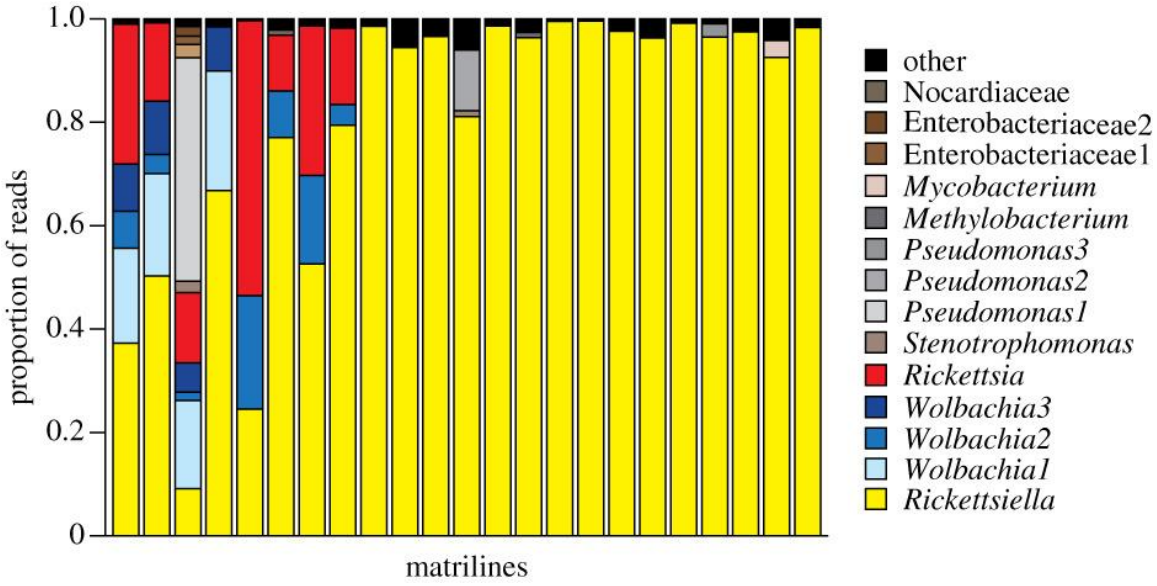
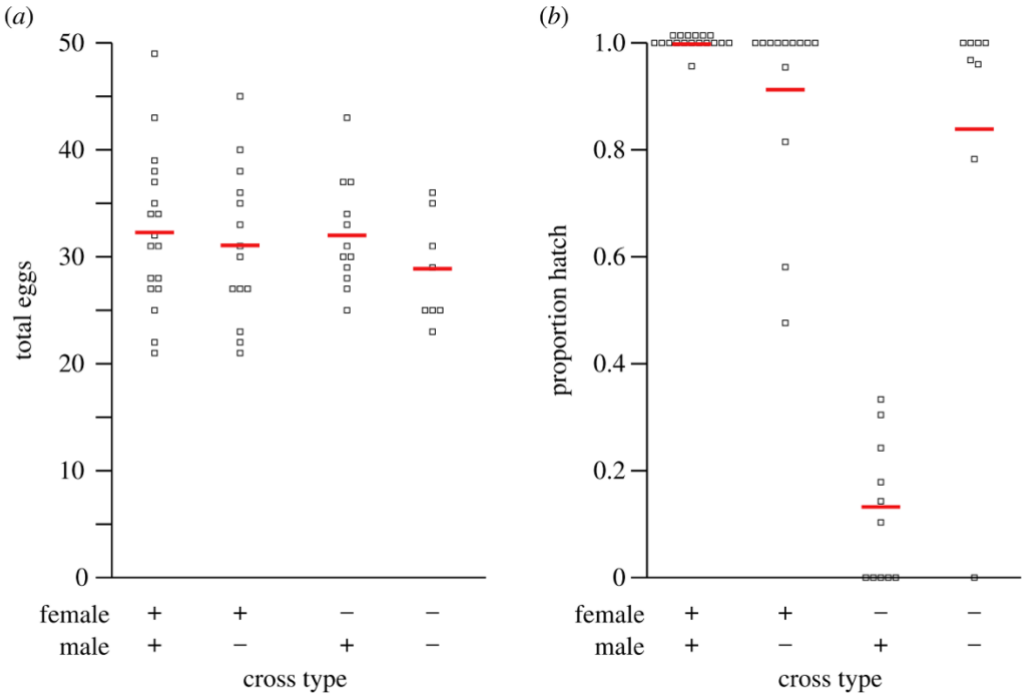


Figure 1.3 Total eggs laid (a) and proportion eggs that hatched (b) in reciprocal crosses between *Mermessus fradeorum* females and males that varied in *Rickettsiella* infection status. Each point represents the outcome from a single cross, horizontal bars represent mean value per treatment. Points above 1.0 in (b) are 1.0.



CHAPTER 2. PERVASIVE AND PERSISTENT: A SURVEY OF SYMBIOTYPES IN REGIONAL POPULATIONS OF *MERMESSUS FRADEORUM*

2.1 Introduction

Endosymbionts are microbes that inhabit the cells of other organisms (Gil et al. 2004), and which can play a diverse set of roles in their arthropod hosts, ranging from essential (Douglas 1998, Moran et al. 2008) to detrimental to the host's survival (Moran et al. 2008, İnce et al. 2018). Facultative endosymbionts, which are not essential to survival of the arthropod host, are common in terrestrial arthropods, but the distribution of these endosymbionts can be irregular in host tissues, individual hosts, and host populations (Moran et al. 2008, Weinert et al. 2015, Sazama et al. 2019). Many of these facultative endosymbionts are vertically transmitted from mother to offspring, and often use a variety of functions to persist in their arthropod hosts (Moran et al. 2008). These functions can be beneficial, such as providing protection from predators (Moran et al. 2008, Tsuchida et al. 2010, Desneux et al. 2018, Ye et al. 2018), pathogens (Brownlie and Johnson 2009, Zindel et al. 2011), or environmental stress (Brownlie and Johnson 2009, Neelakanta et al. 2010, Zindel et al. 2011), or detrimental, such as manipulating host reproduction (Duron et al. 2008a, Moran et al. 2008, Engelstädter and Hurst 2009).

Distribution of facultative endosymbiotic infections among hosts within an arthropod species is driven by multiple factors (Kondo et al. 2005, Mouton et al. 2006, 2007, Fromont et al. 2017, Bockoven et al. 2019). Biotic factors such as the host genotype (Mouton et al. 2007, Tsuchida et al. 2010) as well as the presence of other endosymbiotic infections (co-infections) (Mouton et al. 2003, Thongsripong et al. 2018) are both strong drivers in the persistence of an endosymbiotic infection within a population. Similarly, abiotic factors such as temperature can influence endosymbiotic

infections within arthropod populations (Mouton et al. 2006, Corbin et al. 2017). Symbiont community composition within a particular host species often varies over geographic space. In some cases, frequency of infection of a single symbiont differs among populations, such as *Wolbachia* in bed bugs across North America (Sakamoto and Rasgon 2006), *Wolbachia* in butterflies and moths across the world (Ahmed et al. 2015), and *Rickettsia* in whiteflies across Israel and North America (Cass et al. 2015, Bockoven et al. 2019). In other cases, geographically separated populations are infected by different strains of the same symbiont, as evidenced by the variation of *Wolbachia* strains in ants and Lycaenid butterflies across the world (Russell et al. 2009), funnel-web spiders across North America (Baldo et al. 2008), and in a Linyphiid spider in China (Yun et al. 2011). In yet other cases, multiple symbionts can be present in the same population or even co-infect the same individual, which leads to variation in the composition of differentially infected individuals from population to population. Differentially infected individuals with multiple symbionts have been documented in aphids (Najar-Rodríguez et al. 2009, Sepúlveda et al. 2017), mosquitoes (Thongsripong et al. 2018), fig homotids (Fromont et al. 2017), ticks (Steiner et al. 2008), and whiteflies (Pan et al. 2012b). Variation in infection can have important consequences for both the individual host, and the host population overall.

Inherited bacteria, particularly those that induce reproductive manipulations, can drive the evolution of the arthropod host by providing infected individuals with a selective advantage (Duron et al. 2008a, Engelstädter and Telschow 2009, Duron et al. 2017). Presence or absence of a certain endosymbiont may therefore dictate if that host genotype is able to contribute its genes to the population (Duron et al. 2008a). The

changes induced by facultative endosymbionts in an arthropod host population can often radiate out into the ecosystem and affect the ecological dynamics of the surrounding community (Duron et al. 2010, Fromont et al. 2017).

Spiders (Order Araneae) can harbor some of the highest richness and diversity of endosymbiotic infections (Goodacre et al. 2006, Duron et al. 2008a, White et al. 2020). Direct investigation of the function of endosymbiotic infections in spiders is limited, but we can infer that many are likely causing reproductive manipulations, given that similar bacterial strains cause these effects in insects (Goodacre et al. 2006, Gunnarsson et al. 2009, Perlman et al. 2010, Vanthournout and Swaegers 2011, Chapter 1). Overall, the diversity and richness of endosymbiotic infections in spiders suggest that endosymbionts may influence spider population genetics, biodiversity, and behavioral ecology (White et al. 2020). Most studies on Araneae and their endosymbiotic diversity compare among species, but a few have sought to compare the diversity of endosymbiotic infections among populations within a single species (Busck et al. 2020, White et al. 2020).

Mermessus fradeorum (Linyphiidae) shows remarkable endosymbiotic diversity. From a single population, I documented that this species was infected by up to five endosymbionts: a strain of *Rickettsia*, a strain of *Rickettsiella*, and three strains of *Wolbachia*. *Rickettsiella* proved to be ubiquitous, and was found in every matriline sampled. Further examination of *Rickettsiella* showed that it was inducing cytoplasmic incompatibility in *M. fradeorum* (Chapter 1). Of these five endosymbionts, four combinations of co-infections were found in the original 23 matrilines of *M. fradeorum*, which suggests that some combinations of these endosymbionts may be more stable than others. Interestingly, matrilines that were co-infected with all five endosymbionts, which

represented approximately 15% of the population, were predominantly feminized (Curry et al. 2015) whereas the other observed symbiont combinations were not. This single population therefore encompassed two reproductive manipulation phenotypes as well as five facultative endosymbionts. However, little was known about other *M. fradeorum* populations and their endosymbiotic infections.

The purpose of the present study was to document endosymbiotic infection of *M. fradeorum* across populations in central Kentucky. Specifically, I sought to determine (1) if *Rickettsiella* was similarly pervasive in other populations, (2) if additional facultative endosymbiont strains were present, (3) if different combinations of facultative endosymbionts (symbiotypes) were present in other populations, and (4) if the feminization symbiotype was present in other populations and consistently associated with a female-biased phenotype. This study will provide more context about the dynamics of these endosymbiotic infections in *M. fradeorum* and how they may fluctuate among populations of a host species.

2.2 Materials and methods

Spiders were collected between the months of June-October 2019 across 10 locations in four counties of central Kentucky: Mercer, Woodford, Shelby, and Taylor (Table 2.1). Each sampling location was an alfalfa field, some of which also contained grasses. Fields were sampled at various times relative to cutting, and ranged from the alfalfa being approximately 2 inches tall to the alfalfa being at least 2 feet tall. Locations that yielded high numbers of *M. fradeorum* in the first sampling were sampled a second time at the same sites. At least one location per county was sampled twice during the collection period. All spiders that appeared to be members of Linyphiidae that inhabited

the base of plants in sheetwebs were collected in 1.5mL microcentrifuge tubes. Overall, 408 spiders and one egg mass were collected. Initial *M. fradeorum* identification was based on morphology, and confirmed by viable matings with the resident lab population of *M. fradeorum*. Offspring from these matings were kept and raised in the lab, and were propagated for at least two generations. Sex of offspring that reached adulthood was recorded. Out of the 409 specimens collected, 62 spiders and the egg mass were confirmed to be *M. fradeorum.*

Once in the lab, individual spiders were kept in deli cups (6cm diam.) with hydrated plaster in the bottom for humidity control and maintained in the laboratory at 22^{o}C 24 hour dark conditions. Juvenile spiders were fed symbiont-free collembola (*Sinella curviseta*) until maturity, and adult spiders were fed fruit flies (*Drosophila melanogaster*) at 2-3 day intervals. These flies were infected with a distinct strain of *Wolbachia* (MLST ST1) (Baldo et al. 2006), but this prey symbiont was not detected in our diagnostic assays or microbiome screens of the predators (Chapter 1).

To screen for the symbionts of *M. fradeorum*, DNA from field-caught males was extracted following mating, whereas field-caught females were allowed to lay up to four egg masses before their DNA was extracted. If the female died due to natural causes prior to extraction, her adult offspring were screened instead. Spiders were starved for at least 5 days prior to extraction. Each specimen was surface-sterilized with a series of washes with 0.5% bleach and PCR water. Total DNA was extracted from each whole specimen using DNEasy Blood and Tissue kits (Qiagen, Germantown, MD) according the manufacturer's instructions.

Symbiont community composition for each matriline was determined initially using diagnostic PCR. All extractions were first screened with primers for a portion of the arthropod COI gene (Folmer et al. 1994; Table 2.2), to confirm extraction quality. All extractions yielded a band, indicating that the extraction quality was good. The samples were then screened using diagnostic PCR for the endosymbionts found in the originally sampled population: *Rickettsia, Rickettsiella,* and *Wolbachia* (Table 2.2). To determine the strain types of *Wolbachia*, I used the Restriction Fragment Length Polymorphism (RFLP) technique (Regnery et al. 1991), which uses a restriction enzyme to selectively cleave PCR products. In this case, I digested the *wsp* PCR product from *Wolbachia*-positive samples with the (DdeI) restriction enzyme (New England Biolabs) which differentially cut the three different *Wolbachia* strains (Figure 2.1). Every diagnostic PCR was run at least twice for confirmation of symbiont presence.

I additionally used high throughput 16S sequencing to determine the symbiont community of *M. fradeorum.* The 16S sequencing was used to validate diagnostic PCR results, and to explore whether other endosymbiont strains infected *M. fradeorum*. As described in the previous chapter, the V4 region of bacterial 16S was amplified using dual-indexed 515F/806R primers (Kozich et al. 2013), and a 1μL aliquot of PCR product from each sample was assembled into a multiplexed library for sequencing (Chapter 1). Sequences were then quality filtered and organized into operational taxonomic units (OTUs) using qiime2 (Chapter 1). Overall, 102 spiders were included in the high throughput sequencing screen, representing 63 matrilines, including some offspring from those matrilines to confirm vertical transmission.

The symbiotype for each matriline was designated based on a combination of the diagnostic PCR and high throughput 16S sequencing results. Matrilines used in the analysis were those in which both methods yielded a consistent infection profile. Three matrilines failed to yield results from the high throughput sequencing and did not have additional offspring to screen, and two matrilines produced different results between the high throughput 16S sequencing and diagnostic PCRs that were unable to be resolved. Therefore, 5 matrilines were removed from the analysis, leaving 75 spiders across 58 matrilines that had consistent results between the diagnostic PCRs and the high throughput 16S sequencing.

Based on data from the high throughput 16S sequencing, an apparent second strain of *Rickettsia* was documented in two separate matrilines, originating from two different populations. I used Sanger sequencing to confirm the presence of this second strain of *Rickettsia*. *Rickettsia* DNA was amplified in these samples using RicklongF/RicklongR primers (Table 2.2), and an aliquot of the resulting product was visualized on a 1% agarose gel stained with Gel Red (Biotium). Following purification using GenCatch PCR clean up kit (Epoch Life Sciences, Missouri City, TX), 10μL of PCR product was combined with a 5μL aliquot of 10μM primers and sent to GeneWiz (Plainfield, NJ) for sequencing. Sanger sequencing results were visualized using Geneious R6 (San Diego, CA), and the NCBI BLAST tool (Bethesda, MD).

To compare how the symbiont community composition differed across the regional populations in Kentucky, data from each county (n = 5 counties) was combined across locations and sampling times, as sample sizes were small (Table 2.1). The originally sampled 23 matrilines from Fayette County (Chapter 1) were also included in

the analysis as a county. I then compared the frequency of the most common symbiotype, *Rickettsiella*-only, to a combined category of all other symbiotypes, many of which were rare and precluded more individualized analyses. I used a contingency table analysis with a Chi-Squared test statistic (R Console v. 4.0.2).

To ask whether the five-fold infected symbiotype was consistently associated with a female bias, I compared descendent sex data in the newly sampled counties as well as the original Fayette County population (Chapter 1). Matrilines that were infected with the second strain of *Rickettsia* were excluded from subsequent analyses of female-bias, as one matriline was infected with all 6 potential endosymbiotic infections, and it was not clear which category it should be included in for the statistical analysis. In total, 38 matrilines from the new counties were included, and 29 from Fayette County. The Fayette County matrilines consisted of 22 of the 23 matrilines that were originally collected in 2016 (Chapter 1), as well as 7 additional matrilines that were collected from the same location in 2017-2018. The single matriline from the group of 2016 matrilines was excluded due to inconsistencies of symbiotype between the mother and her offspring. Logistic regression was used to determine whether the five-fold infected symbiotype was more likely to produce females compared to the other symbiotypes (Arc v. 1.06).

2.3 Results

Of the fifty-eight matrilines sampled from four new counties in central Kentucky, all were infected with at least one of the five endosymbionts that were originally found in Fayette County (Chapter 1). All five endosymbionts were found in all counties except for Taylor County (n=15 matrilines) where the third strain of *Wolbachia* was not documented (Figure 2.2). *Rickettsiella* was the most widespread symbiont in all counties sampled, and

infected 57 of the 58 matrilines that were screened. When I compared the frequency of *Rickettsiella*-only versus other symbiotypes among the five counties, I found that the frequency does vary among the counties (χ^2= 13.603, df =4, p = 0.0086). This variation in symbiotype distribution is driven by the contrast between the two counties with the largest sample sizes. In Woodford County (n = 22 matrilines), only 32% of the matrilines were infected with only *Rickettsiella*, and most matrilines had more complicated symbiotypes that had other symbionts in combination with *Rickettsiella*. In contrast, the matrilines in Taylor County (n=15 matrilines) were majority *Rickettsiella*-only, and only two matrilines had co-infections with the other symbiont strains.

High throughput 16S sequencing detected a second strain of *Rickettsia* in two matrilines that were from two separate counties. The high throughput sequences showed that this strain was 98.8% similar to the more common *Rickettsia* strain (3 SNPs over 251 bases), and mapped to a separate clade of *Rickettsia* endosymbionts (Watanabe et al. 2014, Curry et al. 2015). Sanger sequencing of two individuals that had reads for this second *Rickettsia* showed that there were double peaks on the chromatogram, which is consistent with the co-infection of two *Rickettsia* strains. The original *Rickettsia* strain documented in *M. fradeorum* (Curry et al. 2015) was identical to the predominant strain of *Rickettsia* documented in this study, and therefore the second strain of *Rickettsia* could be inferred by choosing the alternative peak at the double peak locations. Over 238 bases, the two *Rickettsia* sequences had 8 SNPs (96.6% similarity). When combined with the high throughput sequencing results, these two *Rickettsia* strains share 97.8% similarity over 489 bases (11 SNPs). Use of the NCBI BLAST tool (Bethesda, MD) suggests that this second strain of *Rickettsia* has fewer similar sequences documented, but that it is

identical over this short sequence to a strain of *Rickettsia* that has been documented in a spider from the same habitat, *Idionella rugosa* (White et al. 2020).

With the additional documentation of the second strain of *Rickettsia*, *M. fradeorum* can be infected with up to six different strains of endosymbiont. However, even with the addition of the sixth potential infection, only ten symbiotypes were documented in the 58 matrilines sampled in this study (Figure 2.2). Half of all the matrilines sampled across all counties were only infected with *Rickettsiella*. The next two most common symbiotypes were matrilines that were co-infected with *Rickettsiella*, *Rickettsia,* and all three strains of *Wolbachia* (19%) and matrilines that were co-infected with *Rickettsiella, Rickettsia,* and the second strain of *Wolbachia* (16%). All of the other symbiotypes represented only 2-3% of sampled matrilines (1-2 matrilines). However, symbiotypes were not consistent among counties, most notably with Taylor County lacking the five-fold infected symbiotype (Curry et al. 2015, Figure 2.2).

Female-bias was associated with the five-fold symbiotype, but the phenotype was not as consistent as observed in the original Fayette County sampling. In Fayette County, the five-fold infected symbiotype produced more females than the other symbiotypes across the 29 matrilines that produced offspring (Wald = 3.673, p = 0.0002; Table 2.3). In the newly sampled counties, the female-bias was still evident in the five-fold infected symbiotype when compared to the other symbiotypes (Wald =2.772, p = 0.0056). The lowest female bias documented in a matriline from Fayette County with the five-fold infected symbiotype was 70% female (n =10 total offspring). However, two matrilines out of the eight from the newly sampled counties did not appear to display the feminization phenotype as strongly: one in Woodford County only produced 63% female

offspring (n =27 total offspring), and one in Mercer County only produced 57% female offspring (n = 7 total offspring). Two other symbiotypes also appeared to be majority female, but had few matrilines available to test for statistical significance: the single matriline infected with all potential symbiont strains except the third strain of *Wolbachia*, and the two matrilines infected with *Rickettsiella* and the second strain of *Wolbachia*. All of the other symbiotypes were evenly split between males and females (Table 2.3).

2.4 Discussion

Endosymbiotic infections of *M. fradeorum* across regional populations in central Kentucky largely shared the same symbionts, but the frequency of those infections differed among the counties sampled. All five of the original endosymbiotic infections were documented in the newly sampled counties, except for *Wolbachia* 3 in Taylor County. This survey of *M. fradeorum* populations across central Kentucky additionally documented the presence of a second strain of *Rickettsia*. These spiders are therefore capable of being infected with up to six different endosymbiont strains. At least one of these (*Rickettsiella*) is a documented reproductive manipulator of *M. fradeorum* (Chapter 1).

Rickettsiella remained the most pervasive symbiont in all the sampled matrilines. *Rickettsiella* infected all but one matriline, suggesting near fixation in the region. This result is not surprising, as endosymbionts that induce cytoplasmic incompatibility are expected to reach high prevalence in a population, provided that the endosymbiotic infection is able to establish in the host population (Engelstädter and Hurst 2009, Engelstädter and Telschow 2009, Jaenike 2009, Shropshire et al. 2020). Therefore, spiders that are infected with *Rickettsiella* appear to be at a selective advantage within

these populations. It is unclear whether or not other endosymbionts in this population are also causing CI or enhancing the CI trait of *Rickettsiella*. Speculatively, with the coexistence of two other genera that are known to induce reproductive manipulations, it is possible that *Rickettsiella* was able to cross the invasion threshold to establish in this population because of the association with the other endosymbiotic strains within this host population. Presence or absence of co-infections can dictate the spread of an endosymbiotic infection through a population, and therefore co-infections of *Rickettsia* and *Wolbachia* may have assisted *Rickettsiella* in the spread through the host population (Kondo et al. 2005, Steiner et al. 2008, Zhu et al. 2012). It is also possible that *Rickettsiella* obtained the CI trait from a co-infection in *M. fradeorum* through a horizontal gene transfer event and then rapidly spread through the host population (Ochman et al. 2000). However, more data is necessary to make conclusions about the origin of cytoplasmic incompatibility induction in *Rickettsiella* and its establishment in *M. fradeorum*.

Overall, the frequency of the various infections in *M. fradeorum* differed among counties. The top three symbiotypes were spiders that were only infected with *Rickettsiella*, spiders that were co-infected with *Rickettsiella, Wolbachia 2,* and *Rickettsia,* and spiders that were co-infected with *Rickettsiella,* all three strains of *Wolbachia,* and *Rickettsia.* These appear to be the most stable combinations of endosymbiotic infections in the regional populations of *M. fradeorum* in central Kentucky. Fluctuation of endosymbiotic infection frequency in a host population can be due to a number of different factors including bacterial strain types, host species and genotypes, and abiotic factors of the surrounding environment, such as temperature

(Kondo et al. 2005, Mouton et al. 2006, 2007, Fromont et al. 2017, Bockoven et al. 2019). The infrequency of the other symbiotypes suggests that they may be generated by imperfect vertical transmission from mother to offspring, and that some combinations of co-infections may be more stable than others (Brownlie and Johnson 2009, Ferrari et al. 2011). Given that all the documented reproductive manipulators that infect *M. fradeorum* are vertically transmitted (Chapter 1), it follows that a failure to transmit most likely shapes the symbiotypes in these populations (Rock et al. 2018).

Regional expansion of the dataset of endosymbiotic infections of *M. fradeorum* led to documentation of a second strain of *Rickettsia* that was present in two separate populations: one matriline in Mercer County and one matriline in Taylor County. Given the infrequency of this infection, we can conclude that the second strain of *Rickettsia* is not a large contributor to the endosymbiotic reproductive manipulations of *M. fradeorum*, at least not within the local area. With the addition of the second strain of *Rickettsia*, these spiders have now been documented to be infected with up to six different strains of potential endosymbiotic reproductive manipulators, and therefore *M. fradeorum* has some of the highest symbiont richness documented thus far in arthropods (White et al. 2020).

The feminized symbiotype (those that were co-infected with *Rickettsiella*, *Rickettsia*, and all three strains of *Wolbachia*), was present in three of the new counties sampled, but was not evident in Taylor County. Taylor County was the furthest county from the others, at a distance of approximately 73 miles from the originally sampled location in Fayette County. This suggests that increasing distances increases differences within the symbiont community, as predicted by other studies that focus on geographic differences among populations and their endosymbiotic infections, but more sampling is

needed (Pan et al. 2012b). In general, cytoplasmic incompatibility can restrict gene flow in populations based on infection, thus potentially leading to reproductive isolation and eventual speciation, but there is currently no evidence of this in *M. fradeorum* (Coyne et al. 1988, Gebiola et al. 2016, Garrick et al. 2017). However, the data suggest that the population documented in Fayette County is fairly representative of the regional populations of *M. fradeorum* that were examined in this study. In particular, the persistence of *Rickettsiella* though almost every single matriline sampled emphasizes the similarities in populations throughout the region.

The five-fold infected symbiotype exhibited a female-bias in comparison to the other symbiotypes. While each county had at least one matriline with the feminization symbiotype Most of the five-fold infected matrilines exhibited strong female bias, but two, originating from separate counties (one from Woodford County and one from Mercer County) had only slightly female biased offspring. The mechanism of feminization is not yet clear, nor is it clear whether a single symbiont or multiple cooperating symbionts cause the phenotype. Additionally, there are not enough representatives of other symbiotypes to fully elucidate which endosymbiont or combinations may be inducing the bias. Speculatively, the inconsistency of the phenotype may be due to differences in titer of one or more of the endosymbiotic bacterial strains (Engelstädter and Hurst 2009, Herren et al. 2013, Bockoven et al. 2019), but further research is necessary.

Endosymbiotic infections are pervasive and persistent in *M. fradeorum*, and are responsible for at least two reproductive manipulation phenotypes in their hosts. This survey of other populations of *M. fradeorum* has documented that these endosymbiotic

infections are largely consistent with our originally sampled population, and that the original population was representative of the region. However, there do appear to be fluctuations in the proportional representation of the dominant symbiotypes from population to population, suggesting dynamic changes in composition over time and space. The data emphasize that these regional *M. fradeorum* populations may be heavily influenced by their endosymbiotic infections. Future studies should focus on expanding sampling to provide more context about *M. fradeorum* and their endosymbiotic infections.

Table 2.1 Collection information for each county. Sites that are listed twice indicate a second collection at the same fields.

Site	Date Collected (2019)	County Collected	Description of location	Males	Females	Egg masses
DeHart	5-Aug	Mercer	Mostly alfalfa, some grass, ~6" cut recently	0	1	0
Ison	5-Aug	Mercer	Mostly alfalfa, some orchard grass, some regular grass, ~2ft	0	3	0
Ison	3-Sep	Mercer	Mostly alfalfa, some orchard grass, some regular grass, ~2ft	1	6	0
Lakes	5-Aug	Mercer	mostly alfalfa but a lot of grass, field not touched for a year	0	1	0
Flowers	11-Jun	Shelby	Mostly alfalfa, ~1ft tall, about to harvest	0	1	0
Tipton	11-Jun	Shelby	Mixture of Alf and grass with some corn, 6" tall	0	2	0
Tipton	3-Oct	Shelby	Mixture of alfalfa and grass with some corn, 6" tall (SUPER DRY)	0	7	0
Cave	3-Jul	Taylor	Mostly alfalfa, 1ft. tall	0	6	0
Cave	17-Oct	Taylor	About 8” tall, sprayed since last visit, also super dry	4	2	0
Cox	3-Jul	Taylor	Mostly alfalfa (round-up ready), some clover ~2ft.	0	4	1
Congleton	29-Jul	Woodford	Very short and stubby alfalfa 3”, just cut	0	1	0
Horn	29-Jul	Woodford	Mixture of alfalfa and grass, mostly grass, ~1ft.	0	3	0
Horn	29-Aug	Woodford	Mixture of alfalfa and grass, mostly grass, ~1ft	5	15	0
LSmith	29-Jul	Woodford	Mixture of alfalfa and grass, mostly grass, 1ft	0	1	0
			Totals	10	53	1

Table 2.2 Diagnostic primers used for analysis of symbiont community composition of *M. fradeorum* populations.

Target	Target gene	Primer name	Primer sequence 5' to 3'	Expected product size (bases)	Annealing temp
Rickettsia[1]	16S	RicklongF RicklongR	ACGTGGGAATCTACCCATCA TAGCCTAGATGACCGCCTTC	~500	60°C
Rickettsiella[2]	16S	RLA16s F1 RLA16s R1	CAGTAAARRTTTCGGYCTTTAYGGG CAAACCTAGTCAACCACCTACACG	~530	56°C
Wolbachia[3]	wsp	wspF1 wspR1	GTCCAATARSTGATGARGAAAC CYGCACCAAYAGYRCTRTAAA	~500	59°C
Spider[4]	COI	lco1490 hco2198	GGTCAACAAATCATAAAGATATTGG TAAACTTCAGGGTGACCAAAAAATCA	~650	53°C

[1]Curry et al. 2015, [2]Duron et al. 2016, [3]Baldo et al. 2006, [4]Folmer et al. 1994

Table 2.3 Offspring sex across F1 and F2 generations across matrilines found in all counties. New counties refer to those sampled in 2019 (Woodford, Mercer, Shelby and Taylor).

	E W_1 W_2 W_3 R_1 R_2	E W_1 W_2 R_1 R_2	E W_1 W_2 W_3 R_1	E W_2 W_3 R_1	E W_2 R_1	E R_1	E W_1 W_3	E W_2	E	R_1
Fayette County										
# matrilines	-	-	5	-	4	-	1	1	18	-
# males	-	-	13	-	30	-	3	20	158	-
# females	-	-	130	-	32	-	8	36	181	-
Total	-	-	143	-	62	-	11	56	339	-
Total proportion female	-	-	0.91	-	0.52	-	0.72	0.64	0.53	-
New counties										
# matrilines	1	1	8	1	4	3	1	2	24	1
# males	7	0	20	8	7	32	14	2	95	4
# females	6	5	71	6	9	38	9	10	78	3
Total	13	5	91	14	16	70	23	12	173	7
Total proportion female	0.46	1.0	0.78	0.42	0.56	0.54	0.39	0.83	0.45	0.42
Total (all counties)	13	5	234	14	78	70	34	68	512	7
Total proportion female (all counties)	0.46	1.0	0.85	0.42	0.48	0.54	0.5	0.68	0.51	0.42

E = *Rickettsiella*, W = *Wolbachia*, R = *Rickettsia*

Figure 2.1 Example of DdeI restriction enzyme digest for *Wolbachia* strain typing.

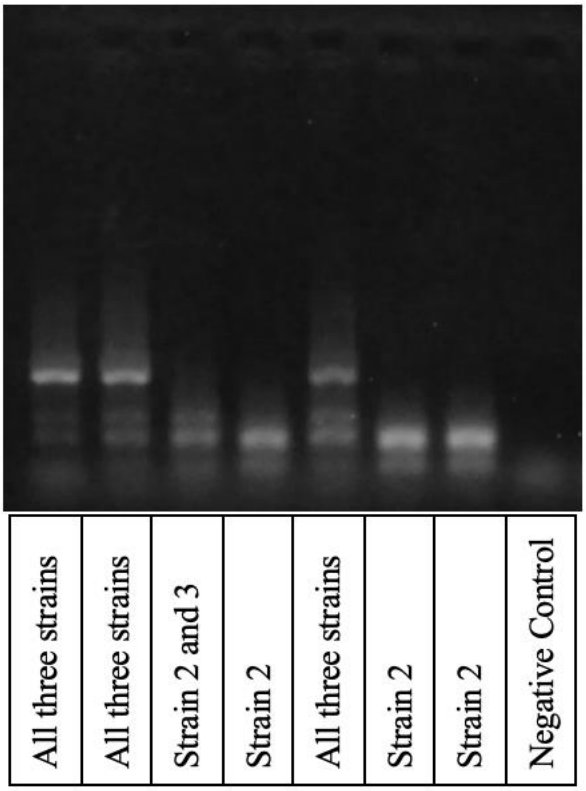
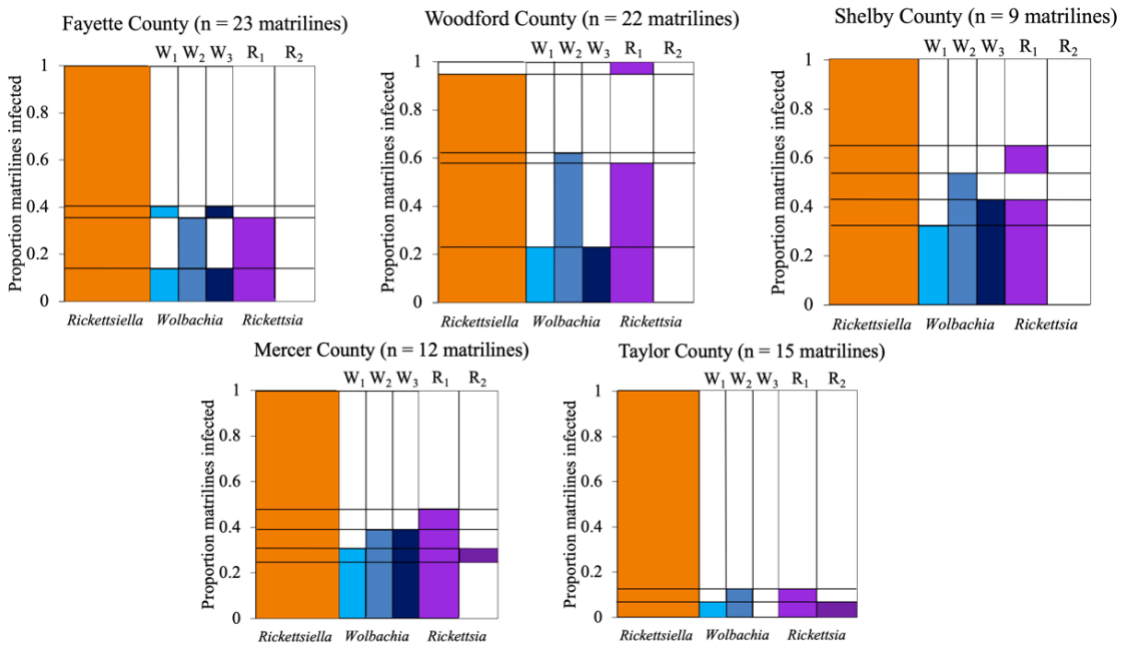


Figure 2.2 Endosymbiotic infections of all sampled populations of *M. fradeorum*. Fayette County represents the population sampled in Chapter 1. Horizontal bands represent co-infecting symbionts in the same host matrilines.



REFERENCES

Ahmed, M. Z., E. V. Araujo-Jnr, J. J. Welch, and A. Y. Kawahara. 2015. *Wolbachia* in butterflies and moths: Geographic structure in infection frequency. Front. Zool. 12: 1–9.

Angelella, G., V. Nalam, P. Nachappa, J. White, and I. Kaplan. 2018. Endosymbionts differentially alter exploratory probing behavior of a nonpersistent plant virus vector. Microb. Ecol. 76: 453–458.

Baldo, L., N. A. Ayoub, C. Y. Hayashi, J. A. Russell, J. K. Stahlhut, and J. H. Werren. 2008. Insight into the routes of *Wolbachia* invasion : high levels of horizontal transfer in the spider genus *Agelenopsis* revealed by *Wolbachia* strain and mitochondrial DNA diversity. Mol. Ecol. 17: 557–569.

Baldo, L., J. C. D. Hotopp, K. A. Jolley, S. R. Bordenstein, S. A. Biber, R. R. Choudhury, C. Hayashi, M. C. J. Maiden, H. Tettelin, and J. H. Werren. 2006. Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. Appl. Environ. Microbiol. 72: 7098–7110.

Beckmann, J. F., J. A. Ronau, and M. Hochstrasser. 2017. A *Wolbachia* deubiquitylating enzyme induces cytoplasmic incompatibility. Nat. Microbiol. 2: 1–7.

Bockoven, A. A., E. C. Bondy, M. J. Flores, S. E. Kelly, A. M. Ravenscraft, and M. S. Hunter. 2019. What goes up might come down: the spectacular spread of an endosymbiont is followed by its decline a decade later. Microb. Ecol. 79: 482-494

Bokulich, N. A., S. Subramanian, J. J. Faith, D. Gevers, J. I. Gordon, R. Knight, D. A. Mills, and J. G. Caporaso. 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods. 10: 57–59.

Bordenstein, S. R., P. F. O'Hara, and J. H. Werren. 2001. *Wolbachia*-induced incompatibility precedes other hybrid incompatibilities in *Nasonia*. Nature. 409: 707–710.

Brownlie, J. C., and K. N. Johnson. 2009. Symbiont-mediated protection in insect hosts. Trends Microbiol. 17: 348–354.

Busck, M. M., V. Settepani, J. Bechsgaard, M. B. Lund, T. Bilde, and A. Schramm. 2020. Microbiomes and specific symbionts of social spiders: compositional patterns in host species, populations, and nests. Front. Microbiol. 11: 1–14.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. Mcdonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods. 7: 335–336.

Cass, B. N., R. Yallouz, E. C. Bondy, N. Mozes-Daube, A. R. Horowitz, S. E. Kelly, E. Zchori-Fein, and M. S. Hunter. 2015. Dynamics of the endosymbiont *Rickettsia* in an insect pest. Microb. Ecol. 70: 287–297.

Corbin, C., E. R. Heyworth, J. Ferrari, and G. D. D. Hurst. 2017. Heritable symbionts in a world of varying temperature. Heredity. 118: 10–20.

Cordaux, R., D. Bouchon, and P. Grève. 2011. The impact of endosymbionts on the evolution of host sex-determination mechanisms. Trends Genet. 27: 332–341.

Cordaux, R., M. Paces-Fessy, M. Raimond, A. Michel-Salzat, M. Zimmer, and D. Bouchon. 2007. Molecular characterization and evolution of arthropod-pathogenic *Rickettsiella* bacteria. Appl. Environ. Microbiol. 73: 5045–5047.

Coyne, J. A., H. A. Orr, and D. J. Futuyma. 1988. Do we need a new species concept? Syst. Zool. 37: 190–200.

Curry, M. M., L. V Paliulis, K. D. Welch, J. D. Harwood, and J. A. White. 2015. Multiple endosymbiont infections and reproductive manipulations in a linyphiid spider population. Heredity. 115: 146–152.

DeSantis, T. Z., P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu, and G. L. Andersen. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72: 5069–5072.

Desneux, N., M. K. Asplen, C. M. Brady, G. E. Heimpel, K. R. Hopper, C. Luo, L. Monticelli, K. M. Oliver, and J. A. White. 2018. Intraspecific variation in facultative symbiont infection among native and exotic pest populations: potential implications for biological control. Biol. Control. 116: 27–35.

Douglas, A. E. 1998. Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera* . Annu. Rev. Entomol. 43: 17–37.

Duron, O., F. Binetruy, V. Noël, J. Cremaschi, K. D. McCoy, C. Arnathau, O. Plantard, J. Goolsby, A. A. Pérez de León, D. J. A. Heylen, A. R. Van Oosten, Y. Gottlieb, G. Baneth, A. A. Guglielmone, A. Estrada-Peña, M. N. Opara, L. Zenner, F. Vavre, and C. Chevillon. 2017. Evolutionary changes in symbiont community structure in ticks. Mol. Ecol. 26: 2905–2921.

Duron, O., D. Bouchon, S. Boutin, L. Bellamy, L. Zhou, J. Engelstädter, and G. D. Hurst. 2008a. The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. BMC Biol. 6: 1–12.

Duron, O., J. Cremaschi, and K. D. McCoy. 2016. The high diversity and global distribution of the intracellular bacterium *Rickettsiella* in the polar seabird tick *Ixodes uriae*. Microb. Ecol. 71: 761–770.

Duron, O., G. D. Hurst, E. Hornett, J. Josling, and J. Engelstädter. 2008b. High incidence of the maternally inherited bacterium *Cardinium* in spiders. Mol. Ecol. 17: 1427–1437.

Duron, O., T. E. Wilkes, and G. D. D. Hurst. 2010. Interspecific transmission of a male-killing bacterium on an ecological timescale. Ecol. Lett. 13: 1139–1148.

Engelstädter, J., and G. D. D. Hurst. 2009. The ecology and evolution of microbes that manipulate host reproduction. Annu. Rev. Ecol. Evol. Syst. 40: 127–149.

Engelstädter, J., and A. Telschow. 2009. Cytoplasmic incompatibility and host population structure. Heredity. 103: 196–207.

Ferrari, J., J. A. West, S. Via, and H. C. J. Godfray. 2011. Population genetic structure and secondary symbionts in host-associated populations of the pea aphid complex. Evolution. 66: 375–390.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3: 294–299.

Fromont, C., M. Riegler, and J. M. Cook. 2017. Relative abundance and strain diversity in the bacterial endosymbiont community of a sap-feeding insect across its native

and introduced geographic range. Microb. Ecol. 74: 722–734.
Garrick, R. C., Z. L. Sabree, B. C. Jahnes, and J. C. Oliver. 2017. Strong spatial-genetic congruence between a wood-feeding cockroach and its bacterial endosymbiont, across a topographically complex landscape. J. Biogeogr. 44: 1500–1511.
Gebiola, M., S. E. Kelly, P. Hammerstein, M. Giorgini, M. S. Hunter, M. Gebiola, S. E. Kelly, P. Hammerstein, M. Giorgini, and M. S. Hunter. 2016. "Darwin's corollary" and cytoplasmic incompatibility induced by *Cardinium* contribute to speciation in *Encarsia* wasps (Hymenoptera : Aphelinidae). Evolution. 70: 2447–2458.
Gil, R., A. Latorre, and A. Moya. 2004. Bacterial endosymbionts of insects: Insights from comparative genomics. Environ. Microbiol. 6: 1109–1122.
Gillespie, J. J., T. P. Driscoll, V. I. Verhoeve, M. S. Rahman, K. R. Macaluso, and A. F. Azad. 2018. A tangled web: Origins of reproductive parasitism. Genome Biol. Evol. 10: 2292–2309.
Goodacre, S. L., O. Y. Martin, C. F. G. Thomas, and G. M. Hewitt. 2006. *Wolbachia* and other endosymbiont infections in spiders. Mol. Ecol. 15: 517–527.
Gotoh, T., H. Noda, and S. Ito. 2007. *Cardinium* symbionts cause cytoplasmic incompatibility in spider mites. Heredity. 98: 13–20.
Gunnarsson, B., S. L. Goodacre, and G. M. Hewitt. 2009. Sex ratio, mating behaviour and *Wolbachia* infections in a sheetweb spider. Biol. J. Linn. Soc. 98: 181–186.
Herren, J. K., J. C. Paredes, F. Schüpfer, and B. Lemaitre. 2013. Vertical transmission of a *Drosophila* endosymbiont via cooption of the yolk transport and internalization machinery. MBio. 4: 1–8.
Hunter, M. S., S. J. Perlman, and S. E. Kelly. 2003. A bacterial symbiont in the Bacteroidetes induces cytoplasmic incompatibility in the parasitoid wasp *Encarsia pergandiella*. Proc. R. Soc. B Biol. Sci. 270: 2185–2190.
İnce, İ. A., O. Özcan, A. Z. Ilter-Akulke, E. D. Scully, and A. Özgen. 2018. Invertebrate iridoviruses: a glance over the last decade. Viruses. 10: 1–25.
Ishmael, N., J. C. D. Hotopp, P. Loanidis, S. Biber, J. Sakamoto, S. Siozios, V. Nene, J. Werren, K. Boutriz, S. R. Bordenstein, and H. Tettelin. 2009. Extensive genomic diversity of closely related *Wolbachia* strains. Microbiology. 155: 2211–2222.
Jaenike, J. 2009. Coupled population dynamics of endosymbionts within and between hosts. Oikos. 118: 353–362.
Koga, R., T. Tsuchida, M. Sakurai, and T. Fukatsu. 2007. Selective elimination of aphid endosymbionts: effects of antibiotic dose and host genotype, and fitness consequences. FEMS Microbiol. Ecol. 60: 229–239.
Kondo, N., M. Shimada, and T. Fukatsu. 2005. Infection density of *Wolbachia* endosymbiont affected by co-infection and host genotype. Biol. Lett. 1: 488–491.
Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander, and P. D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. Appl. Environ. Microbiol. 79: 5112–5120.
Kurtti, T. J., A. T. Palmer, and J. H. Oliver. 2002. *Rickettsiella*-like bacteria in *Ixodes woodi* (Acari: Ixodidae). J. Med. Entomol. 39: 534–540.
Larsson, A. J. M., G. Stanley, R. Sinha, I. L. Weissman, and R. Sandberg. 2018. Computational correction of index switching in multiplexed sequencing libraries. Nat. Methods. 15: 305–307.

LePage, D. P., J. A. Metcalf, S. R. Bordenstein, J. On, J. I. Perlmutter, J. D. Shropshire, E. M. Layton, L. J. Funkhouser-Jones, J. F. Beckmann, and S. R. Bordenstein. 2017. Prophage WO genes recapitulate and enhance *Wolbachia*-induced cytoplasmic incompatibility. Nature. 543: 243–247.

Mains, J. W., C. L. Brelsfoard, R. I. Rose, and S. L. Dobson. 2016. Female adult *Aedes albopictus* suppression by *Wolbachia*-infected male mosquitoes. Sci. Rep. 6: 1–7.

Moran, N. A., J. P. McCutcheon, and A. Nakabachi. 2008. Genomics and evolution of heritable bacterial symbionts. Annu. Rev. Genet. 42: 165–190.

Mouton, L., H. Henri, M. Bouletreau, and F. Vavre. 2003. Strain-specific regulation of intracellular *Wolbachia* density in multiply infected insects. Mol. Ecol. 12: 3459–3465.

Mouton, L., H. Henri, M. Bouletreau, and F. Vavre. 2006. Effect of temperature on *Wolbachia* density and impact on cytoplasmic incompatibility. Parasitology. 132: 49–56.

Mouton, L., H. Henri, D. Charif, M. Boulétreau, and F. Vavre. 2007. Interaction between host genotype and environmental conditions affects bacterial density in *Wolbachia* symbiosis. Biol. Lett. 3: 210–213.

Najar-Rodríguez, A. J., E. A. McGraw, R. K. Mensah, G. W. Pittman, and G. H. Walter. 2009. The microbial flora of *Aphis gossypii:* Patterns across host plants and geographical space. J. Invertebr. Pathol. 100: 123–126.

Neelakanta, G., J. F. Anderson, E. Fikrig, G. Neelakanta, H. Sultana, D. Fish, J. F. Anderson, and E. Fikrig. 2010. *Anaplasma phagocytophilum* induces *Ixodes scapularis* ticks to express an antifreeze glycoprotein gene that enhances their survival in the cold. J. Clin. Invest.120: 3179–3190.

O'Neill, S. L., R. Giordano, A. M. Colbert, T. L. Karr, and H. M. Robertson. 1992. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proc. Natl. Acad. Sci. 89: 2699–2702.

Ochman, H., J. G. Lawerence, and E. A. Groisman. 2000. Lateral gene transfer and the nature of bacterial innovation. Nature. 405: 299–304.

Pan, H., X. Li, D. Ge, S. Wang, Q. Wu, W. Xie, X. Jiao, D. Chu, B. Liu, B. Xu, and Y. Zhang. 2012b. Factors affecting population dynamics of maternally transmitted endosymbionts in *Bemisia tabaci*. PLoS One. 7: 1–8.

Pan, X., G. Zhou, J. Wu, G. Bian, P. Lu, A. S. Raikhel, and Z. Xi. 2012a. *Wolbachia* induces reactive oxygen species (ROS)-dependent activation of the Toll pathway to control dengue virus in the mosquito *Aedes aegypti*. Proc. Natl. Acad. Sci. U. S. A. 109: E23–E31.

Penz, T., S. Schmitz-Esser, S. E. Kelly, B. N. Cass, A. Müller, T. Woyke, S. A. Malfatti, M. S. Hunter, and M. Horn. 2012. Comparative genomics suggests an independent origin of cytoplasmic incompatibility in *Cardinium hertigii*. PLoS Genet. 8.

Perlman, S. J., S. A. Magnus, and C. R. Copley. 2010. Pervasive associations between *Cybaeus* spiders and the bacterial symbiont *Cardinium*. J. Invertebr. Pathol. 103: 150–155.

Regnery, R. L., C. L. Spruill, and B. D. Plikaytis. 1991. Genotypic identification of Rickettsiae and estimation of intraspecies sequence divergence for portions of two Rickettsial genes. J. Bacteriol. 173: 1576–1589.

Rock, D. I., A. H. Smith, J. Joffe, A. Albertus, N. Wong, M. O'Connor, K. M. Oliver,

and J. A. Russell. 2018. Context-dependent vertical transmission shapes strong endosymbiont community structure in the pea aphid, *Acyrthosiphon pisum*. Mol. Ecol. 27: 2039–2056.

Russell, J. A., B. Goldman-Huertas, C. S. Moreau, L. Baldo, J. K. Stahlhut, J. H. Werren, and N. E. Pierce. 2009. Specialization and geographic isolation among *Wolbachia* symbionts from ants and Lycaenid butterflies. Evolution. 63: 624–640.

Russell, J. A., S. Weldon, A. H. Smith, K. L. Kim, Y. Hu, P. Łukasik, S. Doll, I. Anastopoulos, M. Novin, and K. M. Oliver. 2013. Uncovering symbiont-driven genetic diversity across North American pea aphids. Mol. Ecol. 22: 2045–2059.

Sakamoto, J. M., and J. L. Rasgon. 2006. Geographic distribution of *Wolbachia* infections in *Cimex lectularius* (Heteroptera: Cimicidae). J. Med. Entomol. 43: 696–700.

Sazama, E. J., P. Scot, and J. S. Wesner. 2019. Insect-symbiont interactions bacterial endosymbionts are common among, but not necessarily within, insect species. Environ. Entomol. 48: 127–133.

Sepúlveda, D. A., F. Zepeda-Paulo, C. C. Ramírez, B. Lavandero, and C. C. Figueroa. 2017. Diversity, frequency, and geographic distribution of facultative bacterial endosymbionts in introduced aphid pests. Insect Sci. 24: 511–521.

Shropshire, J. D., B. Leigh, S. R. Bordenstein, and M. Initiative. 2020. Symbiont-mediated cytoplasmic incompatibility : What have we learned in 50 years? Elife. 1924: 1–51.

Sinkins, S. P., and F. Gould. 2006. Gene drive systems for insect disease vectors. Nat. Rev. Genet. 7: 427–435.

Steiner, F. E., R. R. Pinger, C. N. Vann, N. Grindle, D. Civitello, K. Clay, and C. Fuqua. 2008. Infection and co-infection rates of *Anaplasma phagocytophilum* Variants, *Babesia* spp., *Borrelia burgdorferi*, and the Rickettsial endosymbiont in *Ixodes scapularis* (Acari: Ixodidae) from sites in Indiana, Maine, Pennsylvania, and Wisconsin. J. Med. Entomol. 45: 289–297.

Takano, S., M. Tuda, K. Takasu, N. Furuya, Y. Imamura, S. Kim, K. Tashiro, K. Iiyama, M. Tavares, and A. C. Amaral. 2017. Unique clade of Alphaproteobacterial endosymbionts induces complete cytoplasmic incompatibility in the coconut beetle. Proc. Natl. Acad. Sci. U. S. A. 114: 6110–6115.

Thongsripong, P., J. A. Chandler, A. B. Green, P. Kittayapong, B. A. Wilcox, D. D. Kapan, and S. N. Bennett. 2018. Mosquito vector-associated microbiota: metabarcoding bacteria and eukaryotic symbionts across habitat types in Thailand endemic for dengue and other arthropod-borne diseases. Ecol. Evol. 8: 1352–1368.

Tijsse-Klasen, E., M. Braks, E. J. Scholte, and H. Sprong. 2011. Parasites of vectors - *Ixodiphagus hookeri* and its *Wolbachia* symbionts in ticks in the Netherlands. Parasites and Vectors. 4: 1–7.

Tsuchida, T., R. Koga, M. Horikawa, T. Tsunoda, T. Maoka, S. Matsumoto, J.-C. Simon, and T. Fukatsu. 2010. Symbiotic bacterium modifies aphid body color. Science. 330: 1102–1105.

Vanthournout, B., and J. Swaegers. 2011. Spiders do not escape reproductive manipulations by *Wolbachia*. BMC Evol. Biol. 11.

Walker, T., P. H. Johnson, L. A. Moreira, I. Iturbe-Ormaetxe, F. D. Frentiu, C. J. McMeniman, Y. S. Leong, Y. Dong, J. Axford, P. Kriesner, A. L. Lloyd, S. A.

Ritchie, S. L. O'Neill, and A. A. Hoffmann. 2011. The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. Nature. 476: 450–455.
Watanabe, K., F. Yukuhiro, Y. Matsuura, T. Fukatsu, and H. Noda. 2014. Intrasperm vertical symbiont transmission. Proc. Natl. Acad. Sci. U. S. A. 111: 7433–7437.
Weinert, L. A., E. V. Araujo-Jnr, M. Z. Ahmed, and J. J. Welch. 2015. The incidence of bacterial endosymbionts in terrestrial arthropods. Proc. R. Soc. B Biol. Sci. 282: 3–8.
Werren, J. H., L. Baldo, and M. E. Clark. 2008. *Wolbachia*: Master manipulators of invertebrate biology. Nat. Rev. Microbiol. 6: 741–751.
White, J. A., S. E. Kelly, S. J. Perlman, and M. S. Hunter. 2009. Cytoplasmic incompatibility in the parasitic wasp *Encarsia inaron*: disentangling the roles of *Cardinium* and *Wolbachia* symbionts. Heredity. 102: 483–489.
White, J. A., A. Styer, L. C. Rosenwald, M. M. Curry, K. D. Welch, K. J. Athey, and E. G. Chapman. 2020. Endosymbiotic bacteria are prevalent and diverse in agricultural spiders. Microb. Ecol. 79: 472–481.
Williams, D. 1982. Extra-binomial variation in logistic linear models. J. R. Stat. Soc. Ser. C. 31: 144–148.
Ye, Z., I. M. G. Vollhardt, N. Parth, O. Rubbmark, and M. Traugott. 2018. Facultative bacterial endosymbionts shape parasitoid food webs in natural host populations: a correlative analysis. J. Anim. Ecol. 87: 1440–1451.
Yun, Y., C. Lei, Y. Peng, F. Liu, J. Chen, and L. Chen. 2011. *Wolbachia* strains typing in different geographic population spider, *Hylyphantes graminicola* (Linyphiidae). Curr. Microbiol. 62: 139–145.
Zhang, L., and Y. Yun. 2018. Insights into the bacterial symbiont diversity in spiders. Ecol. Evol. 8: 4899–4906.
Zhu, L. Y., K. J. Zhang, Y. K. Zhang, C. Ge, T. Gotoh, and X. Y. Hong. 2012. *Wolbachia* strengthens *Cardinium*-induced cytoplasmic incompatibility in the spider mite *Tetranychus piercei*. Curr. Microbiol. 65: 516–523.
Zindel, R., Y. Gottlieb, and A. Aebi. 2011. Arthropod symbioses : a neglected parameter in pest- and disease-control programmes. J. Appl. Ecol. 48: 864–872.
Zug, R., and P. Hammerstein. 2012. Still a host of hosts for *Wolbachia*: Analysis of recent data suggests that 40% of terrestrial arthropod species are infected. PLoS One. 7: 7–9.

VITA

Laura Cecilia Rosenwald

Education

Georgetown University, Bachelor of Science, awarded 2015
Major: Environmental Biology, Minor: Music

Professional Positions

Research Technician, Georgetown University, 2011-2015
Beer Steward and Tour Guide, Flying Dog Brewery, 2015-2016
Senior Laboratory Technician, University of Kentucky, 2016-present

Scholastic and Professional Honors

Presidents Prize for 10-minute graduate student talks, Second Place, at ESA, ESC and ESBC Joint Annual Meeting (2018)
American Arachnological Society Student Travel Grant Recipient (2019)
American Arachnological Society Student Oral Paper Award, 1st Place (2019)
Presidents Prize for 10-minute graduate student talks, Second Place, ESA Annual Meeting (2019)

Professional Publications

Cepero, Laurel C., **Laura C. Rosenwald**, Martha R. Weiss. *The relative importance of flower color and shape for the foraging monarch butterfly (Lepidoptera: Nymphalidae).* 2015. *Journal of Insect Behavior.* 28(4). 499-511.

Rosenwald, Laura C., John T. Lill, Eric M. Lind, and Martha R. Weiss. *Dynamics of host plant selection and host-switching by silver-spotted skipper caterpillars.* 2017. *Arthropod-Plant Interactions*. 11. 833-842.

White, Jennifer A., Alexander Styer, **Laura C. Rosenwald**, Meghan M. Curry, Kelton D. Welch, Kacie J. Athey, Eric G. Chapman. *Endosymbiotic bacteria are prevalent and diverse in agricultural spiders.* 2020. *Microbial Ecology.*79. 472-481.

Rosenwald, Laura C., Michael I. Sitvarin, Jennifer A. White. *Endosymbiotic* Rickettsiella *causes cytoplasmic incompatibility in a spider host*. 2020. *Proceedings of the Royal Society B.* 287 (1930).