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Natural history, behavior, and bacterial microbiomes of a socially polymorphic spider, *Anelosimus studiosus*

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Natural history, behavior, and bacterial microbiomes of a socially polymorphic spider, *Anelosimus studiosus*

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Dedication

This dissertation is dedicated to my parents, for their unending love and support.

Acknowledgements

First and foremost, I have to thank my study species, *Anelosimus studiosus*, whose many lives made all of these projects possible. Truly, though, none of this work would have been possible without the guidance and support of advisors, collaborators, friends, and family, and I want to thank everyone that helped me achieve this goal.

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Abstract

Natural history, behavior, and bacterial microbiomes of a socially polymorphic spider, *Anelosimus studiosus*

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Anelosimus studiosus is a unique spider; throughout its range from Argentina to New England, most adult females are solitary and subsocial (provide maternal care). However, in the northernmost parts of its range in the United States, adult females sometimes live in cooperative aggregations. While this social polymorphism has been well defined in specific regions of *A. studiosus*' range, it is not so thoroughly described elsewhere. Therefore, I first describe the aggregative behavior, natural history, and population sex ratios for *A. studiosus* across a latitudinal gradient in Texas (Chapters 1 and 2). I discovered that *A. studiosus* tend to aggregate at higher latitudes in Texas, but that this tendency was not correlated with female-biased primary sex ratios, suggesting that outbreeding still occurs in these populations. I then compared the social behavior of *A. studiosus* from Texas populations to those further east (Alabama and Tennessee) using tests of aggressive tendency (Chapter 3). Individuals from western and eastern

populations vary in aggressive tendency, but I was unable to corroborate previous findings that different measures of aggressive tendency correlate. Finally, I used 16S rRNA metagenomics to describe the bacterial microbiome of *A. studiosus* (Chapter 4). Similar to some spider species but unlike many other animals, *A. studiosus* do not harbor a consistent, core bacterial community, and instead, their bacterial communities reflect their rearing environment and diet. In all, this dissertation on the socially polymorphic *Anelosimus studiosus* adds to a growing body of literature on the complexity of animal personalities, and their effect on organismal biology.

Table of Contents

List of Tables

List of Figures

CHAPTER 1: NATURAL HISTORY OF A SOCIALLY POLYMORPHIC SPIDER, *ANELOSIMUS STUDIOSUS*, IN TEXAS.

Abstract

Previous studies have shown that the subsocial spider *Anelosimus studiosus* displays variation in aggregative and social behavior across a latitudinal gradient in the southeastern United States. Additionally, researchers have found that *A. studiosus* webs often harbor many foreign visitors, including ectoparasites, heterospecific spiders, and insects. However, the natural history of this socially polymorphic spider has not been described in other areas of North America. Here we have summarized natural history and behavioral observations of *A. studiosus* in Texas, including observations of aggregative behavior, sex ratio behavior, heterospecific interactions, and matriphagy (i.e. when offspring consume their mother). Similar to populations in the Southeastern U.S., we find that aggregations increase in frequency and size as latitude increases, and that *A. studiosus* webs are often home to a diverse group of arthropods. We also describe a perhaps new species of *Baeus* wasp that parasitizes *A. studiosus* egg sacs.

Introduction

Anelosimus studiosus (Araneae: Theridiidae) is a cobweb-building spider that occurs from Argentina to the Southeastern United States (Agnarsson, 2006). Throughout most of its range, adults live alone but display subsocial behavior; females guard their egg sacs, capture prey for their young, and regurgitate food to their young, while juveniles will cooperate in prey capture after their mother dies, and remain in their natal nest until their penultimate molt (Brach, 1977a). Twenty years ago, researchers discovered that in the Southeastern United States, *A. studiosus* occasionally formed aggregations where adult females live together in larger webs, and

cooperate in prey capture, brood care, and web maintenance, while also displaying the maternal care exhibited by solitary females (Furey, 1998). Since that discovery, researchers have documented and described this behavioral variation, or social polymorphism, throughout the Southeastern U.S., namely in Alabama, Georgia, Tennessee, and Florida. Most interestingly, studies have shown that as latitudes increase, the frequency of aggregative, social webs also increases (Riechert and Jones, 2008; Pruitt et al., 2008).

In addition to this behavioral variation, other aspects of *A. studiosus'* life history have been well-documented over the last twenty years. For instance, we know that *A. studiosus* nests are often home to other organisms, including heterospecific spiders, caterpillars, bugs, wasps, and other arthropods (Deyrup et al., 2004, Perkins et al., 2007; Mock, 2008). Though the species makeup of their nests varies across populations, it is clear that *A. studiosus* do not live alone.

Here, we discuss the results of targeted surveys of aggregative behavior and nest associates of *A. studiosus* in a yet unstudied part of its range: the Southcentral United States, or, Texas. We also discuss observations of behavior or species interactions between *A. studiosus* and other organisms that we observed between 2012-2018.

Methods

Collections

To find populations of *A. studiosus* in Texas, we used descriptions of its natural history (Brach, 1977; Furey, 1998), anecdotal sightings by local naturalists, and online blog or website reports of the species in Texas (Lapp, 2007; Quinn, 2007). Populations of *A. studiosus* often become extinct (Pruitt, 2012, 2013), thus the species can be difficult to find based on older reports. Once located, we collected individuals in their webs by bagging the whole web, then cutting the branch it was on. Because *Anelosimus* have short dispersal distances, we collected

webs at least ten meters apart from each other to reduce the chance that they were from offspring of the same mother (Powers and Aviles, 2003; Riechert and Jones, 2008). It should be noted, however, that it was often difficult to determine if *A. studiosus* webs clustered together were part of one nest or multiple different nests, so we only collected webs that were clearly disconnected from other neighboring *A. studiosus* webs. This was not always straight-forward; some locations contained trees or shrubs covered in *A. studiosus* nests, many of which were connected by dense webbing (e.g. Figure 1.5). Whether these connected webs would be considered single multifemale nests or multiple single nests in other studies, we do not know. Therefore, we only collected webs where silk connections between it and neighboring webs were nonexistent or very sparse (few connecting silk threads).

Given the proximity to other *Anelosimus* species (Agnarsson, 2006), we identified *A. studiosus* in the lab using figures, character descriptions, and keys outlined in Agnarsson (2004) and Agnarsson (2006) for spiders from each location. Individuals from some of these populations are deposited at the University of Texas at Austin Entomology Collection under the accession numbers UTIC00253513-UTIC00253519. Coordinates and collection information of all sites containing more than one nest when visited are listed in Table 1.1 and displayed in Figure 1.1. From populations where we were able to collect multiple nests, we counted individuals per nest, and categorized any adults found as male or female (May-September, 2015-2018; N = 231 nests across 9 locations; Table 1.1 and Figure 1.1). It should be noted, however, that adult males do not live as long as females (Dean, 2016), and sex ratios calculated from these collections are likely biased toward females due to the time of year they were collected.

Nest associates

To determine whether spiders associated with nests in Texas are similar to those found in other *A. studiosus* populations in the United States (Deyrup et al., 2004; Perkins et al., 2007; Mock, 2008), we stored heterospecific organisms from twenty-two nests collected in 2014 and 2016 from Wind Point Park, TX (which contains stable, year-round populations of *A. studiosus*) in 70% ethanol for later identification. Individuals were identified to family using the *Spiders of North America: An Identification Manual* (Ubick et al., 2017).

Additionally, in an effort to discover egg sac mites or parasitoids, we allowed egg sacs to hatch unguarded in the lab. Mothers and their egg sacs (N = 91) were collected from three locations with large *A. studiosus* populations (WPP, LRH, and RR), in August, 2017, after which egg sacs were removed from their mothers, visually inspected under the microscope for any signs of parasitism, and placed in small centrifuge tubes to hatch. After hatching, egg sacs were dissected and inspected under the microscope for visible parasites.

Results & Discussion

Collection & Identification of A. studiosus.

From 2015-2018, we identified seventeen previously unknown populations of *A. studiosus* throughout Texas ranging across latitudes 30-33° (Figure 1.1, Table 1.1). We additionally observed countless (unrecorded) nests across the state, particularly in human-disturbed, urban areas (parks, shopping centers, gardens, etc.), where females were often found in either solitary webs or small aggregations of solitary webs in dense vegetation surrounding buildings or walkways. In most of these surveyed areas, *A. studiosus* occurred on ornamental shrubs or trees lining disturbed, sometimes riparian habitats, but prior studies have also found this species in agricultural settings like cotton fields (Dean et al., 1982). These observations, combined with

other documentation of *A. studiosus* populations across Texas (summarized in Dean, 2016), demonstrate that this species is widespread throughout the state, occurs in more counties than previously documented, and may have a continuous distribution throughout the state east of longitude 99° (Figure 1.1, Table 1.1).

We found that the prevalence of multifemale nests (>1 adult female per nest) increased with latitude (Table 1.2; Figure 1.2), with proportions of multifemale nests ranging from 0.00-0.70 across locations and years. Average multifemale nest sizes at each latitude (Table 1.3) were slightly lower than those reported for comparable latitudes in Florida and Alabama by Riechert & Jones (2008), who observed that the average number of females per multifemale nests ranges from 2.38 females per nest at 30° latitude to 3.90 females per nest at 34° latitude. This could be due to increased levels of interindividual tolerance in those locations, or could be a result of sampling bias, if perhaps our definition of a multifemale web is more or less strict than theirs (e.g. if they regarded any connecting silk line between webs to mean those webs were all part of one big web with multiple retreats).

As in previous surveys of *A. studiosus* populations in Tennessee, Georgia, and Alabama (Furey, 1998), we found that sex ratios of adults in Texas populations collected in nests from May-September across years were consistently female-biased (0.62-1.00 ratio of adult females/total adults across years and locations) (Table 1.2). This sex-ratio bias is likely due to time of year and sampling strategy; males have shorter lifespans than females in this species, so are less likely to be collected in late Spring or Summer (Dean, 2016). Additionally, males do not usually build webs of their own, and instead will wander between webs, thus making it more difficult to find them unless males are collected with females in a web that the males are visiting (Furey, 1998). Previous studies of primary sex ratios of *A. studiosus* have found no female bias

at the embryonic stage (Avilés and Maddison, 1991). However, those surveys were in populations of only subsocial females from Ecuador, so it is possible that primary sex ratios in populations with social females may be biased in the egg sac, as it is in other social spiders (Avilés and Maddison, 1991).

Nest Associates

From two surveys of *A. studiosus* webs conducted in 2014 (N=14 webs) and 2016 (N=8 webs) at Wind Point Park in Lone Oak, TX, we found heterospecific spiders from five different families (in order of abundance): Tetragnathidae (in 12 of 22 webs), Araneidae (10/22), Salticidae (7/22), Theridiidae (6/22) and Thomisidae (5/22). Of these, the most commonly co-inhabiting genera were *Tetragnatha* (12/22) and *Metazygia* (6/22). In comparison, a survey of nests from the Archbold Biological Station in central Florida (Deyrup et al., 2004) found that the most common heterospecific spider inhabitants of *A. studiosus* nests (N=39) were in the Anyphaenidae and Tetragnathidae families. When Perkins et al. (2007) investigated common heterospecific spiders of *A. studiosus* webs across a latitudinal gradient in the Southeastern U.S. from Florida to Tennessee (26-36° latitude; N=25 nests per location), they found that *A. studiosus* nests were frequently co-inhabited by Salticidae, Anyphaenidae, Araneidae and Tetragnathidae, were sometimes co-inhabited by Theridiidae, and were never co-inhabited by Thomisidae. They also found that the proportion of each family found in *A. studiosus* nests varied greatly across locations. Another survey of heterospecific inhabitants of *A. studiosus* nests from Bulloch and Evans Counties in Georgia (N = 250) found that Salticidae were the most prevalent spider family in nests, followed by Corinnidae (Mock, 2008). It is possible that Anyphaenidae were the second most prevalent family, however multiple *Hibana* species were labelled as belonging to the Clubonidae family, and many of the Clubonidae are listed as

unknown genera, thus making it impossible to determine if those individuals were Anyphaenidae or Clubonidae. Unlike other studies, Mock (2008) did not find Tetragnathidae in *A. studiosus* webs in Georgia, although Tetragnathidae do occur in that region.

Although we did not find Anyphaenidae or Agelenidae living within *A. studiosus* nests in our surveys from Wind Point Park, we have observed both families living near *A. studiosus* webs in other locations such as TL, E2, and E7 (Table 1.1, Figure 1.1). Interestingly, in these locations, we often found spiders from these families in the retreats of webs that we thought belonged to *A. studiosus*, but contained no *A. studiosus* individuals when dissected in the lab. Perkins et al (2007) found that Anyphaenidae and Agelenidae are common predators of *A. studiosus*, and are more prevalent after extinction of local *A. studiosus* populations. Anecdotally, we witnessed a similar increase in their webs in locations where *A. studiosus* populations decreased dramatically between 2014-2018 (e.g., TL, E2 and E7).

Additionally, we commonly observed the following categories of insects alive in *A. studiosus* webs: dead and living Coleoptera (specifically, Chrysomelidae, Coccinelidae); live Heteropteran nymphs (Figure 1.7); live Metapterini (Figure 1.7); and live *Crematogaster* ants. Metapterini were extremely abundant at Wind Point Park, Lone Oak, Texas, with sometimes dozens of individuals hanging from a single web containing live adult and juvenile *A. studiosus*. We did not witness predation of these assassin bugs on *A. studiosus* individuals in the field or in the lab, nor did we witness interactions between *A. studiosus* individuals and the other insects listed above.

Egg sacs collected and allowed to hatch in the lab occasionally produced tiny *Baeus* wasps (Hymenoptera: Scelionidae; Figure 1.3), but were not visibly parasitized by other potential spider egg sac parasites. *Baeus* are solitary, obligate, endoparasites of spider eggs, and there is one

known *Baeus* parasite of *A. studiosus* egg sacs from Brazil, *Baeus anelosimus* (Margaria et al., 2006). However, the *Baeus* that emerged from *A. studiosus* egg sacs in this study do not fit the description of *B. anelosimus*, suggesting that multiple *Baeus* species parasitize *A. studiosus* across its range. As there is no key for Neartic *Baeus* species, we could not identify these wasps to species, but *Baeus* have been found parasitizing other spider egg sacs in North America (Bowden and Buddle, 2012; Vetter et al., 2012). Of ninety-one dissected egg sacs, five produced *Baeus* wasps, with an average of 16 wasps (SD = 9.6) emerging from each sac (range = 6-27 wasps). We also found hatched juvenile *A. studiosus* within all of the parasitized egg sacs, suggesting that, similar to other *Baeus* wasps (Vetter et al., 2012), this *Baeus* species does not always parasitize all eggs within its host's egg sac. Because *Baeus* males are winged and have long antennae, whereas females are not winged and have clubbed antennae (Figure 1.3, Vetter et al., 2012), we could easily measure the sex ratios of emerged wasps. Sex ratios varied greatly between the five egg sacs (0-83% female; Table 1.4), with one sac producing only male wasps. Two females and two males reared from egg sacs collected in Wind Point Park, Lone Oak, TX on August 19, 2017 are deposited at the University of Texas at Austin Entomology Collection under accession numbers UTIC00213229 (female), UTIC00213230 (female), UTIC00213231 (male), and UTIC00213232 (male).

Additional spurious observations

Matriphagy

In the laboratory, we witnessed juvenile consumption of their mothers, or matriphagy (Figure 1.6), in what we believe is the first reported observation of this behavior in *A. studiosus* from North American populations. There have only been two previous observations of matriphagy in *A. studiosus*, and both observations came from individuals collected in Uruguay that were being

reared in the laboratory (Ghione et al., 2004; Viera et al., 2007). We did not find that matriphagy was common, as it was only seen in two out of twenty-five families we were raising in for an experiment not detailed here. In this experiment, adult females with egg sacs were placed in separate home containers, and juveniles were raised in the lab from these egg sacs, with their mothers. The families had been fed *Drosophila* fruit flies and misted with water ad libitum, and were not purposefully starved at any point. We could not determine in these unplanned observations whether mothers were dying already when their young began to prey on them, as is the case for matriphagy in the subsocial spider *Stegodyphus lineata* (Salomon et al., 2015), or whether the young killed their mothers for food. However, in our observed cases of matriphagy, the mothers were pulling away from attacking juveniles, and attempted to brush off attacking juveniles with their legs for a few minutes before stopping movement completely. In the wild, we have observed the whole, desiccated corpses of adult females in webs containing only juveniles, but we have not observed matriphagy. Foelix (1996) found that instead of pulling prey apart as some spiders do, *A. studiosus* will leave a dry husk of their prey item, so it is possible that juveniles are feeding on their mothers in the wild, either before or after they die. Indeed, Furey (1998) described that juveniles will eat dead *A. studiosus* individuals in the web, though did not specify at what developmental stage those individuals were. However, it is just as possible that desiccation of the mother occurs without predation, or through predation by other neighboring spiders. Further field observations and proper experiments testing conditions leading to matriphagy are necessary to determine if and when this behavior occurs in wild populations of *A. studiosus*.

Aggregation behavior

In riparian areas of Texas, it is not uncommon to find temporary, large aggregations of spiders, including A. studiosus (Figure 1.4). These massive spider aggregations are built primarily by the long-jawed orbweaver, *Tetragnatha guatamalensis*, which are known to be tolerant of other spiders, conspecific or not, and are even known to share web lines, when prey is abundant (Gillespie, 1987). In Texas, these aggregations typically occur after large amounts of rain, and after huge blooms in midge populations (Jackman et al., 2007), but the exact cause is unknown. In these huge aggregations, there exist not only *T. guatamalensis* spiders, but also many other species as well (personal observation; Jackman et al., 2007), including at times, *A. studiosus*. Still, we do not know the relationship between *T. guatamalensis* and these other species, and specifically we have not observed physical interactions between *T. guatamalensis* and *A. studiosus*.

However, we have observed that *A. studiosus* nests found within the *T. guatamalensis* webs seem to serve as a retreat for *T. guatamalensis* individuals. That is, when we collected *A. studiosus* webs (from these aggregations or during typical collections), we often collected *T. guatamalensis* that were retreated from their own orb webs and perched on the outside of an *A. studiosus* web (Figure 1.5B). We found that these are the most common web associates of *A. studiosus* in one Texas population (WPP), and we know that they are also commonly associated with *A. studiosus* in other locations (Perkins et al., 2007; Deyrup et al., 2004; personal observation of Tennessee populations). This co-habitation could be driven by the architecture of both web types and similar plant or web substrate preferences (Figure 1.5), or possibly by a mutualism between the spider species, if they benefit from each other's presence, for example by sharing web lines. Observations of these interconnected webs have found that individuals of the two species do not interact, but perhaps, in times of low prey abundance, *T. guatamalensis* would

prey on *A. studiosus* as has been demonstrated in lab trials by Perkins et al. (2007). In addition, we have not observed interactions between these two species at night, when *A. studiosus* are more active and more likely to leave their retreats (personal observation). Further long-term field experiments are necessary to determine the relationship of these individuals in their natural environment.

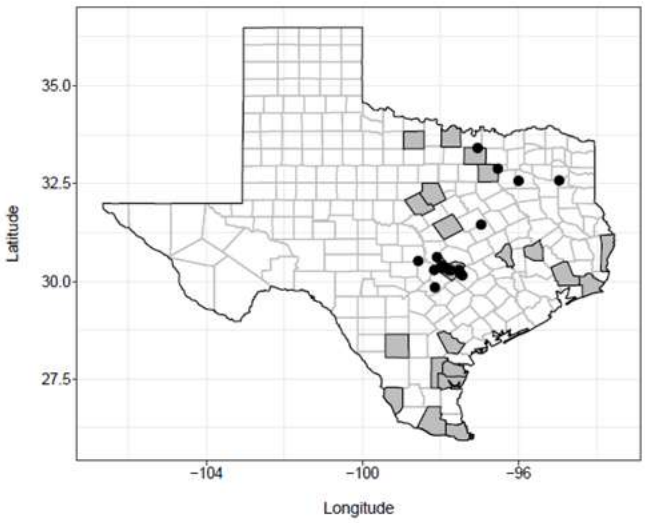

Figure 1.1 Newly identified *A. studiosus* populations (black dots) in Texas, compared to previously documented county presence (grey blocks) by Dean et al (2016). Exact coordinates of new populations are listed in Table 1.1.

Table 1.1 Newly identified A. studiosus populations, their counties, and their coordinates in Texas. Counties where A. studiosus have been previously documented in Dean et al (2016) are marked with an asterisk (*).

Name (Abbrev)	City (County)	N	W
Town Lake (TL)	Austin (Travis*)	30.15	-97.44
Ecolab 1 (E1)	Leander (Travis*)	30.29	-97.52
Ecolab 2 (E2)	Austin (Travis*)	30.20	-97.50
Wind Point Park (WPP)	Lone Oak (Hunt)	32.57	-96.00
Lake Ray Hubbard (LRH)	Rowlett (Rockwall)	32.88	-96.53
UT Campus (UT)	Austin (Travis*)	30.28	-97.73
Brackenridge Field Lab (BFL)	Austin (Travis*)	30.28	-97.78
Ecolab 3 (E3)	Marble Falls (Burnet)	30.52	-98.57
Ecolab 4 (E4)	Bertram (Burnet)	30.62	-98.09
Ecolab 5 (E5)	Dripping Springs (Hays)	30.29	-98.16
Ecolab 6 (E6)	Austin (Travis*)	30.34	-97.93
Ecolab 7 (E7)	Riesel (McClennan)	31.45	-96.96
Ray Roberts Lake (RR)	Denton (Denton*)	33.41	-97.05
Ecolab 8 (E8)	Gladewater (Upshur)	32.58	-94.96
Arkansas Bend State Park (AB)	Lago Vista (Travis*)	30.40	-97.95
Ecolab 9 (E9)	Austin (Travis*)	30.33	-97.83
Ecolab 10 (E10)	New Braunfels (Comal)	29.85	-98.14

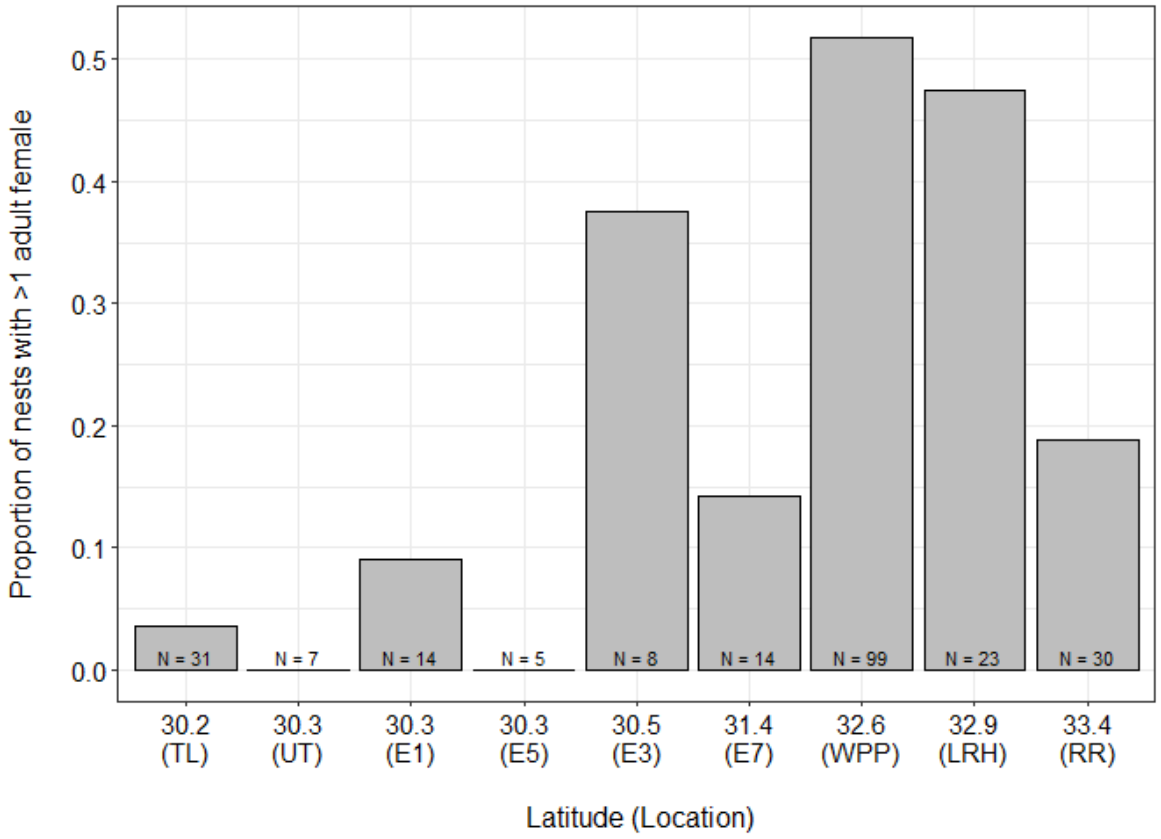

Figure 1.2 Proportion of nests that contained more than one female as a function of latitude. Populations were surveyed June to August, 2015-2018. Proportions are calculated as averages from data combined across years; yearly data are listed in Table 1.2.

Table 1.2 Proportion of multifemale nests and adult sex ratios for collections in different months, years, and locations along a latitudinal gradient from central Texas (latitude 30.2°) to northern Texas (latitude 33.4°).

Location	Latitude	Year Collected	Month Collected	Sample Size	Proportion nests with >1 adult female	N_{female}	N_{male}	Sex Ratio $N_{female}/(N_{female} + N_{male})$
Town Lake	30.15	2015	August	14	0.14	15	2	0.88
		2016	May	4	0.00	4	0	1.00
		2016	August	5	0.00	5	1	0.83
		2017	August	8	0.00	8	0	1.00
University of Texas at Austin	30.28	2016	May	4	0.00	4	0	1.00
		2016	August	3	0.00	3	0	1.00
Ecolab 1	30.29	2015	August	11	0.18	13	4	0.76
		2016	May	3	0.00	3	0	1.00
Ecolab 5		2015	August	3	0.00	3	0	1.00
		2016	May	2	0.00	2	0	1.00
Ecolab 3	30.52	2016	August	8	0.38	12	6	0.67
Ecolab 7	31.45	2017	August	14	0.14	16	0	1.00
Wind Point Park	32.57	2015	August	26	0.58	49	2	0.96
		2016	Sept	8	0.50	20	0	1.00
		2017	August	44	0.61	111	18	0.86
		2018	June	21	0.38	36	11	0.77
Lake Ray Hubbard; Rowlett Park	32.88	2015	August	10	0.70	20	0	1.00
		2016	Sept	5	0.60	13	0	1.00
		2017	August	8	0.13	8	5	0.62
Ray Roberts State Park	33.41	2017	August	24	0.38	34	0	1.00
		2018	June	6	0.00	6	0	1.00

Table 1.3 Number of multifemale (MF) nests, or nests containing more than one female, and the mean, median, and maximum number of females per multifemale nest across collections and years. Sample sizes for each collection listed in Table 1.2.

Location	Latitude	Year	Number MF nests	Mean # females per MF nest	Median # females per MF nest	Max females per nest
TL	30.15	2015	2	2.00 ± 0.00	2	2
E1	30.29	2015	2	2.00 ± 0.00	2	2
E3	30.52	2016	3	2.33 ± 0.58	2	3
E7	31.45	2017	2	2.00 ± 0.00	2	2
WPP	32.57	2015	14	2.50 ± 0.94	2	5
WPP	32.57	2016	4	4.00 ± 2.16	3.5	7
WPP	32.57	2017	25	3.64 ± 2.27	3	10
WPP	32.57	2018	8	2.88 ± 1.72	2	7
LRH	32.88	2015	7	2.43 ± 0.79	2	4
LRH	32.88	2016	3	3.67 ± 2.89	2	7
LRH	32.88	2017	1	2.00	2	2
RR	33.41	2017	8	2.125 ± 0.35	2	3

Figure 1.3 Female (left) and male (right) of the egg-parasite wasp *Baeus sp.*, hatched from *A. studiosus* egg sacs collected in Wind Point Park on August 19, 2017. Photos by Alex Wild at the University of Texas at Austin Biodiversity Center and Entomology Collection.

Table 1.4 Number of male and female *Baeus* wasps emerging from five egg sacs collected from *A. studiosus* females on August 18-19, 2017 across three locations (WPP, LRH, RR; see Table 1.1) in Texas.

Location and Date Collected	**N_{male}**	**N_{female}**	**N_{total}**	**Sex ratio (N_{female}/N_{total})**
WPP 8/19/17	1	5	6	0.83
RR 8/18/17	4	2	6	0.33
LRH 8/18/17	27	0	27	0.00
WPP 8/19/17	19	4	23	0.17
RR 8/18/17	5	13	18	0.72
Average	**11.2**	**4.8**	**16**	**0.41**
Std. Dev.	**11.23**	**4.97**	**9.67**	**0.32**

Figure 1.4 Portions of a massive spider web built in Arkansas Bend State Park, Lago Vista, TX, in October, 2016. Left: *Tetragnatha guatamalensis* settled close together in a small section of the web. Right: A larger view of the web, covering trees in the park next to Lake Travis.

Figure 1.5 A) Mixture of *Tetragnatha guatamalensis* webbing covering a bush containing webs of other spiders, including *A. studiosus*, at Lake Ray Hubbard in Rowlett, Texas, August, 2015. Picture was taken after area had been sprayed for mosquitoes (personal communication with Mike Merchant), so most *T. guatamalensis* orb webs were destroyed, and any live individuals were rebuilding their webs. B) *T. guatamalensis* observed resting on a web containing an adult female *A. studiosus* (arrow) at Wind Point Park in Lone Oak, Texas, April, 2019.

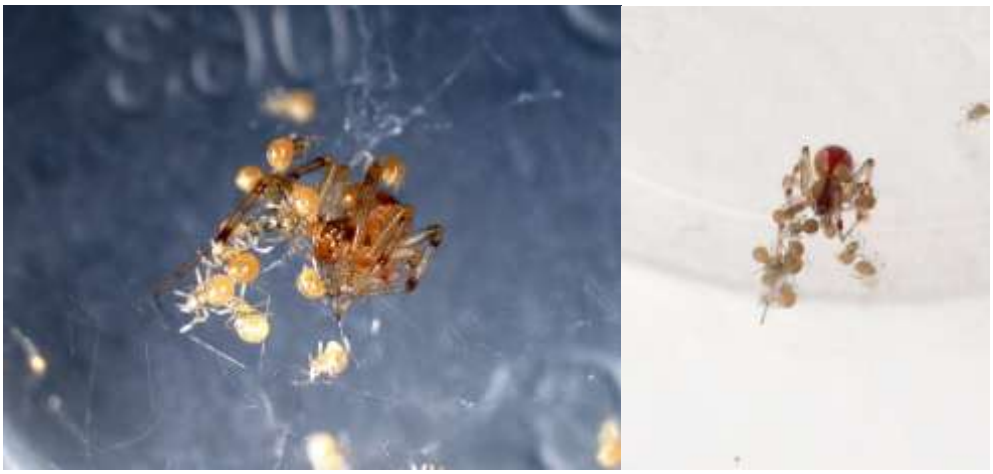

Figure 1.6 *A. studiosus* mother being consumed by her young, June 2016.

Figure 1.7 Commonly observed Heteropera associated with *A. studiosus* webs. Left: a thread-legged bug (Emesinae) hangs on the outside of an *A. studiosus* web. Right: a Miridae nymph crawls along leaves inside an *A. studiosus* web.

CHAPTER 2: NO EVIDENCE FOR FEMALE-BIASED EMBRYONIC SEX RATIOS IN TEXAS POPULATIONS OF *ANELOSIMUS STUDIOSUS*

Introduction

While many spiders show subsocial behavior, or care for their young, adult tolerance of others and cooperation in spiders is extremely uncommon, but has been described for over a dozen spider species, all of which share similarities in their life histories that are sometimes termed a "social syndrome" (Bilde and Lubin, 2011; Settepani et al., 2017). Common behavioral characteristics of this syndrome include cooperative brood care, cooperative prey capture, and cooperative web maintenance amongst adult females within the same web. Furthermore, typical social spider species have overlapping generations, delayed or no juvenile dispersal from the natal web, reproductive skew, and high levels of inbreeding. An additional hallmark of sociality in spiders is a highly female-biased sex ratio (Bilde and Lubin, 2011; Settepani et al., 2017), where the most extremely social colonies contain 10:1 adult female to male ratios (Avilés and Maddison, 1991). To explain this phenomenon, Aviles (1993) outlined three possible selective forces for the evolution of female-biased sex ratios in social *Anelosimus* species; 1) colonies benefit from female-biased sex ratios, since females provide most offspring care and prey capture, 2) colonies go extinct quickly, increasing the strength of group selection for female-biased sex ratios, 3) and there is high within-colony breeding, therefore mate competition between sons would select for higher production of daughters.

Previous studies have found that the sex ratio bias in some social *Anelosimus* spiders occurs in the embryonic stage, while the embryonic sex ratios of subsocial species are equal (Avilés and Maddison, 1991). Though, it has been hypothesized that this embryonic sex ratio bias is the

result of a bias in sperm sorting (Avilés et al., 2000), this has not been confirmed in *Anelosimus*. However, in another social spider genus, *Stegodyphus*, researchers found that males did control offspring sex ratios by producing more female-determining sperm (Vanthournout et al., 2018).

Within social spiders, *Anelosimus studiosus* is atypical; while adult female *A. studiosus* are subsocial and solitary from Argentina to the Southeastern United States, in the most northern edges of its range (Alabama, Tennessee, Texas, and Georgia) adult females will occasionally aggregate in colonies resembling those of social species. That is, *A. studiosus* in some northern populations occasionally display hallmarks of the "sociality syndrome" (Bilde and Lubin, 2011; Settepani et al., 2017), such as cooperation amongst adults (Furey, 1998), delayed juvenile dispersal (Jones and Parker, 2002), and inbreeding (Duncan et al., 2010). Additionally, *A. studiosus* populations, both social and subsocial, often have female-biased sex ratios as adults (Furey, 1998; Chapter 1), but it is unclear whether these biases exist at the embryonic stage.

Studies of the primary sex ratios of *A. studiosus* in Ecuador have found 1:1 ratios of females to males in egg sacs (Avilés and Maddison, 1991) but other studies have found that raising individuals collected from subsocial females in Brazil in the lab, with high offspring survival, resulted in a 1.9:1, female-biased sex ratio (Viera et al., 2007). In another study, researchers again measured the sex ratios of offspring from Tennessee raised in the lab from egg case to their penultimate molt, and found that the proportion of female offspring per egg case ranged from 0.4-0.9 (Lichtenstein et al., 2018). Therefore, it seems that social, and possibly some subsocial, *A. studiosus* populations both have female-biased embryonic sex ratios. In this study, we completed a preliminary survey of embryonic sex ratios of *A. studiosus* from populations in Texas containing either only solitary females, or mixed solitary and aggregative females, to determine if embryonic sex ratios are more female-biased in aggregative Texas populations.

Methods

Collection

In April and May 2019 we collected *A. studiosus* nests from locations known to have distinctly different population structures. Two collection sites, one along a hike and bike trail on Lady Bird Lake, Austin, TX (30°14'51.7"N, 97°43'34.4"W; N = 2) and the other on the University of Texas at Austin campus (30°17'14.4"N 97°44'13.2"W; N = 4), have adult females that are entirely subsocial and solitary, and distribution of *A. studiosus* is sparse and patchy. That is, occasional aggregations of 2-5 webs can be seen on the same plant, but each web contains one female and her brood, and the webs are not connected (Chapter 1). Our third collection site was in Wind Point Park, Lone Oak, Texas (32°57'24.6"N, 96°00'02.7"W; N = 10), where adult females form dense aggregations (Figure 2.1), and can be found sharing a web (Chapter 1). At both sites, we collected females by placing plastic bags over their web and plant substrate, and cutting the plant so that the whole web and all individuals within the web were captured in the bag. Females were then taken to a lab at the University of Texas at Austin and moved to home containers (5.5oz Dart Conex™ Complements soufflé/portion cups). If females had an egg sac or produced an egg sac in the lab, we gently removed it from her grasp or web and stored it in a 0.5ml centrifuge tube until dissection.

Embryo Fixation and Chromosome Staining

A. studiosus females have two pairs of X chromosomes, while *A. studiosus* males have only one pair, therefore embryos can be sexed through chromosome counts (Figure 2.2), where females have twenty-four chromosomes total and males have twenty-two. To prepare embryos for chromosome counting, we used the embryo fixation and chromosome squash procedure outlined in Avilés and Maddison (1991). In summary, this method instructs researchers to fix

embryos in a solution of 25% acetic acid in ethanol, dissect the fixed embryos with fine needles in a 60% acetic acid solution, squash cells using a coverslip, and stain cells for viewing and counting of chromosomes with a microscope, under high magnification. Because there is a short window of time in which embryonic *A. studiosus* cells are undergoing rapid mitosis and capable of being squashed properly (i.e., tissue is not too dense for squashing), we could not feasibly dissect all embryos in each collected egg sac. Therefore, we aimed to dissect at least twenty embryos from each egg sac, which resulted in dissection of 41-100% of all embryos in a sac (Table 2.1).

After preparing the chromosome squashes, cells were stained with a 2% aceto-orcein solution and imaged under 1000x magnification with Leica Application Suite v.4.2 (Leica Microsystems). While conceptually simple, chromosome counts can be difficult or subjective when chromosomes are not spread apart completely. To confirm chromosome counts, we attempted to image the condensed chromosomes of at least two different cells per embryo. To prevent observer bias, images were coded and randomized prior to counting so that researchers were blind to egg sac maternal web ID, collection location, and counts from other cells from the same embryo. For the embryos which we could only obtain one image (i.e., very young or very old embryos with few dividing cells), we asked a second individual to provide a count, blind to the first individual's count. If the counts were different, the embryo was not included in final sex ratio calculations.

Statistical Analysis

Given that previous studies have shown equal primary sex ratios in subsocial *A. studiosus* (Avilés and Maddison, 1991), our null hypothesis was that the primary sex ratios within egg sacs and within populations would be equal. To test this, we used the G-test of Goodness-of-Fit (α =

0.05) in R (R Core Team, 2013) using the *RVAideMemoire* package (Hervé, 2019) on chromosome sex counts summed across solitary and multifemale nests.

Results

We did not find evidence for female-biased sex ratios in egg sacs collected in any of our three collection sites. Instead, we found that sex ratios in all sites were, on average, slightly male-biased (Tables 2.1). From the more southern, solitary populations (combined University of Texas and Lady Bird Lake collections), the proportion of female embryos per egg sac ranged from 0.33-45, with an average female sex ratio of 0.40 across eighty-three embryos (from N = 6 egg sacs). In our more northern, aggregative population in Lone Oak, Texas, the proportion of female embryos per egg sac ranged from 0.25-0.58, with an average sex female ratio of 0.42 across one hundred and forty-two embryos (from N = 10 egg sacs). Summing embryo sex counts across eggsacs from all solitary and all multifemale nests, we found that eggsacs from solitary nests had a female sex ratio of 0.41 while those from multifemale nests had a female sex ratio of 0.42 (Table 2.2, Figure 2.2). These groups did not deviate significantly from the null hypothesis of a 1:1 female to male sex ratio (Table 2.2, Figure 2.2; G-Test of Goodness-of-Fit, p= 0.076 for solitary nests and 0.083 for multifemale nests).

Discussion

Our findings of equal embryonic sex ratios were not surprising for the egg sacs originating in populations containing only solitary, subsocial females, as prior studies have shown that subsocial *A. studiosus* have an equal primary sex ratio (Avilés and Maddison, 1991). However, given the findings from Lichtenstein et al. (2018), and our own observations of female-biased adult sex ratios in Wind Point Park (Chapter 1) we expected some of the females collected from multifemale webs in the northern population to produce egg sacs with a higher proportion of

female embryos. Instead, we found that sex ratios in egg sacs from both southern and northern populations were slightly male-biased, though not significantly different from our null hypothesis of an equal sex ratio.

It is possible that, unlike aggregative populations of *A. studiosus* in Tennessee (Lichtenstein et al 2018), Texas populations of *A. studiosus* may not exhibit some of the outlined conditions necessary for the evolution of female-biased sex ratios. That is, these populations may still be mostly outbreeding, therefore there would be little selective pressure on individuals to produce fewer sons. Though, genetic studies of parentage within webs are necessary to determine if this selective pressure does exist in Texas populations or not. It is also possible that only a small proportion of the aggregative population in this locale produces female-biased sex ratios, and that our study was not large enough to capture that variation. To sample a larger proportion of *A. studiosus* populations, exact chromosome counts as we used in our study could be paired with other techniques used to estimate primary sex ratios, like the flow cytometry method used by Vanthournout et al. (2018) to estimate biased sperm production in *Stegodyphus* spiders. Overall, though, there does not seem to be strong enough selection for all individuals in the population to evolve to produce a higher proportion of female embryos. Therefore, unlike eastern populations of aggregative *A. studiosus*, these populations do not display all of the hallmarks of spider sociality, but instead may represent a level of social behavior intermediate to southern and northeastern populations.

Figure 2.1 Dense cluster of *Anelosimus studiosus* and other spiders' webs on a juniper tree in Wind Point Park, Lone Oak, Texas on May 17, 2019.

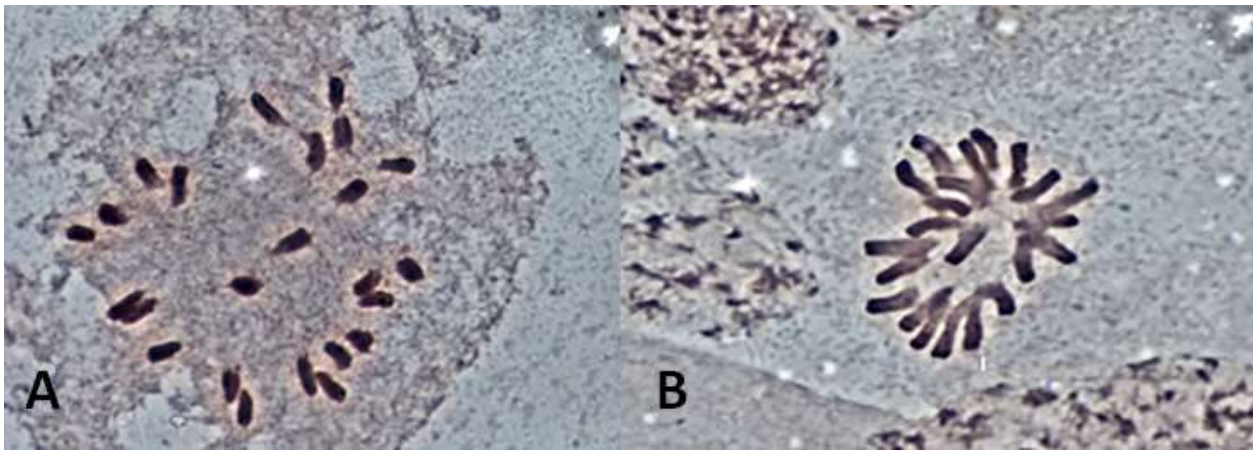

Figure 2.2 Aceto-orcein (2%) stained *Anelosimus studiosus* chromosomes at 1000x magnification. A) Cell of a female embryo, containing twenty-four chromosomes and B) cell of a male embryo, containing twenty-two chromosomes.

Table 2.1 Number of embryos retrieved from egg sacs, dissected, scored, and sexed for each location, and the mean proportion of females per egg sac from each location. ID = Nest identity; MF = whether or not the egg sac was laid by a female that was found in a nest containing other females, i.e. a multifemale nest; LBL = Lady Bird Lake collection site in Austin, TX; UT = University of Texas at Austin collection site in Austin, TX; WPP = Wind Point Park collection site in Lone Oak, Texas.

Location	ID	MF	Total Eggs	Total Dissected	Total Scored	Female	Male	Proportion Female
LBL	A	No	48	20	12	4	8	0.33
LBL	B	No	32	20	7	3	4	0.43
							Average	**0.38**
UT	C	No	45	45	12	5	7	0.42
UT	D	No	40	25	16	5	11	0.31
UT	E	No	45	30	14	6	8	0.43
UT	F	No	43	24	22	10	12	0.46
							Average	**0.40**
WPP	G	No	46	25	21	10	11	0.48
WPP	H	Yes	45	25	13	4	9	0.31
WPP	I	Yes	48	25	14	7	7	0.50
WPP	J	Yes	49	25	18	6	12	0.33
WPP	K	Yes	31	25	21	11	10	0.52
WPP	L	Yes	56	25	7	3	4	0.43
WPP	M	Yes	50	25	17	7	10	0.41
WPP	N	Yes	39	24	12	7	5	0.58
WPP	O	Yes	57	25	12	3	9	0.25
WPP	P	Yes	44	25	7	3	4	0.43
							Average	**0.42**

Table 2.2 The total number and sex of embryos scored across all eggsacs laid by females in solitary or multifemale nests. Multifemale nests are those containing two or more adult females, and were all collected at one site, Wind Point Park in Lone Oak, Texas. Solitary nests were collected across all three sites. For the G-Test of Goodness-of-Fit, sampled sex counts were compared to the sex counts we would expect if proportions of each sex were equal (0.5) with an alpha of 0.05.

	Total Scored	**Female**	**Male**	**Proportion Female**	**G-Test of Goodness-of-Fit**
Solitary	104	43	61	0.41	G = 3.1311 df = 1 p = 0.07681
Multifemale	121	51	70	0.42	G = 2.9959 df = 1 p = 0.08348

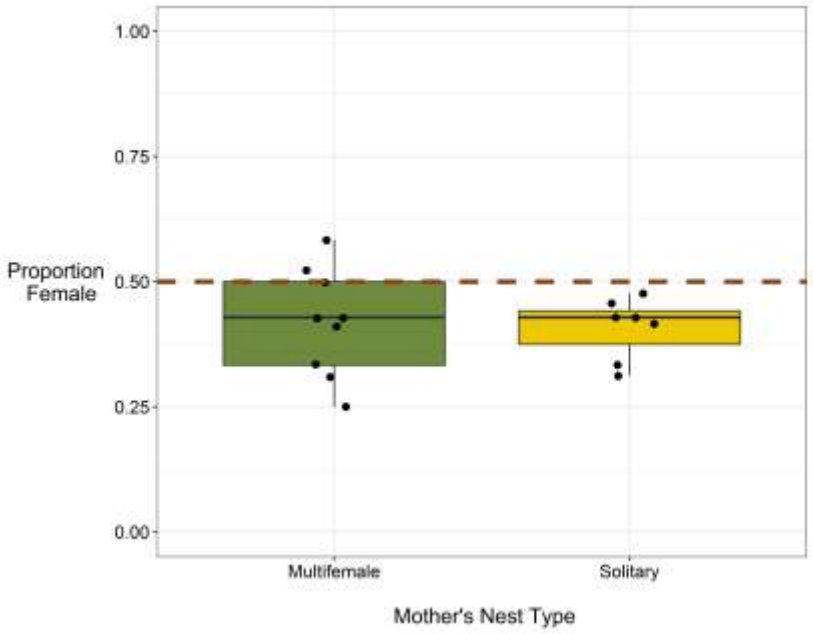

Figure 2.2 Proportion of embryos that were scored as female across all egg sacs collected from females originating in multifemale or solitary nests. Multifemale nests are those containing two or more adult females, and were all collected at one site, Wind Point Park in Lone Oak, Texas. Solitary nests were collected across all three sites.

CHAPTER 3: EVIDENCE OF SOCIAL PHENOTYPE IN TEXAS POPULATIONS OF THE SOCIALLY POLYMORPHIC SPIDER, *ANELOSIMUS STUDIOSUS*.

Abstract

Here we summarize the aggressive tendencies of *A. studiosus* in Texas, and compare their behavior to populations in the Southeastern United States known to show a distinct behavioral polymorphism. Using two animal personality tests, the Interindividual Distance Test and the Huddle Response Test, we found that there exists variation in female aggressive/boldness tendencies, however we found no significant correlation between Interindividual Distance Scores and measures of Huddle Response. Although previous studies have used the same methods and found an association between these tests, we propose that issues with replicability of the tests, along with differences in sample sizes, environmental conditions, and time of year tests were completed, may have contributed to the lack of association between tests of aggressive tendency. Moving forward, we suggest that researchers remain cautious in their categorization of individuals as one personality type or another based on single, unrepeated tests of behavior. Ideally, researchers should use multiple, repeated tests of behavior to type individuals aggressive tendencies before testing the effects of personality on other life history traits of a species.

Introduction

In the last decade, *Anelosimus studiosus* has become a model organism for the study of animal personalities, behavioral syndromes, and other similar methods used to describe consistent correlated variation in behavior. In the most northern latitudes of its range (30-36° latitude in the Eastern United States), some individuals exhibit what has been termed a "social"

(Pruitt et al., 2008b) or "docile" (Pruitt and Riechert, 2009) phenotype, in that such individuals are more likely to be tolerant of other individuals, including conspecifics, predators, and prey (Pruitt et al., 2008b). In northern latitudes, individuals are at times found living in multifemale nests containing up to ~90 adult females (personal observation) and their offspring (Riechert and Jones, 2008). Similar to other social spider species, these females will cooperate in brood care, web maintenance, and prey capture (Furey, 1998). However, throughout the rest of its range from Central America to the Southeastern United States, *A. studiosus* adults exhibit an "aggressive", phenotype, in which adults do not cooperate with other adults, and are not tolerant of heterospecifics (Pruitt et al., 2008b). Both docile and aggressive behavioral types, however, exhibit typical subsocial behavior, where adult females will guard their egg sacs and share prey captures with their young, and juveniles cooperate in their natal nest, but disperse to form new webs before adulthood (Pruitt et al., 2008b). Jones et al. (2007) proposed the brood-fostering model as an explanation for why this behavioral variation across the species range may be maintained in the wild; at higher, colder latitudes, they found that adult females were more likely to die, leaving their egg sac or juvenile offspring to die if no other females are available to provide maternal care. However, at lower latitudes, where the weather is warmer (i.e. the lowest temperatures in a year are typically not as extreme as in higher latitudes), their model predicts that solitary, subsocial females would have a fitness advantage, because they are more aggressive and faster in their response to predation.

The social behavior of *A. studiosus* throughout the rest of its range in the United States has not been explored quite so thoroughly. Given that *A. studiosus* also occurs across a latitudinal gradient and a temperature gradient in Texas, these western populations could serve as an

independent test of the fostering model hypothesis, or could perhaps reveal new behaviors from those seen in the Southeastern U.S. and the rest of the Americas.

In this study, we evaluated the social behavior of *A. studiosus* collected from populations in Central Texas, and compared those results to behavioral tests on populations collected from sites known to have "docile" females in Tennessee and Alabama. To measure social behavior, we used two tests commonly used to determine personality in *A. studiosus*: the Interindividual Distance Test and the Huddle Response Test, which measure, respectively, the tendency to settle with conspecifics, and the time spent in an anti-predator defensive posture after disturbance (Pruitt et al., 2008b). Both tests have been used extensively to study the social tendencies of *Anelosimus studiosus* and other social spiders, and have been shown to be both heritable and repeatable (Pruitt et al., 2008b).

Methods

Collections

In the Southeastern United States, we collected adult, female *A. studiosus* from multiple locations that had previously contained both "docile" and "aggressive" females (Riechert and Jones, 2008; personal communication with TC Jones); Guntersville Lake, Guntersville, AL, (34.4°N, -86.18°W); Warrior's Path State Park, Kingsport, TN (36.49°N, -82.47°W); Weiss Lake, Cedar Bluff, AL (34.24°N, -85.6°W); Wind Creek State Park, Alexander City, AL (32.86°N, -85.92°W); Melton Hill Lake, Oak Ridge, TN (35.99°N, -84.19°W); Boone Lake, Johnson City, TN (36.39°N, -82.36°W); and Norris Dam, Campbell, TN (36.23°N, -84.10°W). Nests containing adult females were collected in July 2017, and brought back to T.C. Jones' lab at Eastern Tennessee State University, a campsite, or other lodging for dissection. Adult females were then placed individually into 5.5oz deli containers (Dart Conex^{TM} Complements

soufflé/portion cups), fed *Drosophila* fruit flies and misted with water, ad libitum. Home containers with females were stored in a cooler in the field car to ensure they did not overheat and die on the way back to a lab at the University of Texas at Austin.

In August 2017 we collected adult females from four locations known to harbor multifemale nests in Texas (personal observation, Ch. 1). These locations were: Wind Point Park, Lone Oak, TX (32.57°N, -96.00°W); Ray Roberts State Park, Denton, TX (33.41°N, -97.05°W); Paddle Point Park, Rowlett, TX (32.88°N, -96.53°W); and a private property in Reisel, TX (31.45°N, -96.96°W). Ziploc bags containing females were brought back to UT in a cooler the same day or the next day, and placed in home containers in the lab. Similarly, they were fed *Drosophila* fruit flies and misted with water, ad libitum. If any female had an egg sac or produced an egg sac while in her home container, the egg sac was removed and the female was not used in behavioral tests for at least twenty-four hours.

Behavioral testing

Whether an individual can be assigned to a "docile" or "aggressive" behavioral type, behavioral syndrome, or personality can be measured using a method known as the Interindividual Distance Test. Earliest versions of this method involve placing two females in a small, square, plastic box, and measuring the distance between them after twenty-four hours (Pruitt et al., 2008; Riechert and Jones, 2008; Pruitt and Riechert, 2009). If two individuals settle in the same corner, both are typed as "docile", and if they settle in adjacent or opposite corners, neither is typed, because one may still be "docile", but would be chased away by an "aggressive" individual. Thus, all individuals are re-tested until they have been paired with a known "docile" female, and are given an Interindividual Distance Score based on their distance from the known "docile" female.

In this study, we used this version of the Interindividual Distance Test to categorize 121 females from Texas and 73 females from control populations as one of three behavioral types based on their Interindividual Distance Scores; if they settled less than 6cm from a known "docile" female, they were typed as "docile", if they settled between 6-8cm apart, they were typed as "unknown", and if they settled more than 8cm apart, they were typed as "aggressive". It has been argued that there is a natural break between the two behavioral types around 7cm (Pruitt and Riechert, 2009), and typically studies consider those with a score under 7cm to be "docile" and those with a score over 7cm to be "aggressive", however as we were using a slightly smaller box for tests than previous studies, we erred on the side of uncertainty in typing those near 7cm. In an effort to replicate earlier studies using this test to measure behavioral variation in populations (i.e., Jones et al., 2007; Pruitt et al., 2008; Riechert and Jones, 2008; Pruitt and Riechert, 2009), we did not use the later adopted criterion (e.g., Pruitt and Riechert, 2011) that known docile females must have a score of zero (touching another female after 24 hours in the same box) before being used to score Interindividual Distances of unknown females. However, we believe this criterion may be necessary for typing individuals rigorously, and we discuss implications of this criterion further in the Discussion section.

Tests for all females were completed in a lab at the University of Texas at Austin, with natural dark-light cycles. Females from each region (Southeast and Texas) were only tested against females from their own region. Tests for females collected in the Southeast were completed in July and August, 2017, and tests for females collected in Texas were completed in August and September, 2017. Throughout the tests, females were misted with water 1-2 times a week, and given 3-4 *Drosophila* flies for food, 24 hours prior to every trial. For each trial, females were paired together randomly in boxes of size 11cm x 11cm x 3.5cm, and each

individual was either marked with a black or yellow Sharpie® on the dorsal abdomen so that we could return females to the correct home containers after distance measurements. Even though some females were typed after each round of tests, experimenters were blind to female's identity (e.g., location collected, whether the female was from a multifemale nest), and type (docile/aggressive, if known) when measuring distance.

A second test, the Huddle Response Test has also been used to estimate aggressive tendency and boldness in *A. studiosus*. This test measures how long females remain in a defense response, or "huddled" position, after the experimenter mimics an approaching predator by aiming two, rapid puffs of air from a newborn nasal aspirator (Safety 1st) at a female approximately 2cm from her dorsal prosoma (Keiser et al., 2016). If the female huddles in response to the puffs, she is timed for how long it takes her from the start of her huddle to a) move out of the huddle, and b) traverse the length of her body. Females that leave their huddle quickly are categorized as more "aggressive" or "bold" whereas those that remain huddled are categorized as less "aggressive". Time spent in the huddle has been shown to be negatively correlated with Interindividual Distance Scores (Pruitt et al., 2008b), suggesting that both tests are measuring some aspect of aggressive tendency in an individual. We completed this test for a subset of females (N = 37 from Southeastern populations; N=36 from Texas populations) from the above collections. Prior to testing, each female was allowed five minutes to explore their arena (a clear, plastic, ethanol-sterilized box), and during testing, experimenters were blind to the female's Interindividual Distance Scores. If a female did not huddle, we waited five minutes before trying to elicit the huddle response again with a second puff of air. If after three tries, no huddle response could be induced, the female was assigned huddle response scores of zero, which is thought to be typical of an extreme "aggressive" or "bold" type (Keiser et al., 2016). If a female

remained huddled for ten minutes, we terminated the test, and did not re-test the female again until they had released their huddle and moved around their test chamber for at least five minutes. Trials were repeated three times during the same day to obtain an average huddle response score for each female, with at least five minutes between trials.

Results

Across all collections in Texas (N = 121 females), we typed 44 females as "docile", 5 females as "unknown" and 72 females as "aggressive" using the Interindividual Distance Test. In comparison, we typed 37 females as "docile", 5 females as "unknown" and 31 females as "aggressive" from the control, Southeastern populations (N = 73 females). The distribution of Interindividual Distance scores (Figure 3.1) was significantly different across the two regions (Wilcoxon Rank Sum Test; W = 5330.5, p = 0.037); Texas populations had an average Interindividual Distance Score of 8.46 +/- 3.64cm and the Southeastern populations had an average score of 7.17 +/- 4.17 cm. Distance scores were also significantly different across all locations (Kruskal-Wallis Rank Sum Test, $p < 0.01$). Figure 3.5 presents the distribution of distance scores at each location. For Texas populations, the proportion of docile individuals decreased with increasing latitude, while there was no apparent latitudinal trend for the Southeastern populations (Figure 3.6).

We also saw much variation in huddle response times across females (Figure 3.2). Out of 36 females sampled from Texas populations, two females showed the extreme "aggressive" behavior of no huddle response. Overall, we saw an average time to move out of huddle of 112.4 sec (range 0-448 sec) and an average time to traverse a whole body length after huddle of 136.5 sec (range 0-448 sec) across Texas populations. Out of 37 females sampled from Southeastern populations, five showed the extreme "aggressive" behavior of no huddle response, and there

was an average time to move out of huddle of 120.5 sec (range 0-552 sec), and an average time to traverse a whole body length after huddle of 181.6 sec (range 0-600 sec). Though we found a slight negative correlation between Interindividual Distance Scores and time to traverse a body length after huddling, these correlations were not significant for either Texas or Southeastern populations (Figure 3.3; Spearman's Rank Correlation Test, $p>0.05$). Comparing huddle response times between behavioral types (based on the Interindividual Distance Test), regions, and whether a female originated from a multifemale nest, we also found no significant difference (Figure 3.4, Figure 3.7; Wilcoxon Rank Sum Test, $p > 0.05$).

Discussion

Although we found variation in aggressive tendencies in Texas populations of *A. studiosus* that is similar to variation seen in Southeastern populations, unlike previous studies, we did not see an association between the two different measures of aggressive tendency as estimated by the Interindividual Distance Test and by the Huddle Response test. There may be multiple, non-exclusive explanations for this discrepancy between our study and earlier studies. First, as stated previously, researchers have occasionally used different or additional criterion to assign behavioral types to females using the Interindividual Distance Test. For example, in Pruitt and Ferrari (2011) and Pruitt et al. (2011), the "docile" females used to designate Interindividual Distance Scores had to be given a score of zero in the first round of tests; that is, they had to be in contact with another female after twenty-four hours. If we had used this strict criterion, none of the females would have been typed as "docile" in our study, as the smallest distance between females we recorded from any location was 0.5cm, and thus we could not have identified "docile" or "aggressive" types at all using this test. Therefore we can conclude that the test we used is only comparable to studies defining population frequencies of behavioral syndromes in *A.*

studiosus that did not use this "zero score" criterion. However, using a less strict definition of "docile" might explain why we do not find longer huddle response times in "docile" females, or a correlation between Interindividual Distance scores and huddle response scores.

Second, individual conditions in this study, such as age, diet, and prior social experience, are different from previous studies. Many studies typing individuals using the Interindividual Distance Test used adult females that had been collected in the field as late-instar juveniles (e.g. Holbrook et al., 2014; Pruitt et al., 2008). Due to various constraints on field collection, we could not collect females before July, therefore the females used in this study were more mature, mated, and reproductive than those used in other studies. Additionally, they had spent more of their life hunting for themselves than females that are fed in the lab from a juvenile stage, meaning that their diet is likely more diverse, and perhaps more or less abundant than a lab diet, depending on their location. These are all factors that could potentially change the aggressive tendencies of adult female *A. studiosus*. For example, a previous study has shown a slight effect of feeding on aggression: Lichtenstein et al. (2016) found that higher feeding rates were associated with reduced "aggression" (latency to attack a prey item) in three spider species, including *A. studiosus*. However, they did not find an effect of feeding on "boldness", as measured using the Huddle Response Test, and they did not measure "aggression" using the Interindividual Distance Test. Older females had also likely experienced more social interactions with female conspecifics, especially if collected from a multifemale nest. Studies in another social spider genus, *Stegodyphus* (Araneae: Eresidae), have found that individuals became more aggressive after social interaction with a shy individual (Hunt et al., 2018) and bolder after repeated social interactions (Modlmeier et al., 2014). While it has yet to be shown whether social experience impacts individual behavior in *A. studiosus*, social history may be an important factor

for why older females are typically more aggressive than younger females, and may explain why we did not find many extreme, docile females, especially in Texas populations that were collected and tested one month later than the Southeastern populations.

Third, if the two tests used in this study do not exactly measure the same behavioral tendency, then our sample size for the Huddle Response Tests may be too small to find a significant correlation between Huddle Response times and Interindividual Distance scores. That is, the Interindividual Distance Test specifically measures whether at least one individual reacts aggressively toward the other (Riechert and Jones, 2008b). In contrast, the Huddle Response Test does not measure aggressive behavior toward another individual, but instead measures the reaction of an individual to an approaching predator, and is often termed a measure of "boldness" rather than aggression. However, if that association is driven by extreme docile individuals, of which we found only a few, or if that association is weak, and our sample size is not large, then the association will not be significant, even if it exists in nature.

In all, while we do not contest previous research demonstrating distinct behavioral syndromes and personalities in *A. studiosus*, we do think that moving forward, researchers should be careful in using single personality tests to deem any individual "docile" or "aggressive" over long periods of time. Not only that, but we suggest researchers test personality multiple times over the course of an experiment, and carefully consider which personality tests are most appropriate to the context of the experiment they are conducting. For example, studies conducted on the effects of intraspecific cooperation on reproductive success in a colony may want to use the Interindividual Distance Test to determine personality of individuals in the group, while the Huddle Response Test would be more appropriate for studies conducted on the effects of personality on evading predation. In addition, researchers must be cognizant of how

spider condition (e.g. age, diet, and social experience) can influence their results, and take these factors into account when comparing their studies to those using spiders collected at a different life stage.

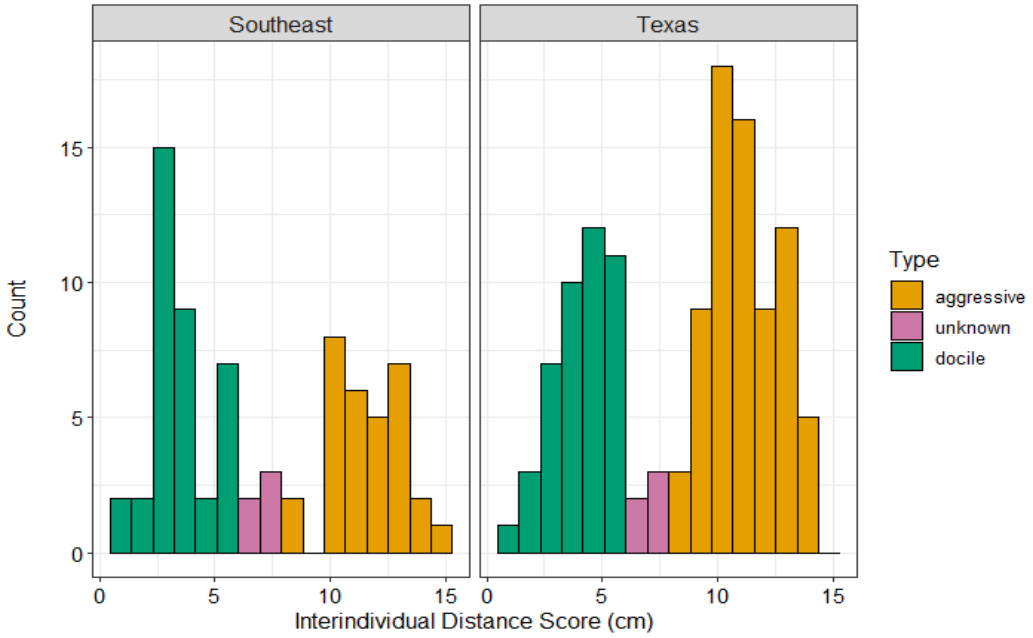

Figure 3.1 Number of females from Southeast (Alabama and Tennessee) U.S. and from Texas, U.S. categorized as "docile", "unknown", or "aggressive" according to their Interindividual Distance Score, as determined by testing each female against a known "docile" female.

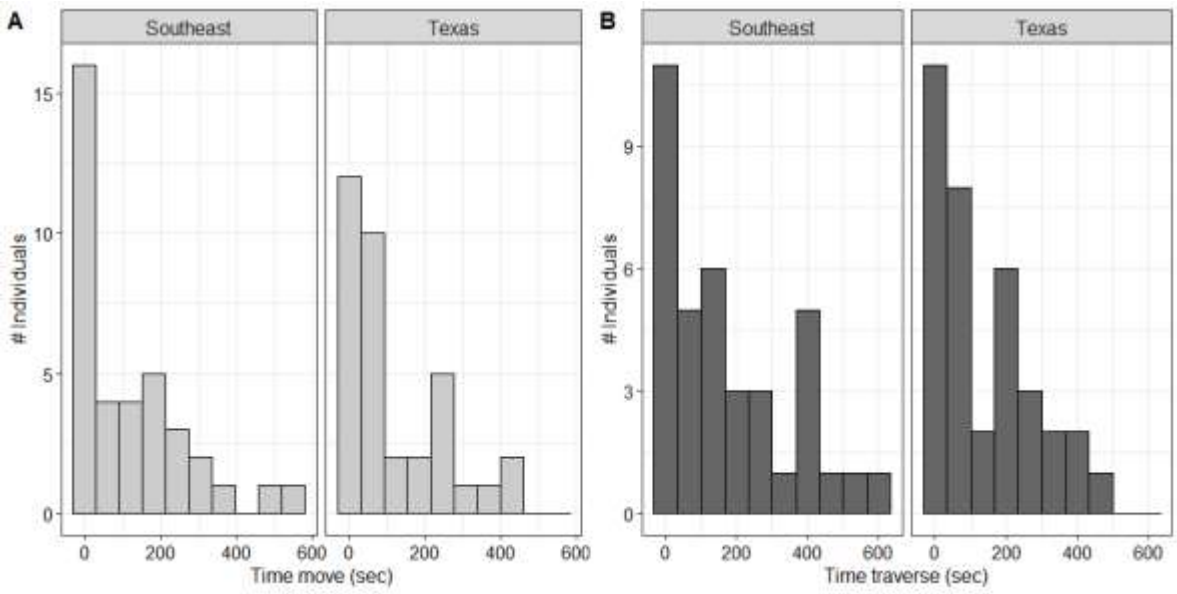

Figure 3.2 Distribution of huddle response times for each region. **A)** The time between huddling and when the female first moved out of the huddle, and **B)** the time between huddling and when the female first traversed a whole body length.

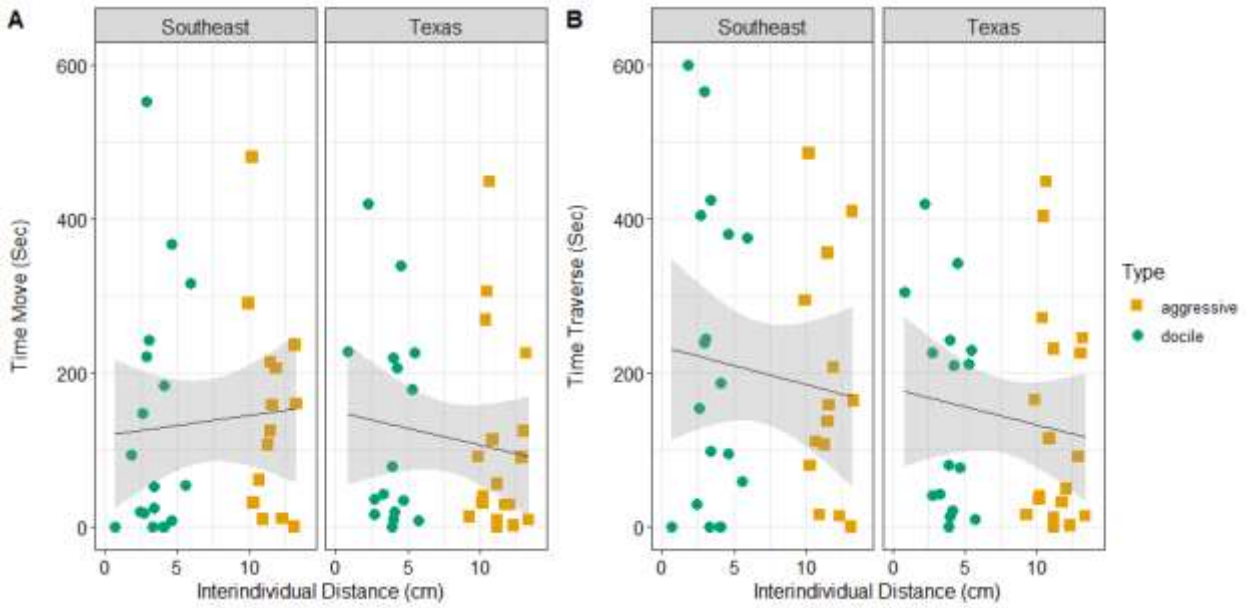

Figure 3.3 Average huddle response times by Interindividual Distance Score for each region; **A)** The time between start of huddle and first movement out of huddle, and **B)** the time between start of huddle and traversing a whole body length. The association between Interindividual Distance Scores and huddle response is not significant ($p>0.05$ Spearman's Rank Correlation Test) for any huddle response or region.

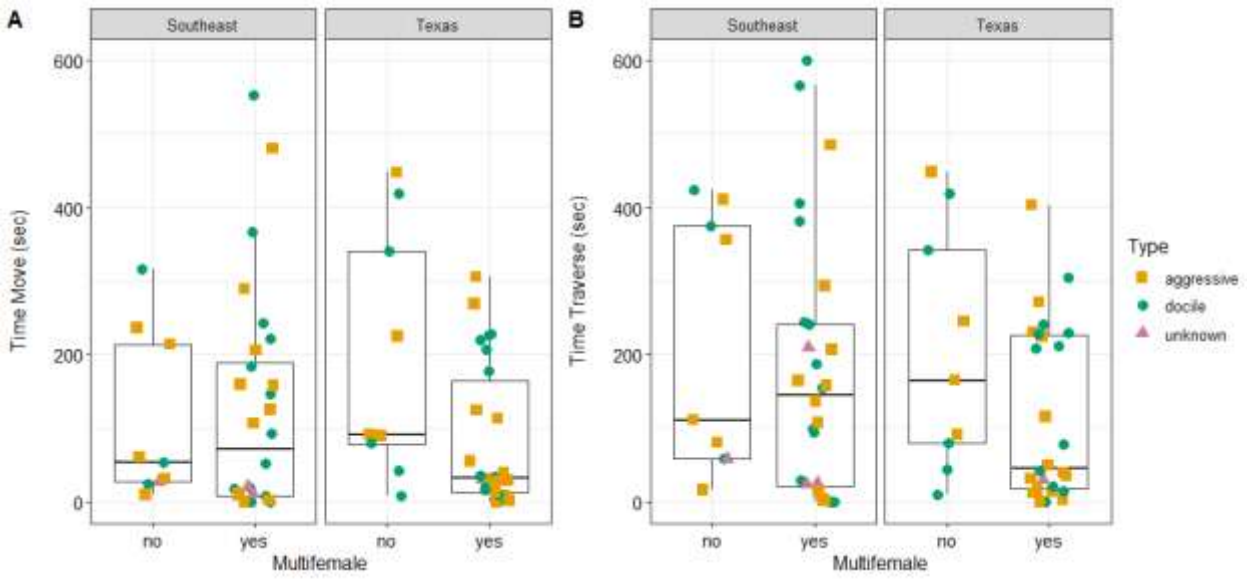

Figure 3.4 Huddle response scores as a result of whether females originated from a multifemale nest or not, and according to behavioral "type" as determined by the Interindividual Distance Test. There were no significant differences in huddle response times between regions, types, or multifemale nest types (Wilcoxon Rank Sum Tests, $p > 0.05$).

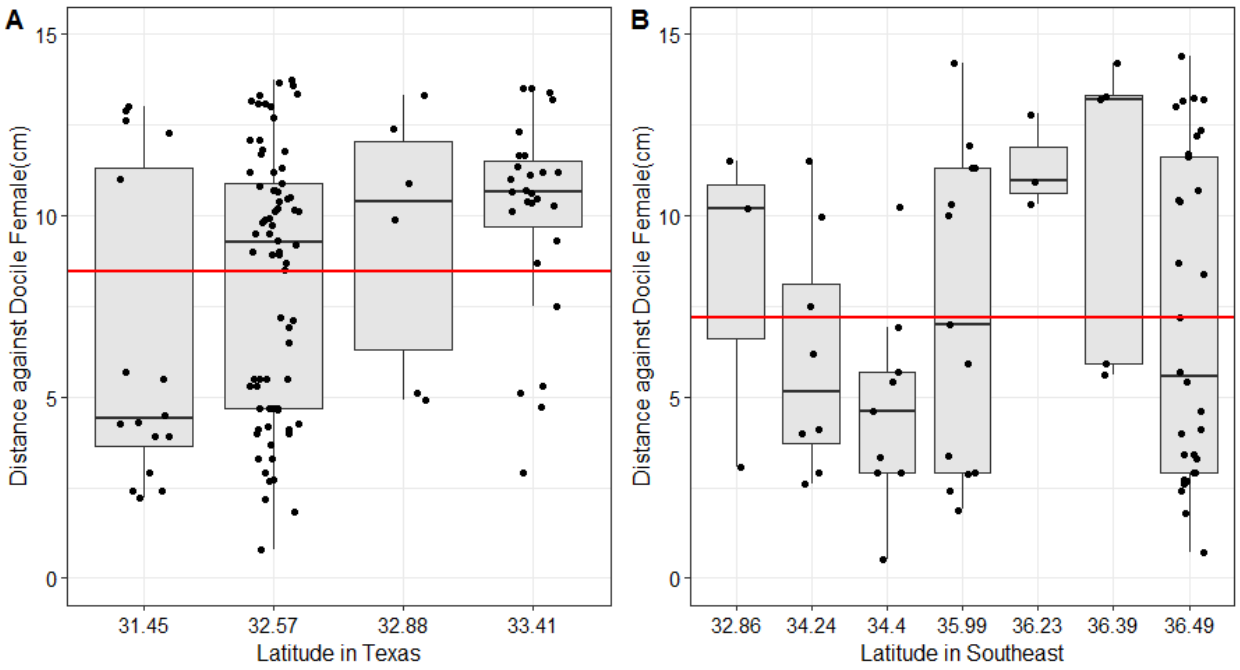

Figure 3.5 Individual distance scores based on the Interindividual Distance Test by latitude of each sampled location. Average for each region indicated by red line.

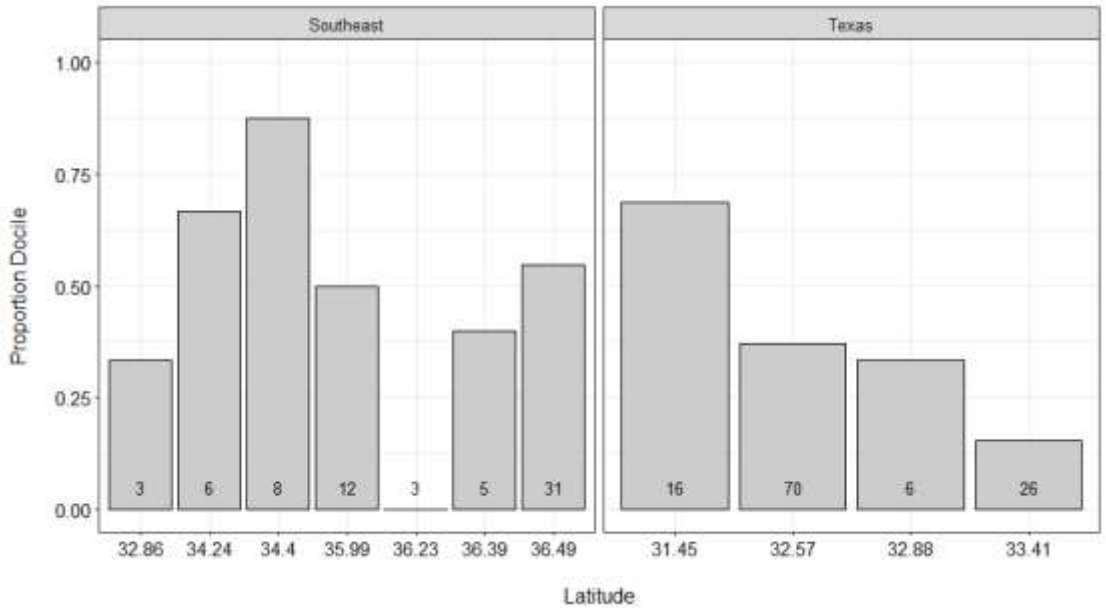

Figure 3.6 Proportion of females typed as "docile" using the Interindividual Distance Test by latitude of females' collection site. Sample size for each location indicated by value within bars.

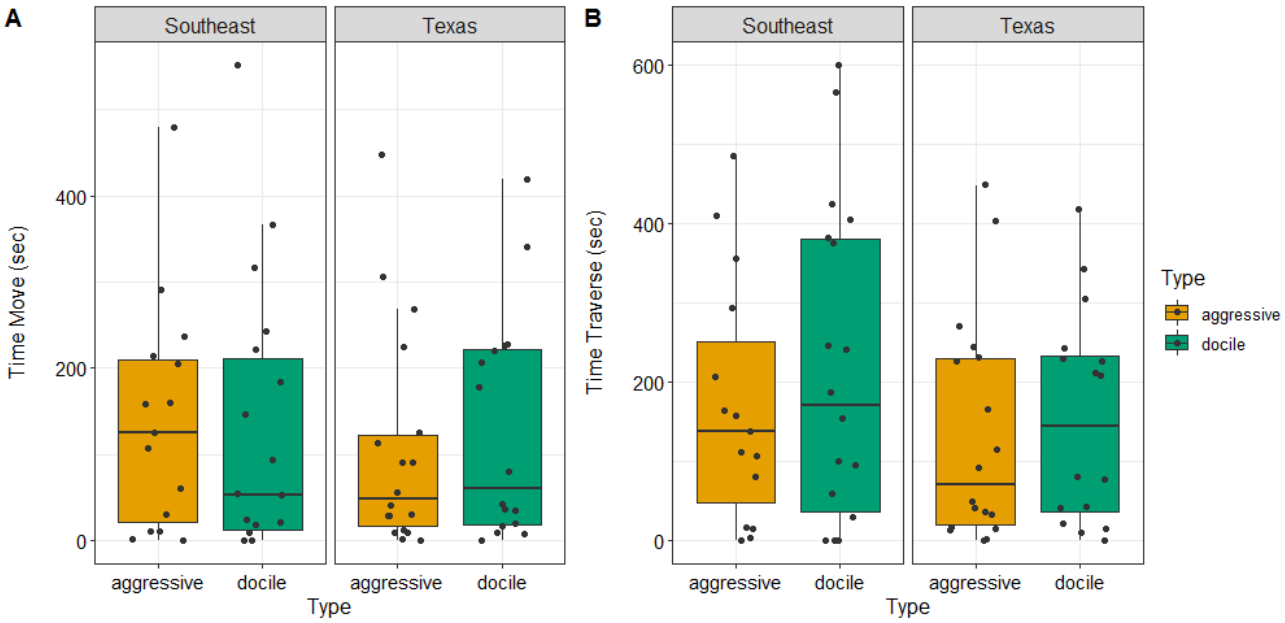

Figure 3.7 Results of the huddle response test for each region and social type (as determined by the Interindividual Distance Tests); specifically, the time it took a female from the beginning of a huddle to **(A)** move out of the huddle and **(B)** traverse her whole body length. There was no significant difference between types for any time measurement (Wilcoxon Rank Sum Test, $p>0.05$).

CHAPTER 4: PATHOGENS DOMINATE THE BACTERIAL MICROBIOME OF THE SOCIALLY POLYMORPHIC SPIDER *ANELOSIMUS STUDIOSUS*.

Abstract

Studies completed thus far on spider microbiomes have found spiders have a simple bacterial microbiome that is either dominated by maternally inherited endosymbionts or dependent on diet. This study attempts to answer multiple remaining questions regarding spider bacterial microbiomes: 1) do spiders always harbor a low diversity of bacterial species, 2) are spider bacterial microbiomes transient or stable, and 3) is there overlap in the bacterial communities of spiders and their silk? To answer these questions, we completed 16S rRNA metagenomics of bacterial communities of wild-caught adult females, juveniles, egg sacs, and silk samples of the socially polymorphic spider, *Anelosimus studiosus* (Araneae: Theridiidae). We also performed an experiment on lab-raised individuals, to test whether single feeding events or molting alter the bacterial microbiomes of *A. studiosus*. Across assays, we found that *A. studiosus* raised in the lab harbored much lower bacterial diversity within and between samples than wild-caught individuals, suggesting that environment, and potentially diet, plays a large role in *A. studiosus* bacterial communities. Additionally, we found *A. studiosus* individuals in both assays, but especially in the wild-caught assay, were often infected with pathogenic bacteria, including *Borrelia* and *Ehrlichia*. Despite observing lower bacterial diversity in adult females than egg sacs or juvenile *A. studiosus* collected in the wild, we found that molting significantly increased bacterial diversity. Overall, our results were in accordance with previous studies findings' that individual spiders do not harbor diverse bacterial microbiomes, but we show that diet and

environment can nevertheless influence these communities. We also found that, the unclean cobwebs of *A. studiosus* silk may harbor many bacteria, though further studies are needed to decide if these bacteria are dead or alive.

Introduction

Countless studies have demonstrated the importance of bacterial microbiomes to the health and survival of their hosts (Berendsen et al., 2012; Cho and Blaser, 2012; Engel and Moran, 2013; Raymann and Moran, 2018). Nevertheless, while many species depend on specific bacteria for particular functions or benefit from harboring diverse bacterial communities, other studies have found that not all species depend on, or retain, a diverse, core bacterial community (Hammer et al., 2017). For example, a study in *Ixodes* ticks found that, while ticks may occasionally harbor diverse bacterial communities, these communities are not constant, and only a few, transient, pathogenic, bacterial genera may stably colonize the gut during a tick's lifetime (Ross et al., 2018). Likewise, in the first culture-independent study of spider microbiomes in the dwarf spider *Oedothorax gibbosus*, Vanthournout and Hendrickx (2015) found that more than 99% of bacterial taxa were assigned to only four operational taxonomic units (OTUs) representing *Candidatus* Rhabdochlamydia porcellionis, *Wolbachia sp.*, *Rickettsia sp.*, and *Candidatus* Cardinium hertigii, bacterial genera that are all arthropod endosymbionts. Therefore, the authors hypothesized that the spider microbiome is dominated by, or even restricted to, these maternally inherited endosymbionts (Vanthournout and Hendrickx, 2015). It has been shown that spiders often do harbor such endosymbionts, but this is not true for all species or individuals (Goodacre et al., 2006; Martin and Goodacre, 2009), thus raising the question of how the composition of the spider bacterial microbiome is structured in the absence of maternally inherited endosymbionts.

Surveys across the bee family (Apidae) have not shown that the presence of maternally inherited endosymbionts (e.g. *Wolbachia*) necessarily predicts lower overall diversity in bacterial communities, demonstrating that these specialized endosymbionts may not always be the dominant species in their host, even when present (Martinson et al., 2011; McFrederick et al., 2012, 2014). Indeed, a recent study of the bacterial communities of eight spider species found that though the spiders' bacterial communities were always dominated by maternally inherited endosymbionts (e.g., *Wolbachia*, *Rickettsia*, etc.), they also contained many common environmental bacteria (e.g., *Pseudomonas*, *Sphingomonas* etc.) (Zhang et al., 2018). However, a survey of the bacterial communities of multiple *Stegodyphus* spiders demonstrated that maternally inherited bacteria are not always common in spiders, and proposed that diet may be highly influential in determination of spider microbiomes when endosymbionts are not prevalent (Vanthournout et al., 2018). It is possible, though, that both diet, and the presence of maternally inherited bacteria can shape the bacterial communities of spiders, but it is unknown to what extent maternally inherited bacteria and other life-history factors influence spider microbiomes.

Here, we further investigate the question of whether spiders harbor a low diversity of bacteria by surveying the bacterial communities of multiple wild populations of the socially polymorphic spider, *Anelosimus studiosus*, at different life stages (egg sac, juveniles, adult males and females). We then compare bacteria found in these individuals to the bacterial communities of their silken nests, in an effort to determine if their environment influences their microbiome. In addition to these surveys, we set up a lab experiment testing whether feeding behavior, molting, or maternally inherited bacteria had large effects on the bacterial communities of lab-raised *A. studiosus*.

Study species

Anelosimus studiosus is a socially polymorphic spider that lives throughout the Americas from Argentina to the United States, where it is primarily subsocial in most of its range; adult females provide care for their young, but do not cooperate with other adults, and juveniles spend extended periods of time in their natal nests, but disperse before adulthood. In the northernmost parts of its range in the Southeastern United States, *A. studiosus* occasionally exhibits social behaviors similar to that seen in the quasi-social spider species, in which adults live in colonies containing dozens of individuals, with females cooperating in brood care, web maintenance, and prey capture (Furey, 1998; Riechert and Jones, 2008). In all *A. studiosus* nests, adult females capture and share prey with their juvenile offspring, not only by degrading tissue and allowing juveniles to consume the liquefied prey (Figure 4.1B), but also through direct trophallaxis from mother to offspring. In subsocial nests, penultimate juvenile females will usually leave their natal nest to build their own dense, solitary cobwebs in nearby disturbed, riparian habitats (Figure 4.1A). Occasionally, late-instar females may take over their natal nest as their other siblings disperse (Brach, 1977b). Adult males may build smaller, solitary webs near females' webs, or will build retreats in an adult female's web (Brach, 1977b). Females do not groom their webs extensively; consequently, over the course of a web's lifetime spanning sometimes multiple generations and years if an offspring inherits the nest, a web will collect much debris like dead insect carcasses, fallen leaves, soil particles, and stream/lake particles if overhanging a waterway.

Methods

Wild-caught Assay: Collection

Webs of *Anelosimus studiosus* were collected in Central Texas, USA, from June-August 2015 (n = 4 locations; 70 webs across locations; collection site coordinates in Table A4.1). Prior to collection, Ziploc 1-gallon bags used to collect females in their webs were sterilized by placing them under UV light in a laminar-flow hood for twenty minutes. After bagging in the field, webs were dissected in the lab using sterile technique; lab benches and buckets were sterilized with 10% bleach then 70% ethanol, utensils were flame-sterilized, and clean gloves were worn at all times when collecting spiders and webs. If present, adults (females and males), juveniles of unknown developmental stage, egg sacs, and spooled silk samples were placed into separate, sterile, centrifuge tubes, and frozen at -60C. Silk samples were not cleaned or altered in any way, except to remove them from any large pieces of plant material they were stuck to; thus they often contained visible soil particles, moisture, sap, small pieces of plant material and/or dead insect carcasses.

Diet and Molting Assay: Collection and Experimental Design

Given the lack of highly prevalent bacteria (see Results), we set up lab experiments to test whether development, feeding, and molting (shedding of the spider exoskeleton), altered the bacterial communities of individual *A. studiosus*. Females with their egg sacs were collected in July-August 2016 throughout central Texas (n = 6 locations; see Table A4.1 for coordinates), then placed into sterile home containers in the lab (5.5 oz. Dart Conex^{TM} Complements soufflé/portion cups) at UT-Austin. Females were fed live *Drosophila melanogaster* fruit flies ad libitum, and containers were misted with Epure water once a week. Once juveniles hatched, 3-5 of these second-instar individuals were collected and stored in sterile 1.5 ml centrifuge tubes in a

-60°C freezer. These individuals were assigned to the "Emerged" group as they had recently emerged from their egg sac. After this, the remaining juveniles and their mothers were fed *D. melanogaster*, until mothers died and juveniles matured to their third or fourth instar. At this point – before their final molt, but after they were large enough to catch prey alone – juveniles from each family were separated into individual containers and assigned one of three different treatments: Before Feeding, After Feeding, and After Feeding + Molting.

Individuals in the Before Feeding group were starved for one week, at which point they were frozen at -60°C in 1.5ml sterile centrifuge tubes. Individuals in the After Feeding group were also starved for one week, then allowed to feed for twenty-four hours on a meal of *Drosophila melanogaster* fruit flies, then placed in a 1.5ml sterile centrifuge tube and frozen at -60°C. All individuals in this group were observed feeding on a fruit fly. The After Feeding + Molting group were starved for a week, then fed multiple meals of *D. melanogaster* fruit flies until they molted, which took from one week to two months across individuals. After each feeding of the After Feeding + Molting individuals, we removed *D. melanogaster* carcasses from the web, to ensure the spiders did not molt and feed again on preserved prey in their webs. These fly carcasses were frozen and included in microbiome analyses (Flies Fed treatment) to see if their microbiomes were altered by the spider feeding behavior. Within twenty-four hours of molting, we placed spiders and their molted exoskeletons (Molts) in separate sterile centrifuge tubes and stored them at -60°C. Additionally, we froze live *D. melanogaster* samples from the fruit fly stock each time we fed the spiders. If individuals died, they were removed from the experiment. To ensure balanced blocking of treatments by family (offspring from the same mother), if we did not have individuals in each of the spider treatments from the same family by the end of the

experiment, the family was not included in further analyses. By the end of the experiment, we had samples across all treatments from fourteen different families.

Sample Preparation and Sequencing

We extracted DNA of all samples for both experiments, including negative controls, using MoBIO Powersoil kits following the instruction manual. After extraction, we quantified DNA using Qubit and we used PCR amplification of the 16S ribosomal gene to check that samples contained bacteria. We submitted samples for sequencing if they contained at least 1ng/µl of DNA (Qubit) and positive 16S rDNA results. We also submitted all negative controls (extraction kit reagents only) for sequencing, although they always had DNA concentrations of <0.05 ng/µl and did not show any bands after 16S gene amplification. Unfortunately, the "Emerged" and "Molts" samples from the diet and molting assay did not consistently contain more than 0.05 ng/µl of DNA, and thus were not submitted for sequencing. While it is possible these samples contain small amounts of bacteria, submitting such low quantities of DNA could result in spurious amplification of lab, DNA extraction kit, or sequencing kit contaminant bacterial DNA (Eisenhofer et al., 2019). We submitted samples to the Genome Sequencing and Analysis Facility (GSAF) at the University of Texas, Austin, for library preparation with the NebNext kit. The GSAF completed metagenomic sequencing of the variable V4 region of the 16s rRNA gene (primers: forward - Hyb8F_rRNA: 5'-TCGTCGGCAGCGTCA GA TGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTA -3' and Hyb338R_rRNA and reverse - 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGT WTC TAAT-3") on the Illumina MiSeq V3 Platform. Sequencing for the wild-caught assay and the diet and molting assay were completed in different runs, in different years, therefore the below quantitative analyses were completed only within assays.

Quality Filtering and Denoising

We used QIIME2 (Bolyen et al., 2018) and DADA2 (Callahan et al., 2016a) to remove primers, perform quality filtering, join reads, and remove chimeras to produce a community of amplicon sequence variants (ASVs) for each sample. Taxonomy was subsequently assigned against the Silva 132 database (Glöckner et al., 2017) using a Naïve Bayes classifier trained on Silva 132 99% OTUs from 515F/806R region of the 16S rRNA gene. We created a phylogenetic tree for input to Unifrac using FastTree 2 (Price et al., 2010) in QIIME2.

Preprocessing Amplicon Sequence Variant (ASV) Tables

From this point forward, we completed all analyses in R 3.5.1. (R Core Team, 2013), and roughly followed the workflow described in Callahan et al. (2016b). All plots were made in base R, or using the packages *ggplot2* (Wickham, 2016) with *ggpubr* (Kassambara, 2018). First, we identified and removed non-bacterial ASVs (chloroplast, mitochondrial, and Archaeal DNA) and low abundance ASVs (present in <1 % of individuals, which for both assays approximately meant present in two or fewer samples per assay) using the *phyloseq* package (McMurdie and Holmes, 2013). Although the negative controls always contained <0.05ng/µl DNA and did not show bands in 16S rDNA screens before sequencing, they still contained ASVs after sequencing, many of which have been identified as common kit contaminants in multiple studies (Salter et al., 2014; de Goffau et al., 2018; Eisenhofer et al., 2019), and some of which are likely an artifact of cross-contamination during sequencing (e.g. the intracellular bacteria *Ehrlichia* sp.; [Eisenhofer et al., 2019; Gu et al., 2019]). To remove potential contaminant ASVs, we used the *decontam* package (Davis et al., 2018) , which identifies ASVs that are more prevalent in negative samples than samples of interest. We used a conservative prevalence threshold of 0.1 for both assays after investigating probability score distributions (Figure A4.3), removing all

ASVs identified as contaminants at this threshold. We also removed individual ASVs that were present in our negative controls, and not removed by decontam, but known to be highly abundant in MoBIO Powersoil Extraction Kits (e.g. *Propionibacterium* sp.; Glassing et al., 2016), but we did not remove all ASVs belonging to these genera (i.e. ASVs belonging to genera common to MoBIO kits that were not found in negative controls, but found in other samples).

Estimating Alpha Diversity

We calculated Shannon's richness and Pielous' evenness using *phyloseq* (McMurdie and Holmes, 2013), again. We tested for significant differences in alpha diversity between specimen types (for wild-caught assay) and treatments (for diet and molting assay) using the Wilcoxon Rank Sum Test ($p = 0.05$). All alpha diversity measures were calculated after removing potential contaminating bacteria ($n = 54$ ASVs for wild-caught assay; $n = 56$ ASVs for diet and molting assay), unassigned ASVs, and ASVs assigned to Kingdoms outside of Bacteria, but before filtering low prevalence (present <1% of samples) taxa.

Estimating Beta Diversity

Given the variation in sampling depth, we used natural log transformations before calculating and plotting ordinations for both assays. This transformation successfully normalized data across both ASV tables (Figure A4.4). For the wild-caught assay, we used the *vegan* package (Oksanen et al., 2015) to estimate beta diversity using Bray-Curtis and weighted Unifrac distances. We then plotted both diversity estimates in PCoA ordinations, and used *vegan* to perform permutational analysis of variation (PERMANOVA) to test for significant differences between groups (with Bray-Curtis distances only), with 999 permutations. For the diet and molting assay we used Bray-Curtis distances for PERMANOVA analyses, and plotted community differences using a DPCoA ordination of patristic distances. Additionally, for each categorical variable in

both assays we analyzed whether within group dispersions were significantly different using ANOVA ($p = 0.05$), as heterogeneity in within group dispersion can cause significant differences between groups as analyzed by PERMANOVA.

Differential Abundance Analysis

To test for significant differences in ASV abundance across variables, we used hierarchical multiple testing implemented through the *structSSI* (Sankaran and Holmes, 2014) and *phyloseq* (McMurdie and Holmes, 2013) packages. Because the majority of ASVs in our assays were not abundant, we first transformed ASV abundance tables using the variance stablizing transformation available through *DESeq2* (Love et al., 2014) on geometric means of ASV abundance across factors. After this, we performed hierarchical multiple testing with the *structSSI* package, which produces corrected p-values utilizing the Hierarchical False Discovery Rate Procedure described in Benjamini and Yekutieli (2001). For the wild-caught assay, we tested the following factors: specimen type, collection location, web/nest, collection location for adult females only, and collection location for silk only. For the diet and molting assay, we tested the following factors: treatment (include *D. melanogaster* samples), treatment (without *D. melanogaster* samples, Before Feeding vs After Feeding treatments, After Feeding vs After Feeding + Molting treatment, family, and maternal collection location.

Random Forest Analysis

We used the *randomForest* package (Liaw and Wiener, 2002) in R to generate random forest models on natural log-transformed ASV abundance data to determine if ASV abundances could predict specimen type or location collected for the wild-caught assay, and treatment, family, or maternal collection site for the diet and molting assay. For cross-validation of all models except the family model (diet and molting assay), we randomly sorted 80% of samples into a training

set, and 20% of samples into a testing set. For cross-validation of the family model (diet and molting assay), we had to increase the size of our training set (90% of samples, not including *D. melanogaster* controls) so that all fourteen families were represented. For the diet and molting assay, we completed random forest modelling for treatment with and without *D. melanogaster* samples, and we completed random forest modelling for family and maternal collection site without *D. melanogaster* samples.

Results

Wild-Caught Assay

Alpha diversity

Sequencing the V4 region of the 16S rRNA gene of 181 samples resulted in 7,810,451 total reads, with a mean of 43,151.7 reads per sample, a median of 27,968 reads per sample, and a range of 897 to 1,094,962 reads per sample (see Figure 4.6 for reads across specimen types). After removing reads mapped to eukaryotic DNA, taxa unidentified to Kingdom, and potential contaminants, we found that there were 12,174 ASVs across 172 samples, with a mean of 92.4± 115.0 ASVs per sample. Adult males had the lowest median Shannon diversity, while silk samples had the highest, and were significantly more diverse than all other sample types (Wilcoxon Rank Sum, all $p < 0.05$ for all comparisons between sample types; Table 4.1, Figure 4.2). Similarly, community evenness was highest for silk samples, lowest for adult males, and there was a wide range of evenness across adult female samples (Table 4.1, Figure 4.2). After removing taxa not identified to Phylum, and taxa that were present in less than 1% of samples, we found that there were 945 ASVs across 172 wild-caught samples.

Beta Diversity and Community Overlap

We found ASV communities significantly differed across all specimen types, (Table 4.2; PERMANOVA, $p < 0.05$), but this result may be due to higher community dispersion in adult male samples than other specimen types (Figure A4.5). Removing adult male samples from analysis, we found that communities remained significantly different across specimen types (adult females, silk, egg sacs, and juveniles; Table 4.2; PERMANOVA, $p<0.05$). However, with and without the inclusion of adult males, specimen type only predicted approximately 5-6% of the total variation across all sample communities, indicating that there is much variation within each specimen type. Therefore, we also tested a model including location (where samples were collected) and family (whether samples originated from the same nest), where family was nested in collection location, and specimen type was nested in family (Table 4.2). All terms and interactions were significant, with the double-nested term explaining 39% of the variation across samples (PERMANOVA, $p<0.05$), and 9% of variation unexplained. However, beta dispersion estimates across family and location variables were also significantly different (ANOVA, $p<0.05$; see beta dispersion estimates for location in Figure A4.6), suggesting that variation within these variables could be responsible for significant differences between groups (Figure A4.7). Accordingly, PCoA plots of Bray-Curtis and weighted Unifrac distances showed some grouping of specimen types and locations (Figure 4.3), but no distinct separation between categories within each factor.

Although bacterial communities were significantly different across specimen types and locations, we also found some bacteria were shared across samples. For instance, within nests, females and their silk samples shared, on average, 11% (SD = 0.14; Table 4.3) of bacterial ASVs. To determine if silk was a potential source of bacteria for female bacterial communities, we calculated the proportion of female ASVs found in silk samples taken from the same nest (as

opposed to ASVs found in *both* silk and female). On average, 29% of bacteria in adult females were identical to ASVs from their silk nests, but this proportion varied widely, with the proportion of female ASVs found in their silk ranging from 0-100% (Table 4.3 and Figure A4.8). We also investigated if adult females were a large source of bacteria for their egg sacs, but found that less than 15% of the ASVs in all egg sacs (n=15) were also found in their mother's ASV communities (Table 4.3; mean = 0.07 ± 0.09; Figure A4.9), with five egg sacs harboring none of the ASVs found in their mother's sample, despite harboring much higher ASV diversity (Table 4.1).

Prevalence & Abundance Analyses

No single ASV was present in more than 50% of all samples, across specimen types (adult females, egg sacs, adult males, juveniles, and silk). The most prevalent ASV in females and males was an ASV assigned to an unidentified Anaplasmataceae species which was present in 42.5% of adult female samples (n=80) and 86% of adult male samples (n=7). Ten other ASVs assigned to *Ehrlichia* and unidentified Anaplasmataceae species were also abundant in 86% of male samples. The most prevalent ASV in silk was an unidentified Anaplasmataceae species that was present in 39.3% of silk samples, while the most prevalent bacterial genus was *Methylobacterium*, which was present in 73.8% of silk samples (n=61). The most prevalent ASV in juvenile samples was a *Borrelia* species which was present in 67% of samples (n = 9). *Pseudomonas* and *Propionibacterium* were the most prevalent (49.4%) bacterial genera across all specimen types.

Differential abundance analysis on variance-stabilizing transformed data found eighty-eight ASVs with significantly different abundance across all specimen types (Table A4.2). No ASVs had significantly different abundance across collection locations when all specimen types were

included, but within adult females, *Carnimonas* ASVs and various Xanthomonadales ASVs were differentially abundant across collection sites (Table A4.3). Surprisingly, when we investigated silk samples, alone, no ASVs had significantly different abundance across locations. The relative abundance of fourteen genera and two families containing differentially abundant ASVs is plotted in Figure 4.4. The relative abundance of all genera present in >2% of samples is plotted in Figure A4.7. While a few *A. studiosus* samples contained ASVs corresponding to the maternally inherited endosymbionts *Wolbachia*, *Rickettsiella*, and *Spiroplasma*, many of these same ASVs were also found in silk samples from the same location, suggesting that they perhaps originated from consumption of prey items infected with these bacteria (i.e., horizontal transfer), rather than through maternal inheritance (i.e., vertical transfer). ASVs for *Rickettsiella* were occasionally found in egg sac samples, but they were not abundant (Figure 4.4) and were not also found in their mothers (Figure A4.9). Potentially pathogenic bacteria were also abundant in *A. studiosus* samples (individuals and silk), particularly *Borrelia* and *Ehrlichia* species (Figures 4.4 and 4.9). While some common environmental bacterial were abundant across all or most samples (*Propionibacterium*, *Methylobacterium*, and *Stenotrophomonas*), a few genera had ASVs that were more abundant in silk, namely: *Pseudonocardia*, *Friedmanniella*, *Sphingomonas*, *Actinomyspetora*, and *Massilia* (Figure 4.4).

Random Forest Models

Our random forest model had a maximum accuracy of 0.602 (kappa = 0.332) when classifying samples across specimen types. The ASV with the highest importance for predicting specimen type was assigned to an unidentified Anaplasmataceae species that was significantly more abundant in adult female samples than other samples (Figure A4.10). When we classified samples by collection location, our model reached maximum accuracy of 0.556 (kappa = 0.331).

An *Ehrlichia* species was the most important predictor of location. Though *Ehrlichia* was present in all locations and across all specimen types, it was more abundant in specimens collected from Location D (Figure A4.11). When classifying only adult female samples by collection location, our model had a maximum accuracy of 0.673 (kappa = 0.486) and the most important ASV was a *Carnimonas* species that was more abundant in females from Location G (Figure A4.12). We additionally classified silk samples by location, but the model had a lower accuracy of 0.454 (kappa = 0.235). The ASV that best predicted location where the silk samples were collected from was an *Ehrlichia* sp. which was significantly more abundant in silk samples from Location G (Figure A4.13).

Diet and Molting Assay

Because newly-emerged individuals did not provide enough DNA for 16S rRNA gene sequencing, we unfortunately could not investigate how bacterial communities change across all developmental stages in *A. studiosus*. However, we could still investigate the effects of single feeding events and of molting on microbiomes.

Alpha Diversity

Sequencing the V4 region of the 16S rRNA gene of 147 samples resulted in 6,942,037 total reads, with a mean of 47,225 reads, a median of 47,507 reads, and a range of 614 to 355,748 reads (see Figure A4.2 for reads across treatments). After removing reads mapped to eukaryotic DNA, taxa unidentified to Kingdom, and potential contaminants, we found that there were 2,959 ASVs across 136 samples, with an average of 63.8±40.5 ASVs per sample. After Feeding and After Feeding + Molting treatments had significantly higher median ASV Shannon diversity than the Before Feeding treatment, though the After Feeding + Molting treatment had a large range in Shannon diversity across samples (Table 4.1; Figure 4.5; Wilcoxon Rank Sum Test, $p < 0.05$).

Interestingly, flies fed on by spiders (Flies Fed treatment) had significantly higher Shannon diversity than all other treatments, except the After Feeding & Molting treatment (Wilcoxon Rank Sum Test. $p < 0.05$). Evenness estimates found that ASVs were not evenly distributed within samples, though values ranged widely across treatments (Table 4.1). After removing taxa that were unidentified to Phylum and taxa present in less than 1% of samples, we found that there were 472 ASVs across 136 samples.

Beta Diversity and Ordination

ASV communities significantly differed across treatments, families and location of mother's collection (PERMANOVA, $p<0.05$; Table 4.5). Within locations and families, beta dispersion estimates were not significantly different. However, Before Feeding samples showed significantly lower dispersion estimates than other treatments (Figure A4.14; ANOVA $p<0.05$), meaning that significant differences across treatments could be due to differences in beta dispersion within treatments. While PERMANOVA across all treatments explained the most variation of each individual categorical variable (approximately 30%), family and mother's collection location also explained significant amounts of variation (19% and 8%, respectively; Table 4.5). After removing *D. melanogaster* samples, treatment did not explain as much variation across ASV communities (Table 4.5). This is reflected in our DPCoA ordination of all treatments, which shows a clear separation between *A. studiosus* and *D. melanogaster* samples, but less clear separation within each animal species (Figure 4.6). The bacterial Classes Alphaproteobacteria, Bacilli, Betaproteobacteria, and Gammaproteobacteria varied most across axis 1, while Actinobacteria, Alphaproteobacteria, and Gammaproteobacteria varied most across axis 2. We also investigated differences in ASV communities using a model where treatment (using spider samples only) was nested in family, which was nested in location of mother's

collection. This resulted in a significant interaction term of all three variables which explained 35% of variation across spider ASV communities (PERMANOVA, $p < 0.05$).

Prevalence & Abundance Analyses

Unlike the wild-caught assay, there were highly prevalent ASVs (ASVs present in all or most samples) in the diet and molting assay. An ASV belonging to *Acinetobacter* was present in 100% of samples, including *D. melanogaster* samples (n = 136), while an *Achromobacter* ASV and an *Enhydrobacter* ASV were prevalent in 97.9 and 95.1% of all samples, respectively (Figure 4.8). In spiders, alone (n = 106), *Acinetobacter* and *Achromobacter* were both in 100% of samples.

Hierarchical multiple testing returned thirty-eight ASVs across twelve taxonomic families and nineteen genera (with six unidentified genera) with significantly different abundance across all treatments (Table A4.4). Of these, twenty-one ASVs were still significantly different across treatments after *D. melanogaster* treatments were removed. Those that differed across *A. studiosus* treatments only were primarily environmental bacteria like *Pseudomonas* species, *Comamonas* species, *Stenotrophomonas* species, and *Acinetobacter* species (Figures 4.7 and 4.8; Table A4.5). In contrast, when *D. melanogaster* samples were included, *Wolbachia* species, *Acetobacter* species, Shingomonadaceae species, and a *Serratia* species were also differentially abundant (Figures 4.7 and 4.8; Table A4.4). No ASVs had significantly different abundance across families or maternal collection location, or between treatments comparing only Before Feeding with After Feeding, or comparing only After Feeding with After Feeding and Molting.

Random Forest Models

Using random forest analysis to classify all samples across treatments, we found a model with a maximum accuracy of 0.524 (kappa = 0.363). The most important predictor was

Acetobacter sp. which is a well-known member of the core microbiome in *D. melanogaster* (Broderick and Lemaitre, 2012), and was more abundant in the Flies Fed and Drosophila treatments (Figures 4.8 and A4.16). Removing *D. melanogaster* samples to see if we could accurately classify samples across the three *A. studiosus* treatments, alone (no *D. melanogaster* controls), we found a model with lower accuracy of 0.486 (kappa = 0.224). The ASV contributing most to classification was a *Pseudomonas* species (Figures 4.8 and A4.17), which was more abundant in *A. studiosus* in the Before Feeding treatment. Trying to predict spider family by ASV abundance resulted in a model with very low accuracy (0.201, kappa = 0.128). Our random forest model for classifying spider samples based on location also had a low accuracy of 0.394 (kappa = 0.175). The ASVs of largest importance for classifying samples based on family and maternal collection location were *Borrelia* species that were more abundant in the D2 and D3 families, and maternal collection location D (Figures A4.18 and A4.19).

Discussion

Previous studies have shown that spider bacterial microbiomes are largely dominated by maternally inherited bacteria (Vanthournout and Hendrickx, 2015; Zhang et al., 2018), if present, and otherwise contains few bacteria, potentially obtained through feeding on infected insects (Vanthournout et al., 2018). In this study, we were interested in describing the bacterial microbiome of a spider that is widespread across the Americas, *Anelosimus studiosus*, and interested in determining if their bacterial microbiome is structured similarly to other spiders, and how large of an effect diet and molting has on the structure of their bacterial communities. Though we found that, occasionally, *A. studiosus* are infected with maternally inherited endosymbionts like *Spiroplasma*, *Wolbachia* and *Rickettsiella*, it is unlikely that these bacteria are being passed from mother to offspring in this spider. Instead, we found that potential

pathogens like *Borrelia* and *Ehrlichia* had high relative abundance in some individuals, and that rearing environment, including diet, influenced bacterial diversity and bacterial community composition.

Pathogens are frequent components of the microbiome of A. studiosus

Individuals in both assays were often infected with *Borrelia* ASVs, where in some individuals, *Borrelia* had the highest relative abundance of all bacterial genera (Figure A4.9). In total, we found sixteen different, but closely related ASVs assigned to *Borrelia* in the wild-caught assay (Figure 4.9) and two different ASVs assigned to this genus in the diet and molting assay. While it is well known that *Borrelia* is an obligate parasite of arachnids in the superorder Parasitiformes (Fikrig and Narasimhan, 2006), no one has reported its infection of spiders (Suffridge et al., 1999). Outside of the Arachnid class, studies have found that various *Borrelia* species infect mosquitoes (Halouzka et al., 1999; Melaun et al., 2016), fleas (Teltow et al., 1991), and horse flies (Magnarelli and Anderson, 1988). Interestingly, we found *Borrelia* was quite common in our silk samples (Figures 4.4 and 4.9), and *A. studiosus* webs capture a variety of Diptera and other flying insects (personal observation), therefore *Borrelia* in silk may reflect the infection of arthropods *A. studiosus* has fed upon and that are captured by webs of *A. studiosus*.

Much of what we know about the biology and life cycle of *Borrelia* in arthropods comes from studies of *Borrelia burgdorferi*, the causative agent of Lyme disease, in ticks. For instance, while some ticks become infected with *B. burgdorferi* transovarially (Magnarelli et al., 1987; Rollend et al., 2013), *B. burgdorferi* is best known for its ability to infect individuals transstadially; that is, when larval or nymphal *Ixodes* ticks feed on infected animal reservoirs, *B. burgdorferi* enters the digestive system, attaches to the gut lining of the individual, and remains

in the gut even after molting, before migrating back to the salivary glands of the now adult tick during a feeding event (Lane et al., 1991). In the diet and molting assay, we saw that *Borrelia* was present in a few flies that had been preyed upon by *A. studiosus* (specifically, three *D. melanogaster* samples collected from D2 and D3 home containers), but it was not present in any other *D. melanogaster* controls, despite being prevalent in *A. studiosus* individuals throughout the assay (Figure 4.8). In addition, it was only present and abundant in families D2, D3 and A10 (Figure 4.10), despite juveniles from those families living separately for at least one molt. Given this family-specific infection rate, along with the lack of *Borrelia* found in egg sacs (Figure 4.4) or in the *D. melanogaster* live stocks (Figures 4.8 and 4.10), we hypothesize that *Borrelia* may not only be passed to *A. studiosus* individuals through their prey items, but also through prey sharing/trophallaxis in early life stages. That is, it is possible that mothers of the D2, D3, and A10 families may have obtained *Borrelia* while feeding outside of the lab before collection, and passed it to their offspring during early food sharing events.

We also found *A. studiosus* from both assays were commonly infected with bacteria in the genus *Ehrlichia* (Figures 4.4, 4.8-4.10), though there was a higher diversity of *Ehrlichia* and closely related but unidentified Anaplasmataceae ASVs in the wild-caught assay. These obligate, intracellular bacteria are infamous for causing a suite of vertebrate illnesses, known as ehrlichioses (Dumler and Walker, 2001), after they are passed to vertebrates from a tick host. In ticks, *Ehrlichia* is not transmitted transovarially, but, like *Borrelia*, it remains in individuals across developmental stages (Dumler and Walker, 2001; Hodzic et al., 1998). While a few studies have found *Ehrlichia* infections in invertebrates outside of ticks (e.g. in mosquitoes [Guo et al., 2016]), it is very rare. Given this, we were surprised to find that some *D. melanogaster* stock samples were infected with *Ehrlichia*. Though we took what measures we could to reduce

contamination and cross-contamination in the lab, it is not uncommon for reads from samples in the same sequencing run cross-contaminate other samples (Eisenhofer et al., 2019; Gu et al., 2019), which could explain the presence of *Ehrlichia* at very low abundance in *D. melanogaster* samples (Figures 4.8 and 4.10), and some silk samples (Figures 4.4 and 4.9). However, some silk samples had as high abundance of *Ehrlichia* as *A. studiosus* individuals (Figures 4.4 and 4.9), so perhaps these silk samples contained arthropods infected with *Ehrlichia*.

Our assays also found evidence of other potential pathogens *A. studiosus* may commonly interact with. In the wild-caught assay, *Carnimonas* was particularly abundant in specific locations, but it was not present at all in the diet and molting assay. Typically, *Carnimonas* is not associated with arthropods, but one study detected *C. nigrificans* in *Varroa destructor* mites collected from honeybee colonies (Hubert et al., 2015). In both assays, *A. studiosus* was occasionally infected with the common environmental pathogen, *Serratia*, and entomopathogen, *Bacillus*. All of these potential pathogens, including *Borrelia* and *Ehrlichia*, often had the highest relative abundance of any bacteria in adult females (Figure A4.8). However, further studies should investigate the effects of these bacteria within *A. studiosus* to determine if they are pathogenic or not.

Lifetime diet and environment influence the bacterial microbiome of A. studiosus.

Similar to *Stegodyphus* spiders (Vanthournout et al., 2018), and omnivorous insects (Yun et al., 2014), we found that diet can play a role in shaping the bacterial communities of *A. studiosus*. Though we did not see significant overlap in the bacterial communities of fed spiders (After Feeding treatment) and their *D. melanogaster* meals (Figure 4.6), and we did not see significantly different bacterial abundances between the Before Feeding and After Feeding treatments, we did see a significant increase in bacterial diversity after feeding (Figure 4.5). The

combination of these findings may mean that a random assortment of some, but not all, bacteria from the *D. melanogaster* meal stay in individuals' guts after feeding. That is, *A. studiosus* feeding behavior may filter random bacteria from their meal, resulting in an overall increase in bacterial diversity, but no significant overlap in bacterial communities with their meal, and no bacteria that are consistently more abundant right after feeding. For example, *Acetobacter*, *Serratia*, and *Commensalibacter* genera that were all abundant in the *D. melanogaster* samples, were all more prevalent in the After Feeding and After Feeding + Molting treatments than the Before Feeding treatment (Figure 4.8). In addition, the variable but sometimes high overlap between silk samples and adult female samples from the wild-caught assay (Table 4.3) suggest that adult females may sometimes obtain most of their bacteria from their environment, including bacteria present in or on their prey.

Furthermore, though maternally-inherited bacteria (*Wolbachia*, *Spiroplasma*, and *Rickettsiella*) were abundant in a few individuals in the wild-caught assay (Figure 4.4), we did not observe these bacteria in high abundance in lab-raised individuals, suggesting that they may be present in wild-caught individuals as a result of diet alone, rather than via maternal inheritance. For example, *Wolbachia*, which was not prevalent in the wild-caught assay, and did not infect egg sacs at all, was more abundant in the diet and molting assay, where *A. studiosus* fed on *D. melanogaster* infected with the bacteria. Though we did find three egg sacs with *Rickettsiella* in the wild-caught assay (Figure 4.4), the mother of only one of these egg sacs was also infected with *Rickettsiella*, the bacteria was not abundant in the egg sacs, and we did not find any *A. studiosus* infected with *Rickettsiella* in the diet and molting assay, though we collected adult females with egg sacs from the same location. *Spiroplasma*, though prevalent in the diet and molting assay, only infected five adult females in the wild-caught assay, and was

also present in some of the *D. melanogaster* samples in the diet and molting assay, suggesting, again, that individuals likely obtain infection (probably temporary) with this bacteria through feeding.

Overall, there was very little similarity in the most abundant taxa and bacterial community structure across the wild-caught and diet and molting assays. For example, comparing the relative abundance of bacterial genera in juveniles from both assays (Figure A4.20), we found some common taxa, but more diversity between groups from the wild-caught assay. Likewise, we saw higher ASV diversity but lower ASV prevalence in samples collected from the wild-caught assay than samples used in the diet and molting assay (Table 4.1). For example, five ASVs in the diet and molting assay were prevalent in more than 75% of samples, while no ASVs were as prevalent in the wild-caught assay. While we would expect the presence or absence of common lab/environmental bacteria to vary across the two sequencing runs, we do not think that run differences can explain the low ASV prevalence across samples in the wild-caught assay. Instead, we propose that *A. studiosus* individuals from the diet and molting assay most likely have overall lower diversity across samples because they were all raised in the same environment, and fed the same, consistent lab diet from birth.

Does molting influence the microbiome?

After viewing a decrease in bacterial diversity from egg sac to adult in the wild-caught assay, we hypothesized that molting may remove many of the bacteria associated with *A. studiosus* individuals. Ecdysis typically includes the shedding of all parts of the exoskeleton, including the entire lining of the gut, and *A. studiosus* molt 6-7 times before adulthood (Viera et al., 2007). Moreover, spiders often stop feeding or feed less in the few days leading up to ecdysis (Turnbull, 1973), meaning that if bacteria in the gut survive on the spiders' meals, they may also be starved

for a period of time, and thus may be less abundant or absent after molting. Instead, we found that bacterial diversity was overall higher in *A. studiosus* after one round of molting, though the range of bacterial diversity across molted individuals was wide (Table 4.1; Figure 4.5). It is possible that bacterial diversity is higher in some of the After Feeding + Molting individuals than in the After Feeding individuals because they were older, and had fed more often since the one-week starvation period all treatments had undergone. It is also possible that some bacteria are better adapted to remain in individuals after molting; if those bacteria are in some but not all individuals after feeding, we could see increased overall diversity but also a range of diversity estimates across individuals in the After Feeding + Molting group. Though we did not see significant differences in specific ASV abundances between the After Feeding and the After Feeding + Molting treatments, there are some trends in ASV prevalence across treatments. For instance, *Borrelia* was less prevalent in the After Feeding + Molting treatment than it was prior to molting. Some observed differences across families within *A. studiosus* treatments in the diet and molting assay (Table 4.5; Figure A4.14) may also explain which bacteria obtained at an early age are not expelled or removed through the molting process. For example, multiple individuals from family A10 had a high relative abundance of *Spiroplasma* bacteria; whether individuals in this family obtained the bacteria from communal feeding events before they were separated, or through maternal inheritance is not known. Similarly, individuals of all three treatments in families D2 and D4 had higher relative abundance of *Ehrlichia*, suggesting that *Ehrlichia* likewise remains in individuals through molting. However, the lack of any larger, significant trends between the After Feeding and After Feeding + Molting treatments makes it difficult to make strong claims about the effects of molting on spider microbiomes.

Bacterial communities of silk.

In the wild-caught assay, silk samples had the highest bacterial ASV diversity, with a few ASVs consistently more abundant in silk samples than spider samples (Figure 4.4). These bacteria consisted mostly of water-borne, soil-borne, and other environmentally prevalent bacteria (e.g. Microbacteriaceae [Evtushenko and Takeuchi, 2006], *Massilia* [Gallego et al., 2006], and *Sphingomonas* [White et al., 1996]). Given that *A. studiosus* build their cobwebs on plants in disturbed, riparian habitats, and that their webs collect environmental debris like soil and water particles for long periods of time, it is not surprising that their silk samples contain an abundance of bacteria common to their environment. Whether these bacteria are dead or alive is unclear; culture-based studies have found evidence for the antimicrobial activity of silk suggesting that silk may kill specific bacteria (Keiser et al., 2015; Roozbahani et al., 2014; Wright and Goodacre, 2012), but other studies have shown that bacteria can grow from silk plated in the lab (Iwai et al., 2009). Given the abundance of actinomycetes on our silk samples (Figure 4.4), it is possible that bacteria that grows on silk contributes to silk's antimicrobial properties. However, given the plethora of pathogens (e.g. *Bacillus*, *Serratia*) infecting adult and juvenile *A. studiosus*, it is clear that the potential antimicrobial properties of silk webs do not always protect their inhabitants.

Although adult females sometimes shared large proportions of their bacterial communities with their silk, silk did not share large proportions of their bacterial communities with adult females (Table 4.3). This disparity in community overlap could signify that silk is a source for the bacterial communities of adult female *A. studiosus*, if females obtain bacteria from their silken cobwebs, but not vice versa. However, it is also likely that the overlap in adult female bacterial communities with their silk comes from the dead prey items caught in silk samples.

That is, prey items may be a direct source of the same bacteria for both silk and adult females from the same web, and as a source for both would contribute to overlap between the communities. Given that adult females harbor fewer bacteria (Figure 4.2), contributions from prey items to these bacterial communities would make up a larger proportion of their entire bacterial community, potentially leading to the observed imbalance in community overlap between silk and adult females.

Figure 4.1 A) *A. studiosus* web (adult female upper left) with dead plant debris and prey exoskeletons caught in silk. B) *A. studiosus* mother sharing a prey item with her newly emerged offspring.

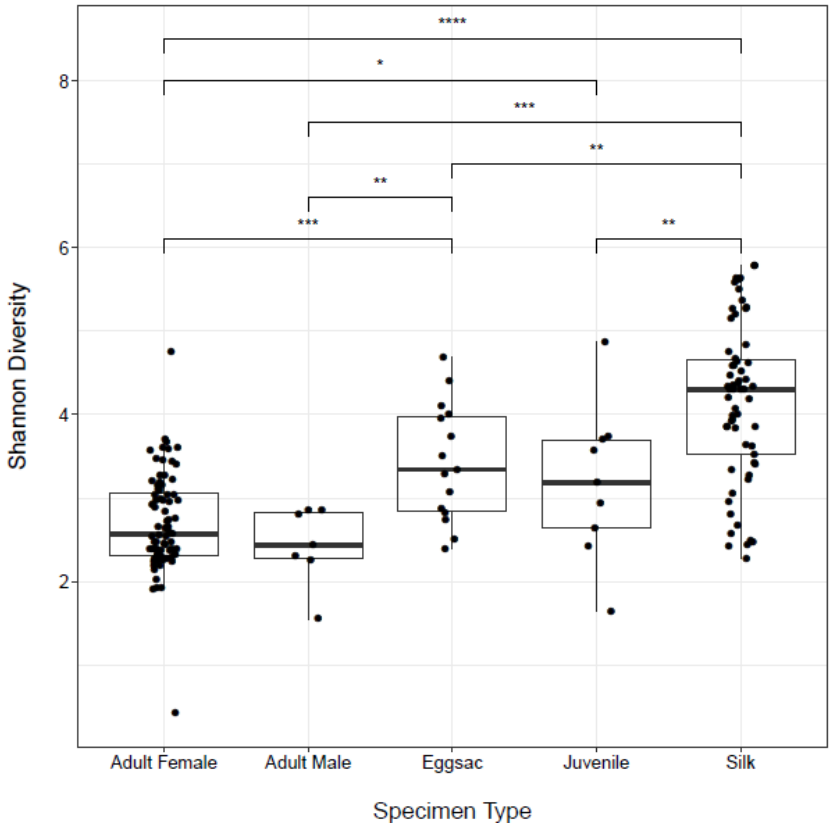

Figure 4.2 Shannon diversity for different specimen types in the wild-caught assay. Significance of Wilcoxon Rank Sum tests indicated by bars and asterisks (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$).

Table 4.1 ASV richness and evenness of different specimen types or treatments for each assay. Richness = Shannon diversity. Evenness = Shannon Diversity/ln(Observed Diversity).

	Specimen Type	Sample Size	Shannon (min)	Shannon (median)	Shannon (max)	Evenness (min)	Evenness (median)	Evenness (max)
Wild-caught Assay	Adult Female	83	0.42	2.57	4.75	0.19	0.81	0.97
	Adult Male	7	1.55	2.43	2.86	0.38	0.76	0.93
	Egg sac	15	2.39	3.34	4.69	0.72	0.88	0.94
	Juvenile	10	1.64	3.19	4.88	0.66	0.88	0.94
	Silk	66	2.27	4.30	5.79	0.63	0.89	0.96
	Treatment*	**Sample Size**	**Shannon (min)**	**Shannon (median)**	**Shannon (max)**	**Evenness (min)**	**Evenness (median)**	**Evenness (max)**
Development Assay	BF	41	0.20	0.99	2.12	0.08	0.23	0.45
	AF	41	0.12	1.44	3.91	0.05	0.34	0.76
	AFM	24	0.04	1.97	2.51	0.01	0.54	0.73
	FF	22	0.26	1.77	2.21	0.10	0.42	0.59
	D	8	0.52	1.03	1.76	0.13	0.28	0.49

* BF = Before Feeding; AF = After Feeding; AFM = After Feeding and Molting; FF = dead *D. melanogaster* flies fed upon by spiders; D = live *D. melanogaster* flies from stock.

Table 4.2 PERMANOVA results for wild-caught assay using Bray-Curtis dissimilarity measurements and permutations = 999. Our first model (ST) investigated ASV community differences across all specimen types (adult females, adult males, silk, juveniles, and egg sacs). Our second model (ST no males) investigated ASV community differences across specimen types with adult males removed from the dataset. Our third model (L[F[ST]]) investigated ASV community differences across specimen types nested in family (if all samples came from the same nest) and location (where individuals were collected).

Model*		**df**	**sums of squares**	**mean squares**	**F model**	**R^2**	**Pr(>F)**
~ ST	ST	4	4.35	1.09	2.46	0.06	0.001
	residuals	167	73.64	0.44		0.94	
~ ST no males	ST	3	3.59	1.20	2.69	0.05	0.001
	residuals	161	71.52	0.44		0.95	
~ L[F[ST]]	L	3	6.35	2.12	7.44	0.08	0.001
	L:F	67	34.00	0.52	1.81	0.44	0.001
	L:F:ST	77	30.53	0.40	1.39	0.39	0.001
	residuals	25	7.11	0.28		0.09	

*ST = specimen type; L = location; F = family

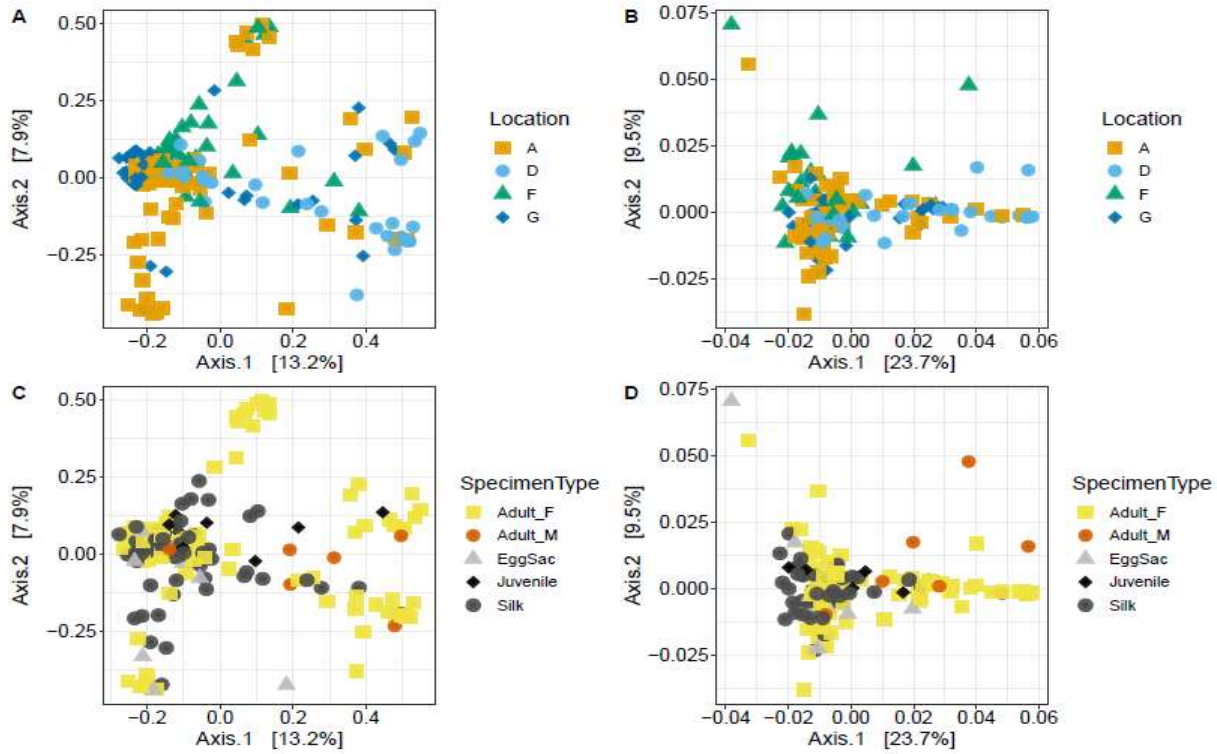

Figure 4.3 PCoA ordinations of Bray-Curtis (A, C) and weighted Unifrac (B,D) distances for the wild-caught assay colored by sample collection location (A, B) and specimen type (C,D).

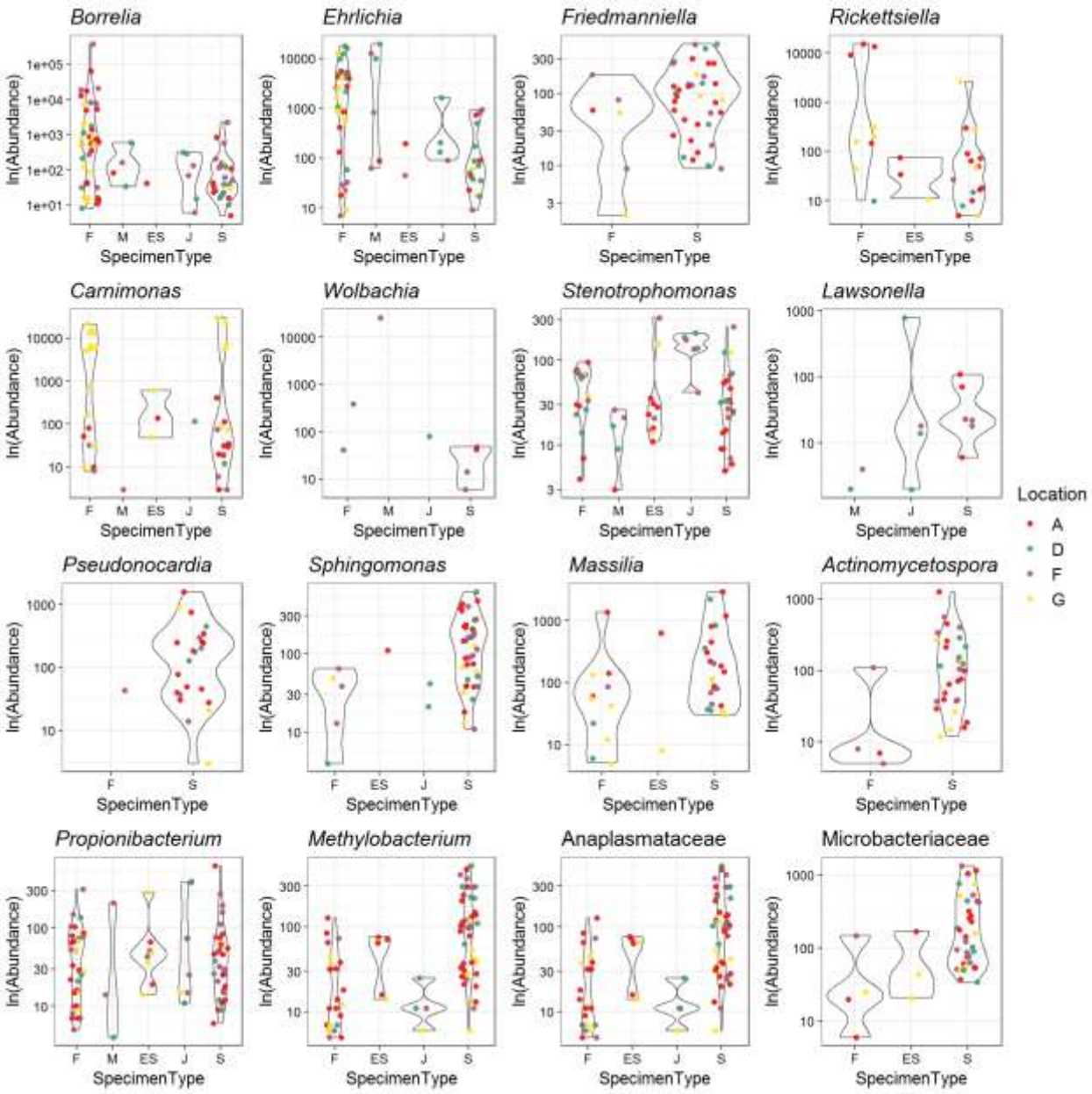

Figure 4.4 Logged abundance per sample of each bacterial genus with ASVs determined to be differentially abundant across specimen types (if present) and collection sites (except *Carnimonas*, which was only differentially abundant within adult females across locations). F = adult females; M = adult males; ES = egg sacs; J = juveniles (instar unknown); S = silk.

Table 4.3 Proportion of ASVs adult females shared between silk and adult females collected from the same nest as measured two ways (where F = ASV community of adult female sample and S = ASV community of the corresponding silk sample): 1) the proportion of all silk and adult female ASVs shared between silk and the corresponding adult female ($F \cap S / F \cup S$); and 2) the proportion of all adult female ASVs shared between silk and the corresponding adult female ($F \cap S / F$).

Nest	$\frac{F \cap S}{F \cup S}$	$\frac{F \cap S}{F}$	Nest	$\frac{F \cap S}{F \cup S}$	$\frac{F \cap S}{F}$	Nest	$\frac{F \cap S}{F \cup S}$	$\frac{F \cap S}{F}$
A2	0.07	0.40	A27	0.11	0.47	F10	0.18	0.24
A4	0.03	0.11	A28	0.05	0.11	F11	0.03	0.25
A6	0.16	0.95	A29	0.02	0.07	F12	0.03	0.03
A7	0.01	0.07	A30	0.00	0.00	F13	0.09	0.76
A8	0.46	0.74	D2	0.05	0.08	F15	0.03	0.04
A11	0.05	0.19	D3	0.12	0.43	F16	0.03	0.12
A12	0.18	0.29	D4	0.29	0.64	G2	0.04	0.06
A15	0.06	0.15	D7	0.06	0.91	G3	0.41	0.56
A16	0.01	0.08	D8	0.07	0.24	G4	0.80	0.95
A17	0.04	0.10	D11	0.22	0.63	G5	0.22	0.36
A19	0.20	0.75	F1	0.08	0.26	G6	0.09	0.12
A20	0.01	0.13	F3	0.26	0.36	G7	0.13	0.20
A21	0.03	0.14	F4	0.11	0.30	G8	0.13	0.25
A23	0.04	0.05	F6	0.04	0.07	G10	0.06	0.21
A24	0.03	0.04	F7	0.00	0.00	G11	0.13	0.79
A25	0.00	0.00	F8	0.05	0.07	G12	0.28	0.42
A26	0.02	0.05	F9	0.14	0.36	**Avg±SD**	**0.11±0.14**	**0.29±0.26**

Table 4.4 Proportion of ASVs in egg sac samples that were also found in mother's bacterial community. E = ASV community of egg sac sample and M = ASV community of corresponding adult female sample.

Egg sac	$\frac{E \cap M}{E}$	Egg sac	$\frac{E \cap M}{E}$
A21	0.00	F6	0.02
A23	0.00	F9	0.00
A24	0.12	F12	0.01
A25	0.00	G7	0.11
A26	0.03	G8	0.02
A28	0.00	G10	0.07
	Average	**0.07±0.08**	

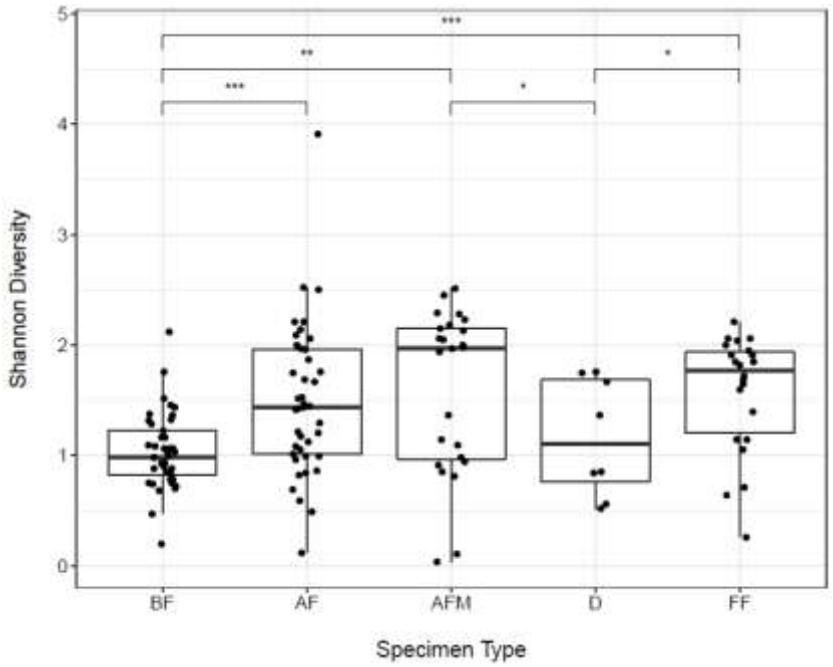

Figure 4.5 Shannon diversity for different treatments in the diet and molting assay. BF = Before Feeding; AF = After Feeding; AFM = After Feeding + Molting; FF = dead *D. melanogaster flies* fed upon by spiders; D = live *D. melanogaster* flies from stock. Significance of Wilcoxon Rank Sum tests indicated by bars and asterisks (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$).

Table 4.5 PERMANOVA results for diet and molting assay using Bray-Curtis dissimilarity measurements and 999 permutations. Our first model (Treatment with Dros) investigated ASV community differences across all Treatments (Before Feeding, After Feeding, After Feeding + Molting, Fed Flies, and Drosophila). Our second model (Treatment) investigated ASV community differences across treatments without the Fed Flies and Drosophila (*D. melanogaster*) treatments. Our third model (Location) investigated ASV community differences across locations where individuals' mothers were collected. Our fourth model (Family) investigated ASV community differences across families (family indicates individuals originated from the same egg sac). And finally, our fifth model (L[F[Treat]]) investigated ASV community differences across Before Feeding, After Feeding, and After Feeding + Molting treatments nested in family and location.

Model*		**df**	**sums of squares**	**mean squares**	**F model**	**R^2**	**Pr(>F)**
~ Treatment (with Dros)	Treatment	4	9.42	2.35	14.2	0.30	**0.001**
	Residuals	131	21.67	0.17		0.70	
~ Treatment	Treatment	2	2.51	1.25	6.76	0.12	**0.001**
	Residuals	103	19.11	0.19		0.88	
~Location	Location	5	1.81	0.36	1.83	0.08	**0.007**
	Residuals	100	19.81	0.20		0.92	
~Family	Family	13	4.15	0.32	1.68	0.19	**0.001**
	Residuals	92	17.47	0.19		0.81	
~ L[F[Treat]]	L	5	1.81	0.36	2.40	0.08	**0.001**
	L:F	8	2.34	0.29	1.93	0.11	**0.003**
	L:F:Treat	27	7.64	0.28	1.87	0.35	**0.001**
	residuals	65	9.83	0.15		0.46	

*Treat = treatment; L = location; F = family; Dros = *D. melanogaster* samples

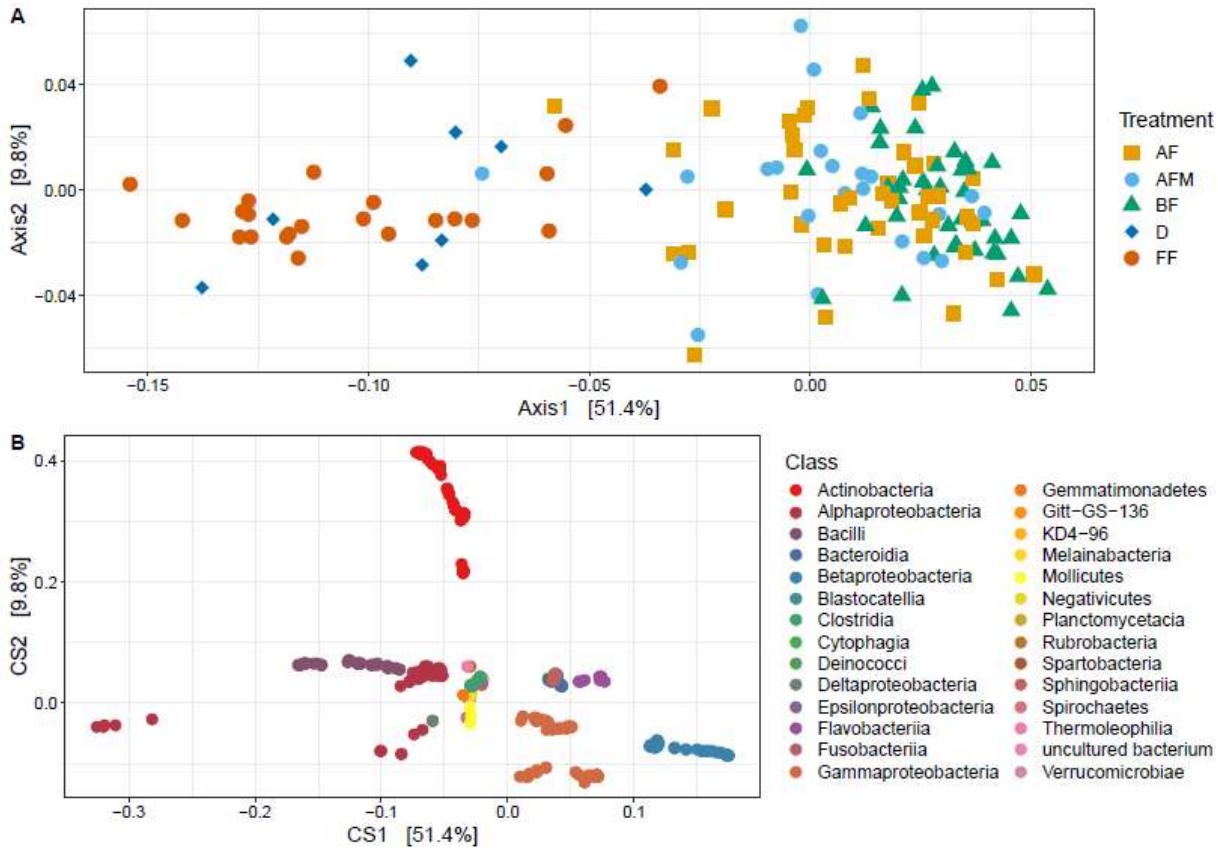

Figure 4.6 DPCoA of A) treatments and B) ASVs in the diet and molting assay. That is, axes represent percent variation explained by differences across treatments (A) or differences across bacterial ASVs (B), and differences across ASVs are associated with differences within and between communities. BF = Before Feeding; AF = After Feeding; AFM = After Feeding and Molting; FF = dead *D. melanogaster flies* fed upon by spiders; D = live *D. melanogaster* flies from stock.

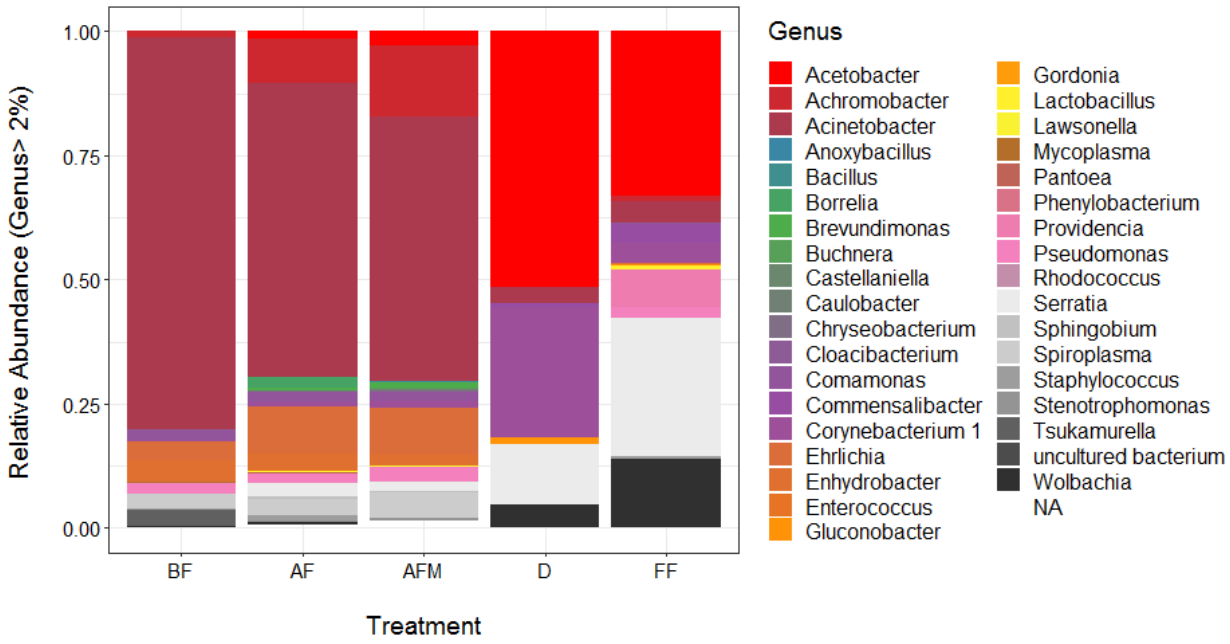

Figure 4.7 Relative abundance of bacterial genera present in more than 2% of samples across treatments in the diet and molting assay. BF = Before Feeding; AF = After Feeding; AFM = After Feeding and Molting; FF = dead *D. melanogaster flies* fed upon by spiders; D = live *D. melanogaster* flies from stock

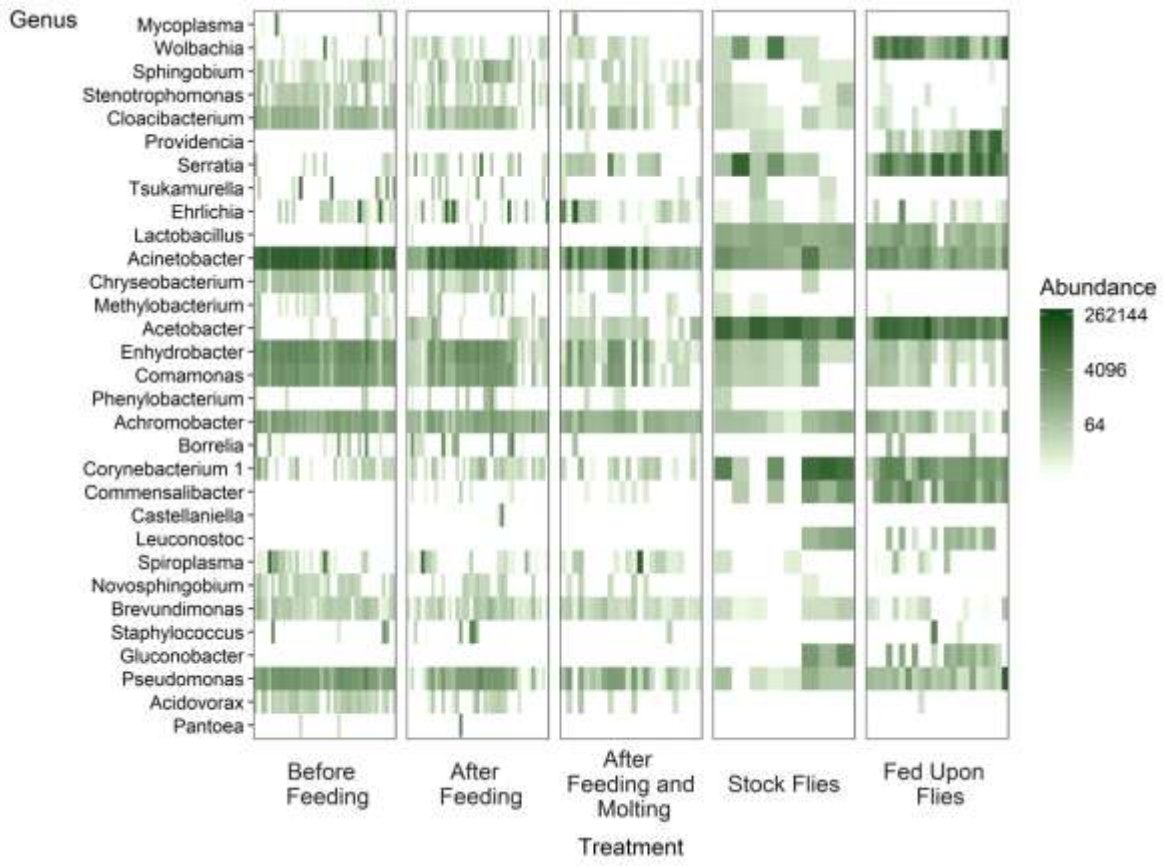

Figure 4.8 Heatmap of the abundance of the fifty most abundant ASVs across all samples in the diet and molting assay, pooled by bacterial genus. Each column represents one microbiome sample. White indicates not present, light green indicates slightly higher abundance, and darker green indicates high abundance.

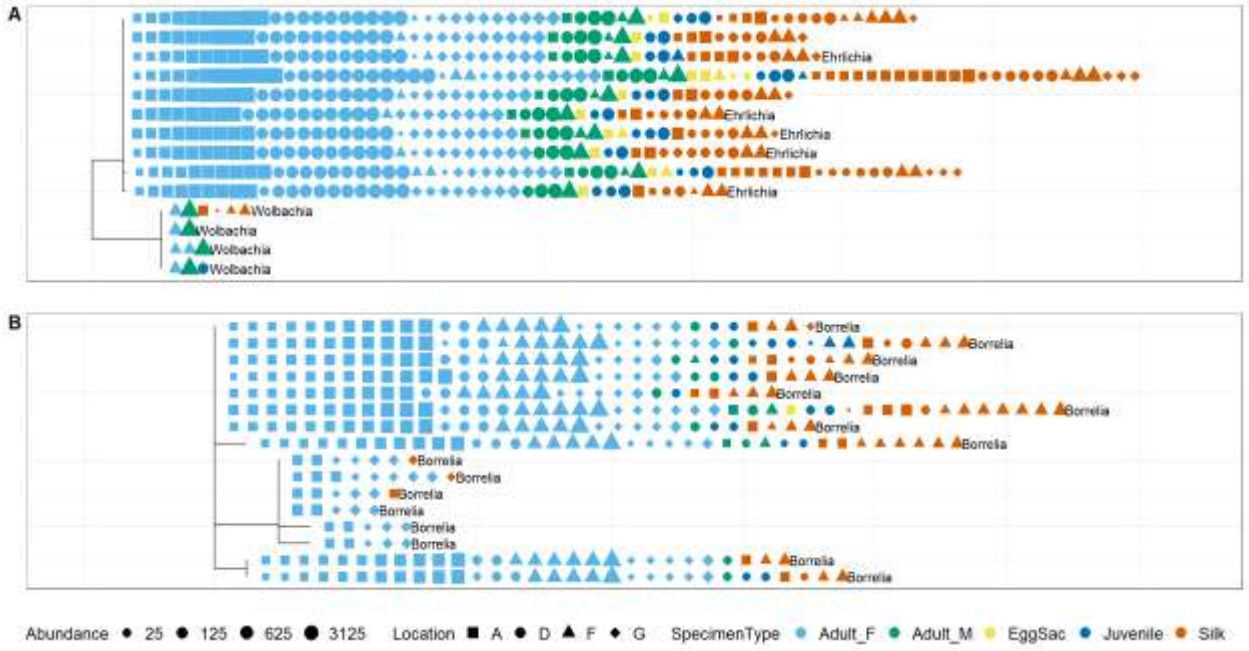

Figure 4.9 Phylogenetic relationship between all (A) Anaplasmataceae and (B) *Borrelia* ASVs from the wild-caught assay, and their abundance across locations and specimen types.

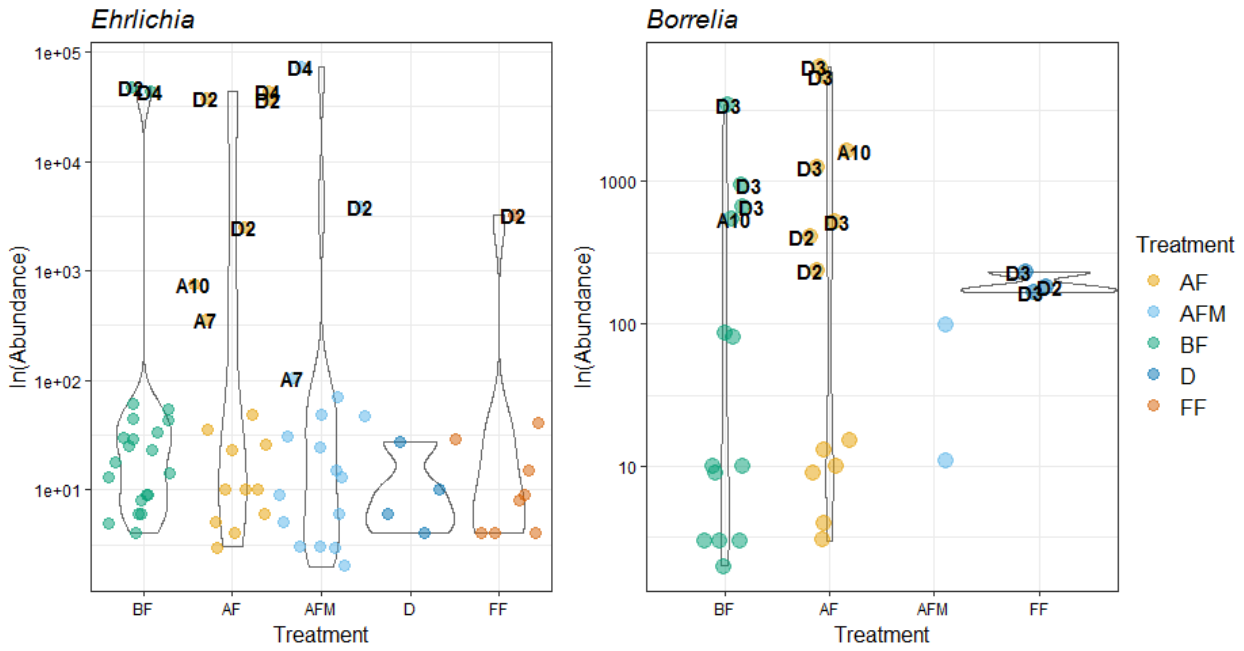

Figure 4.10 Natural log-transformed abundance of *Ehrlichia* and *Borrelia* ASVs across treatments in the diet and molting assay. Point labels denote family and location for all samples with abundance greater than ln(100) reads for each respective bacteria. For example, label D3 is an individual whose mother was collected at location D, and all individuals labelled D3 are from the same family. Treatment key: BF = Before Feeding; AF = After Feeding; AFM = After Feeding and Molting; FF = dead *D. melanogaster flies* fed upon by spiders; D = live *D. melanogaster* flies from stock.

APPENDICES

Appendix A: Supplementary Tables for Chapter 4

Table A4.1 Coordinates of collection sites used in each assay. WC=wild-caught assay; DM = diet and molting assay; Y= yes, used in assay; N = no, not used in assay.

Location	Coordinates		WC	DM
A	32.57 N	-96.00 W	Y	Y
B	30.28 N	-97.78 W	N	Y
C	30.34 N	-97.93 W	N	Y
D	30.52 N	-98.57 W	Y	Y
E	30.28 N	-97.73 W	N	Y
F	30.15 N	-97.44 W	Y	Y
G	32.88 N	-96.53 W	Y	N

Table A4.2 Amplicon sequence variants (ASVs) from wild-caught assay, with significantly different abundance across specimen types from all locations. Unadjusted and adjusted p-values calculated using hierarchical multiple testing across variance stabilizing transformed read counts. Adjusted p-values incorporate the Hierarchical False Discovery Rate Procedure described in Benjamini and Yekutieli (2001).

Class	**Order**	**Family**	**Genus**	**unadjp**	**Adjp**
Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Lawsonella*	5.45E-05	0.000206
Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Lawsonella*	0.000103	0.000206
Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Lawsonella*	0.00071	0.000947
Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Lawsonella*	0.048233	0.048233
Actinobacteria	Corynebacteriales	Tsukamurellaceae	*Tsukamurella*	0.018157	0.018157
Actinobacteria	Micrococcales	Intrasporangiaceae	*Ornithinicoccus*	0.01749	0.034981
Actinobacteria	Micrococcales	Microbacteriaceae	*Amnibacterium*	0.009857	0.029571
Actinobacteria	Micrococcales	Microbacteriaceae	NA	0.000908	0.004538
Actinobacteria	Micrococcales	Microbacteriaceae	NA	0.003668	0.006113
Actinobacteria	Micrococcales	Microbacteriaceae	NA	0.003289	0.006113
Actinobacteria	Micrococcales	Microbacteriaceae	NA	0.009245	0.011556
Actinobacteria	Micrococcales	Microbacteriaceae	NA	0.009282	0.046409
Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Friedmanniella*	1.94E-06	1.55E-05
Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Friedmanniella*	0.000241	0.000963
Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Friedmanniella*	0.00051	0.001359
Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Friedmanniella*	0.009633	0.015801
Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Friedmanniella*	0.009875	0.015801
Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Friedmanniella*	0.009174	0.027521
Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Friedmanniella*	0.022515	0.030021
Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Propionibacterium*	1.23E-05	2.45E-05
Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Propionibacterium*	0.00318	0.022258
Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Propionibacterium*	0.01136	0.033676
Actinobacteria	Propionibacteriales	Propionibacteriaceae	*Propionibacterium*	0.014433	0.033676
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Actinomycetospora*	0.001401	0.004202
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Actinomycetospora*	0.001405	0.005167
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Actinomycetospora*	0.001476	0.005167
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Actinomycetospora*	0.003688	0.006455
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Actinomycetospora*	0.003006	0.006455
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Actinomycetospora*	0.010508	0.010508
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Actinomycetospora*	0.009278	0.012989
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Pseudonocardia*	0.000187	0.000747
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Pseudonocardia*	0.00053	0.002122
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Pseudonocardia*	0.003682	0.007364
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Pseudonocardia*	0.009198	0.012265
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Pseudonocardia*	0.009451	0.018902
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Pseudonocardia*	0.022249	0.022249
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	*Pseudonocardia*	0.022205	0.029607
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Cloacibacterium*	3.99E-08	1.99E-07
Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Methylobacterium*	6.63E-06	3.31E-05

Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Methylobacterium*	4.36E-05	0.000109
Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Methylobacterium*	0.004608	0.007679
Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Methylobacterium*	0.009559	0.011949
Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Methylobacterium*	0.013999	0.027998
Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Uncultured*	0.010659	0.021319
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Ehrlichia*	2.02E-05	6.57E-05
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Ehrlichia*	3.28E-05	6.57E-05
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Ehrlichia*	0.000134	0.000214
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Ehrlichia*	0.000249	0.000249
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Ehrlichia*	0.001605	0.001834
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	NA	2.52E-05	6.57E-05
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	NA	2.09E-05	6.57E-05
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	NA	0.00034	0.000454
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	NA	0.002691	0.002691
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	NA	0.042142	0.042142
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Wolbachia*	0.002635	0.00527
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Wolbachia*	0.002178	0.00527
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Wolbachia*	0.006912	0.009216
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Wolbachia*	0.042911	0.042911
Alphaproteobacteria	Sphingomonadales	NA	NA	0.004165	0.005553
Alphaproteobacteria	Sphingomonadales	NA	NA	0.032994	0.032994
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	0.000558	0.001115
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	0.000582	0.001163
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	0.00032	0.001163
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	0.026978	0.026978
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingopyxis*	3.95E-05	7.89E-05
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia*	0.009488	0.018976
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia*	0.009613	0.02274
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia*	0.009552	0.02274
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia*	0.01137	0.02274
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia*	0.028077	0.033692
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia*	0.023236	0.033692
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia*	0.012212	0.036637
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia*	0.026239	0.039359
Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia*	0.043515	0.043515
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	1.53E-08	1.23E-07
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	2.84E-06	2.84E-06
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	9.18E-07	3.67E-06
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	0.000266	0.00071
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	0.01694	0.033879
Spirochaetes	Spirochaetales	Spirochaetaceae	*Borrelia*	0.000123	0.000246
Spirochaetes	Spirochaetales	Spirochaetaceae	*Borrelia*	8.10E-05	0.000567
Spirochaetes	Spirochaetales	Spirochaetaceae	*Borrelia*	0.001483	0.001483
Spirochaetes	Spirochaetales	Spirochaetaceae	*Borrelia*	0.001143	0.00287
Spirochaetes	Spirochaetales	Spirochaetaceae	*Borrelia*	0.00164	0.00287
Spirochaetes	Spirochaetales	Spirochaetaceae	*Borrelia*	0.001292	0.00287
Spirochaetes	Spirochaetales	Spirochaetaceae	*Borrelia*	0.00543	0.007603
Spirochaetes	Spirochaetales	Spirochaetaceae	*Borrelia*	0.009394	0.009394
Spirochaetes	Spirochaetales	Spirochaetaceae	*Borrelia*	0.011945	0.013936

Table A4.3 ASVs from adult females in wild-caught assay, with significantly different abundance across locations. Unadjusted and adjusted p-values calculated using hierarchical multiple testing across variance stabilizing transformed abundance. Adjusted p-values incorporate the Hierarchical False Discovery Rate Procedure described in Benjamini and Yekutieli (2001).

Class	**Order**	**Family**	**Genus**	**unadjp**	**adjp**
Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Carnimonas*	2.63E-11	5.27E-11
Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Carnimonas*	1.93E-10	1.35E-09
Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Carnimonas*	7.04E-10	2.47E-09
Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Carnimonas*	3.11E-09	3.59E-09
Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Carnimonas*	3.59E-09	3.59E-09
Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Carnimonas*	6.06E-08	6.06E-08
Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Carnimonas*	6.12E-08	8.57E-08
Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Carnimonas*	5.78E-08	8.57E-08
Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Carnimonas*	6.06E-08	8.57E-08
Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Carnimonas*	3.25E-07	3.79E-07
Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Carnimonas*	3.96E-07	3.96E-07
Gammaproteobacteria	Xanthomonadales	Uncultured	NA	2.63E-11	5.27E-11
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Luteibacter*	2.63E-11	5.27E-11
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Lysobacter*	2.63E-11	2.63E-11
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Thermomonas*	2.63E-11	2.63E-11

Table A4.4 ASVs from diet and molting assay, with significantly different abundance across treatments, including *D. melanogaster* samples. Unadjusted and adjusted p-values calculated using hierarchical multiple testing across variance stabilizing transformed read counts. Adjusted p-values incorporate the Hierarchical False Discovery Rate Procedure described in Benjamini and Yekutieli (2001).

Class	**Order**	**Family**	**Genus**	**unadjp**	**adjp**
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Chryseobacterium*	6.59E-13	1.32E-12
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Cloacibacterium*	1.98E-10	3.96E-10
Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Brevundimonas*	3.43E-08	6.85E-08
Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Acetobacter*	3.95E-37	3.95E-37
Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Acetobacter*	5.13E-37	1.03E-36
Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Acetobacter*	1.63E-28	1.63E-28
Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Commensalibacter*	2.63E-31	2.63E-31
Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Gluconobacter*	3.68E-17	3.68E-17
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Wolbachia*	6.91E-20	1.38E-19
Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Wolbachia*	0.031506	0.031506
Alphaproteobacteria	Sphingomonadales	NA	NA	0.00019	0.00019
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Novosphingobium*	8.90E-09	1.78E-08
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingobium*	5.99E-06	1.20E-05
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	6.72E-07	1.34E-06
Betaproteobacteria	Burkholderiales	Alcaligenaceae	*Achromobacter*	4.52E-12	9.04E-12
Betaproteobacteria	Burkholderiales	Comamonadaceae	*Acidovorax*	5.36E-13	1.07E-12
Betaproteobacteria	Burkholderiales	Comamonadaceae	*Comamonas*	1.21E-09	2.43E-09
Betaproteobacteria	Burkholderiales	Comamonadaceae	*Comamonas*	0.035619	0.035619
Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	1.27E-14	2.55E-14
Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	0.026749	0.026749
Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	0.024906	0.049812
Betaproteobacteria	Methylophilales	Methylophilaceae	NA	0.002673	0.005345
Betaproteobacteria	NA	NA	NA	2.19E-05	4.37E-05
Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Providencia*	2.85E-25	5.70E-25
Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Serratia*	1.49E-19	2.99E-19
Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	5.96E-16	1.19E-15
Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	2.99E-15	5.98E-15
Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	1.95E-11	1.95E-11
Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Enhydrobacter*	7.30E-13	1.46E-12
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	4.95E-17	9.89E-17
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	3.13E-10	6.25E-10
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	5.42E-09	5.42E-09
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	2.11E-06	2.11E-06
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0.000651	0.001302
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0.00263	0.00526
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	1.02E-07	2.04E-07
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	3.81E-05	7.61E-05
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	0.010346	0.010346

Table A4.5 ASVs with significantly different abundance across treatments in the diet and molting assay, not including *D. melanogaster* samples. Unadjusted and adjusted p-values calculated using hierarchical multiple testing across variance stabilizing transformed read counts. Adjusted p-values incorporate the Hierarchical False Discovery Rate Procedure described in Benjamini and Yekutieli (2001).

Class	**Order**	**Family**	**Genus**	**unadjp**	**adjp**
Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Acetobacter*	0.000249	0.000498
Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	*Commensalibacter*	0.014037	0.014037
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	2.14E-05	4.28E-05
Betaproteobacteria	Burkholderiales	Comamonadaceae	*Acidovorax*	8.79E-08	1.76E-07
Betaproteobacteria	Burkholderiales	Comamonadaceae	*Comamonas*	9.46E-07	1.89E-06
Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	3.78E-05	7.55E-05
Betaproteobacteria	NA	NA	NA	0.003225	0.006449
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Chryseobacterium*	5.64E-06	1.13E-05
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Cloacibacterium*	7.64E-05	0.000153
Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	8.41E-10	1.68E-09
Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	3.82E-09	7.64E-09
Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter*	4.66E-08	4.66E-08
Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Enhydrobacter*	3.00E-08	5.99E-08
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	3.58E-09	7.16E-09
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	9.42E-06	9.42E-06
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	6.49E-06	9.42E-06
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0.000195	0.000195
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	0.020308	0.040616
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	7.65E-05	0.000153
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	0.003321	0.003321
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	0.002549	0.003321

Appendix B: Supplementary Figures for Chapter 4

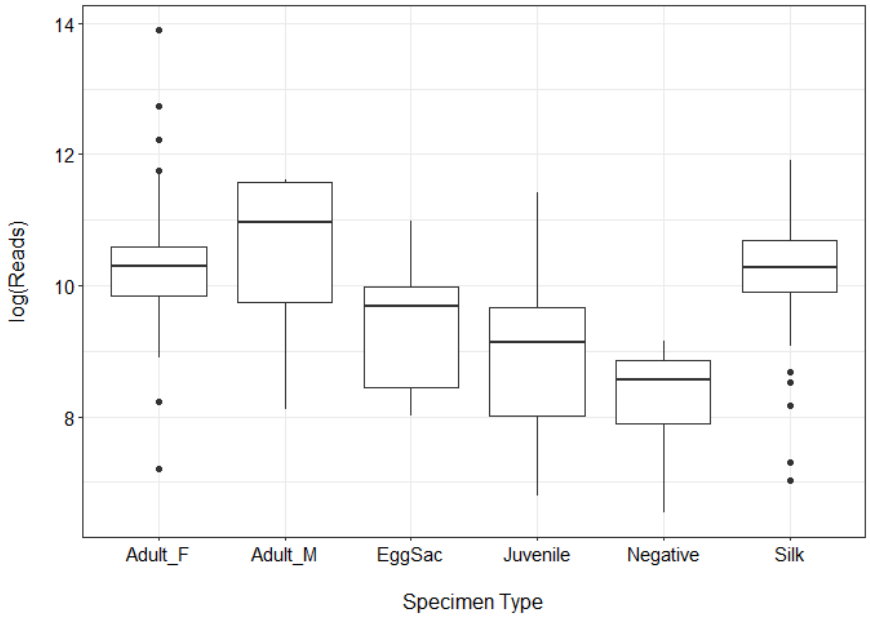

Figure A4.1 Natural log-transformed read counts of sequenced samples from the wild-caught survey. Counts were not significantly different across specimen types (ANOVA, $p>0.05$). Adult_F = adult female *A. studiosus*; Adult_M = adult male *A. studiosus*; EggSac = whole *A. studiosus* eggsacs; Juvenile = single *A. studiosus* juveniles of varying developmental stages; Negative = no sample added to DNA extraction tubes; Silk = silk samples spooled from the webs of *A. studiosus* females.

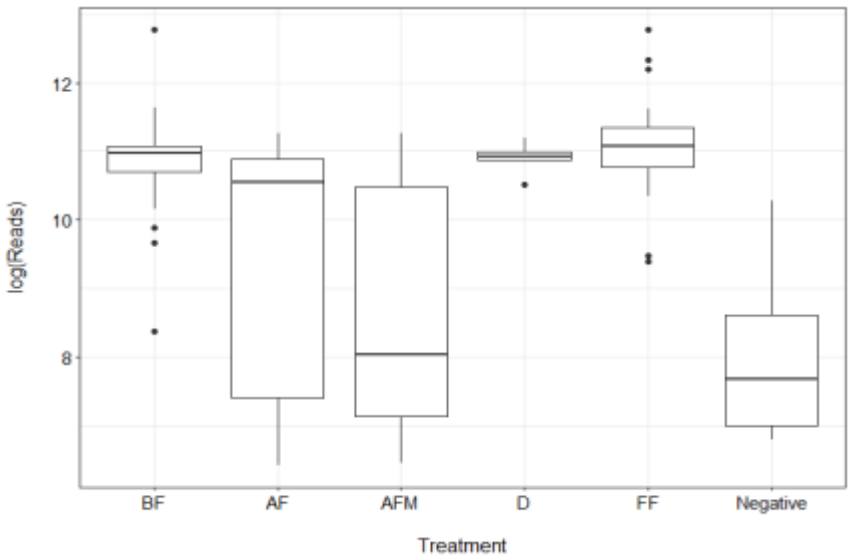

Figure A4.2 Logged read counts for samples from the diet and molting assay. Read counts were significantly different across samples (ANOVA, $p < 0.05$), with and without inclusion of negative controls. BF = before feeding; AF = after feeding; AFM = after feeding and molting; FF = flies fed upon by spiders; D = *D. melanogaster* from stock; Negative = no DNA added to extraction kit tubes.

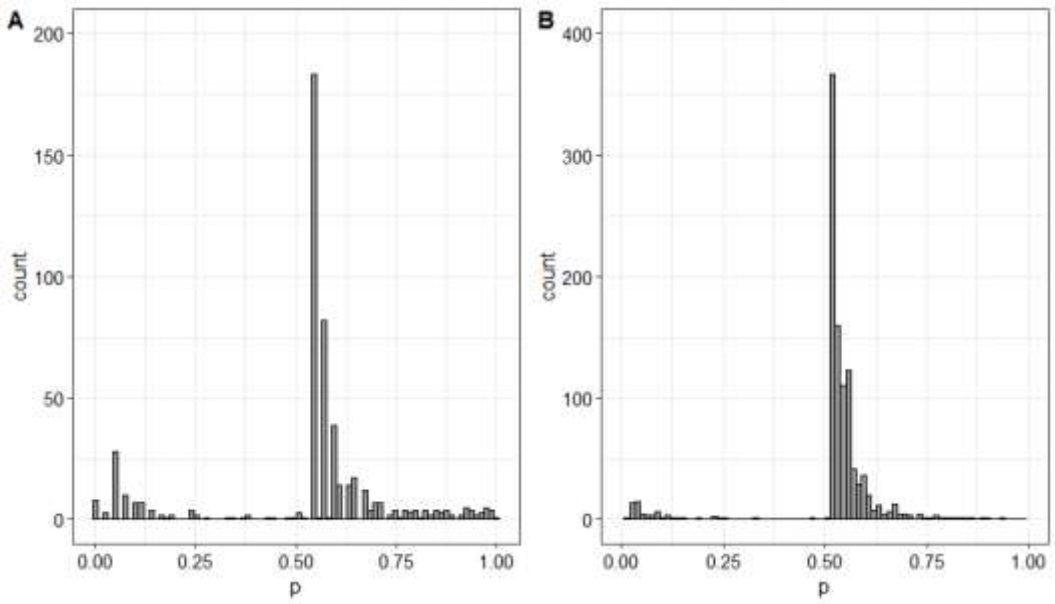

Figure A4.3 Prevalence score statistic (p) used to identify amplicon sequence variants (ASVs) as possible contaminants or not, as calculated by *isContaminant* function in the *decontam* package (further methods described in Davis et al. [2018]). A) Scores for the diet and molting assay and B) scores for the wild-caught assay.

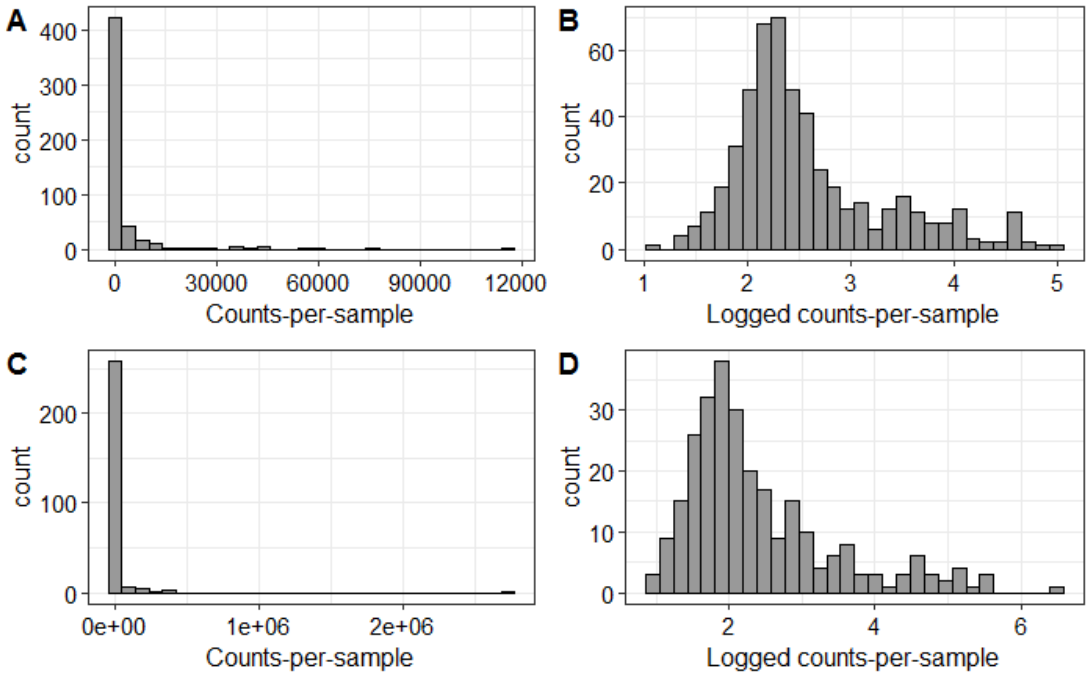

Figure A4.4 Distribution of number of samples with a given number of reads per sample (after preprocessing) for A) the wild-caught assay, B) the wild-caught assay, with reads per sample log-transformed C) the diet and molting assay, and D) the diet and molting assay, with reads per sample log-transformed.

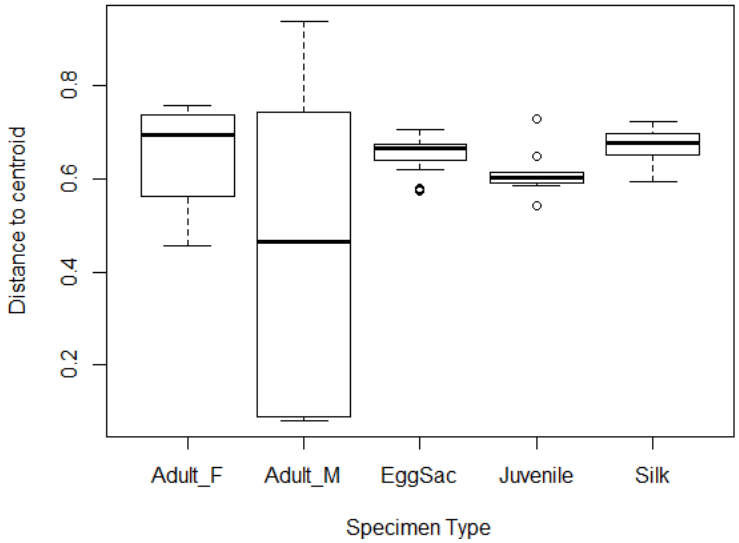

Figure A4.5 Beta dispersion estimates for different specimen types from the wild-caught assay. Adult males show significantly different distance to the types' centroid than other types (ANOVA and Tukey tests, $p < 0.05$). Adult_F = adult female *A. studiosus*; Adult_M = adult male *A. studiosus*; EggSac = whole *A. studiosus* eggsacs; Juvenile = single *A. studiosus* juveniles of varying developmental stages; Silk = silk samples spooled from the webs of *A. studiosus* females.

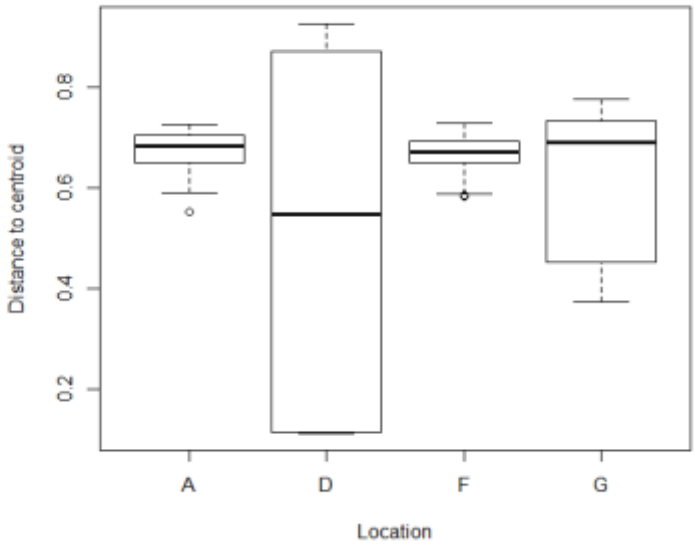

Figure A4.6 Beta dispersion estimates for samples from four collection sites from the wild-caught assay. Estimates were significantly different across locations (ANOVA, $p < 0.05$).

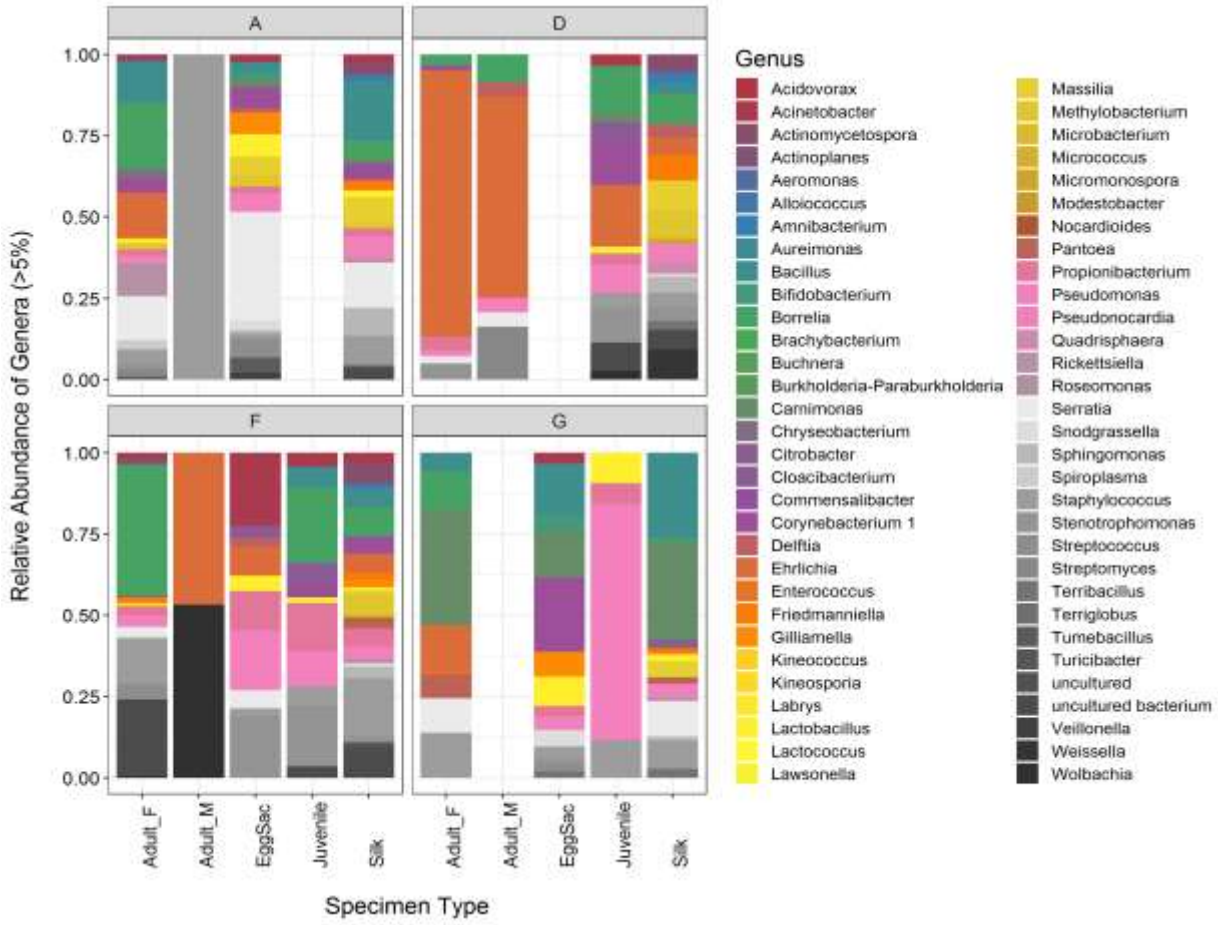

Figure A4.7 Relative abundance of bacterial genera that were present in >5% of taxa across specimen types and collection locations in the wild-caught assay. Adult_F = adult female *A. studiosus*; Adult_M = adult male *A. studiosus*; EggSac = whole *A. studiosus* eggsacs; Juvenile = single *A. studiosus* juveniles of varying developmental stages; Silk = silk samples spooled from the webs of *A. studiosus* females.

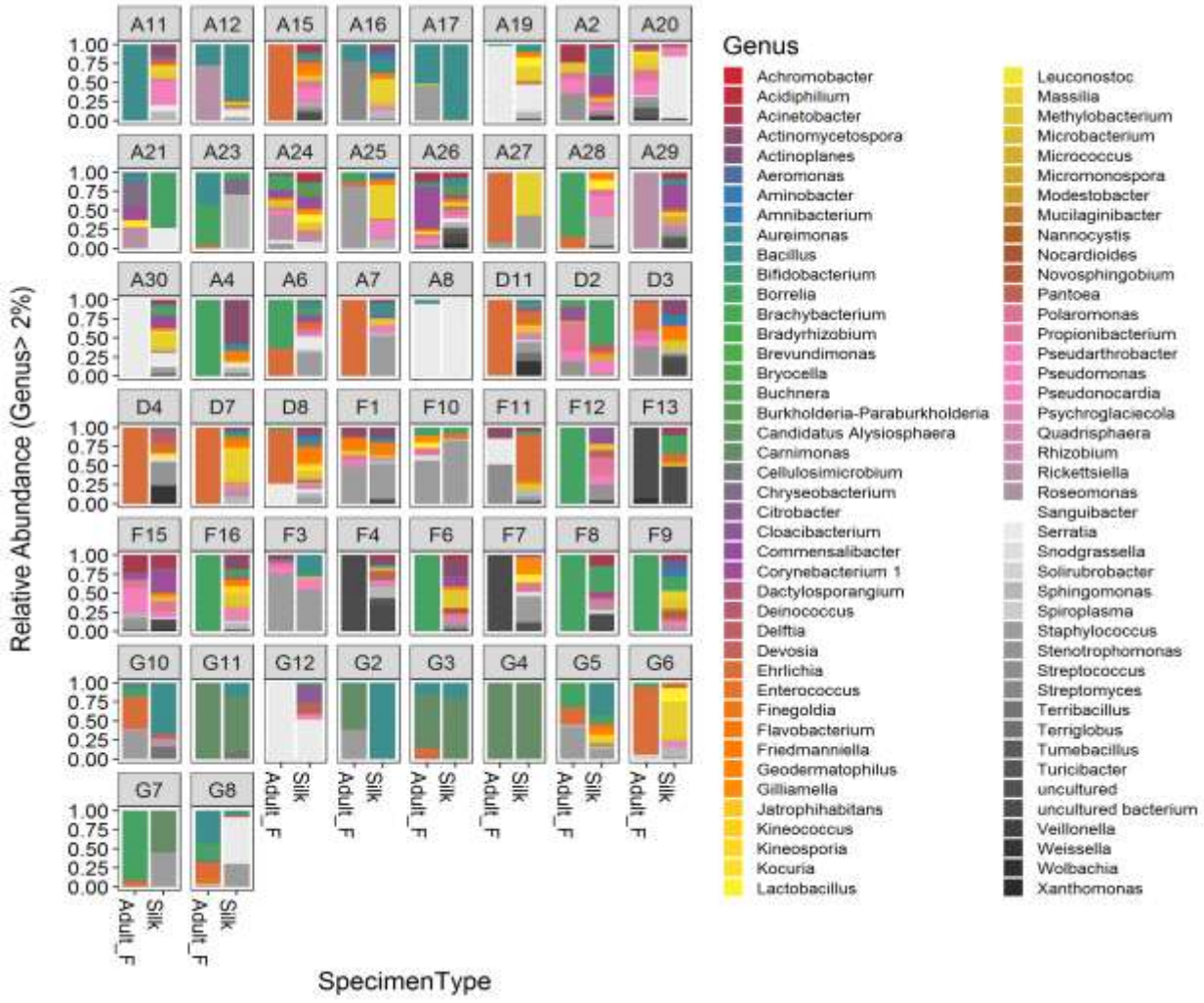

Figure A4.8 Relative abundance of bacterial genera in adult females and their corresponding silk samples. Shown are genera that were present in >2% of samples in the wild-caught assay. Adult_F = adult female *A. studiosus*; Silk = silk samples spooled from the webs of *A. studiosus* females.

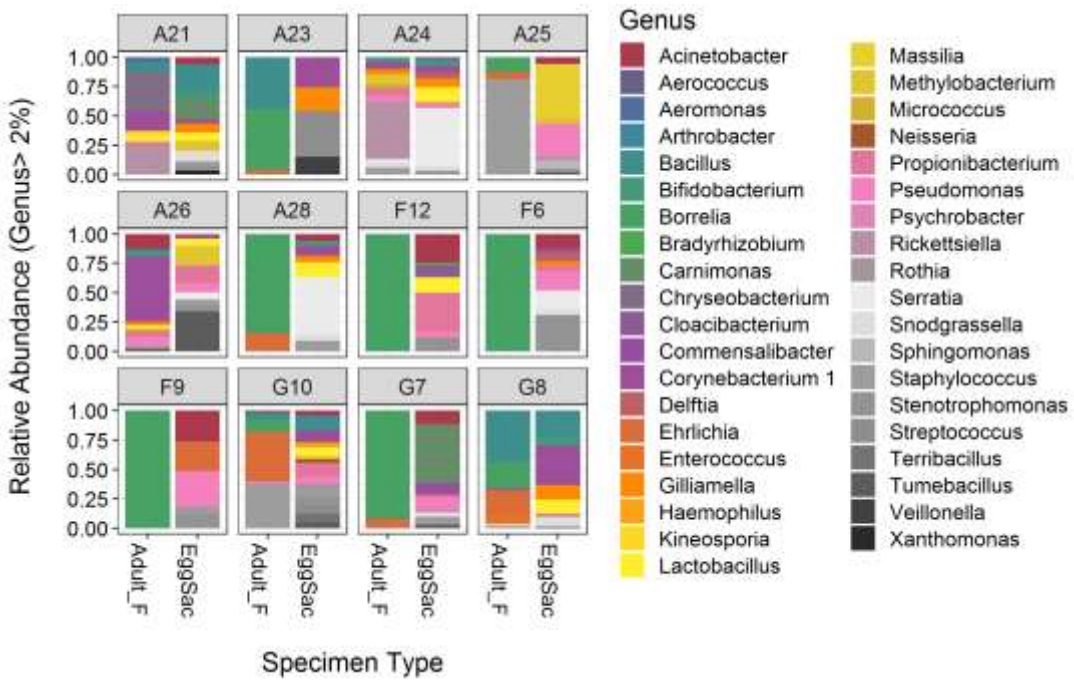

Figure A4.9 Relative abundance of bacterial genera in adult females and their corresponding eggsacs. Shown are genera that were present in >2% of taxa in the wild-caught assay. Adult_F = adult female *A. studiosus*; Silk = silk samples spooled from the webs of *A. studiosus* females.

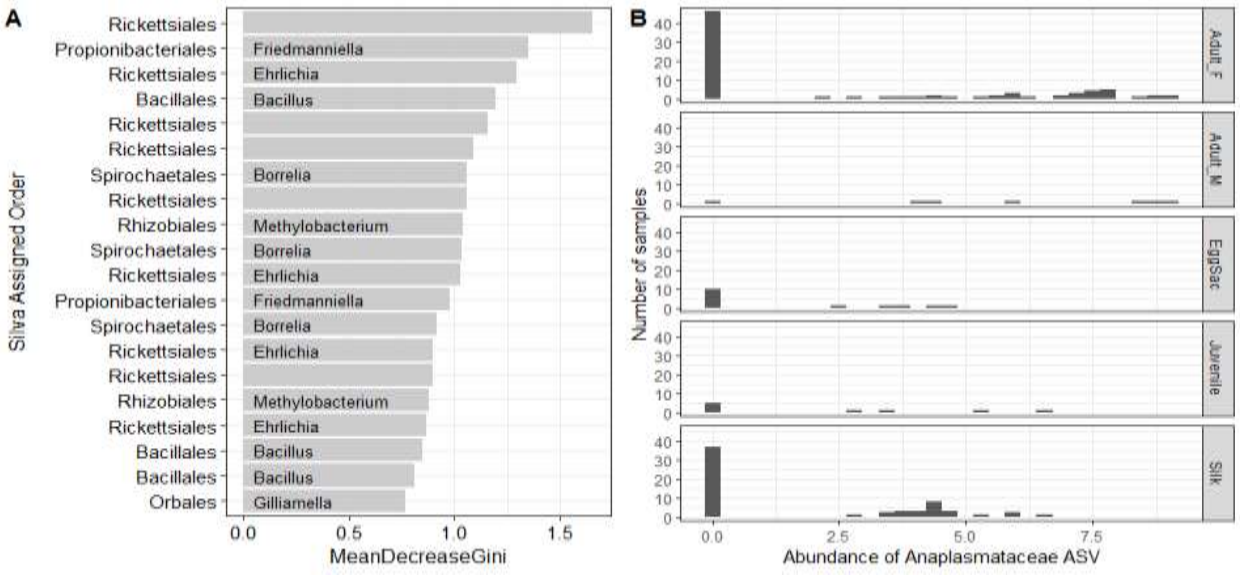

Figure A4.10 A) Most important predictors by specimen type determined by random forest analysis. B) Abundance of the Anaplasmataceae ASV across specimen types, that was found to be the most important predictor for specimen type in random forest analyses. Adult_F = adult female *A. studiosus*; Adult_M = adult male *A. studiosus*; EggSac = whole *A. studiosus* eggsacs; Juvenile = single *A. studiosus* juveniles of varying developmental stages; Silk = silk samples spooled from the webs of *A. studiosus* females.

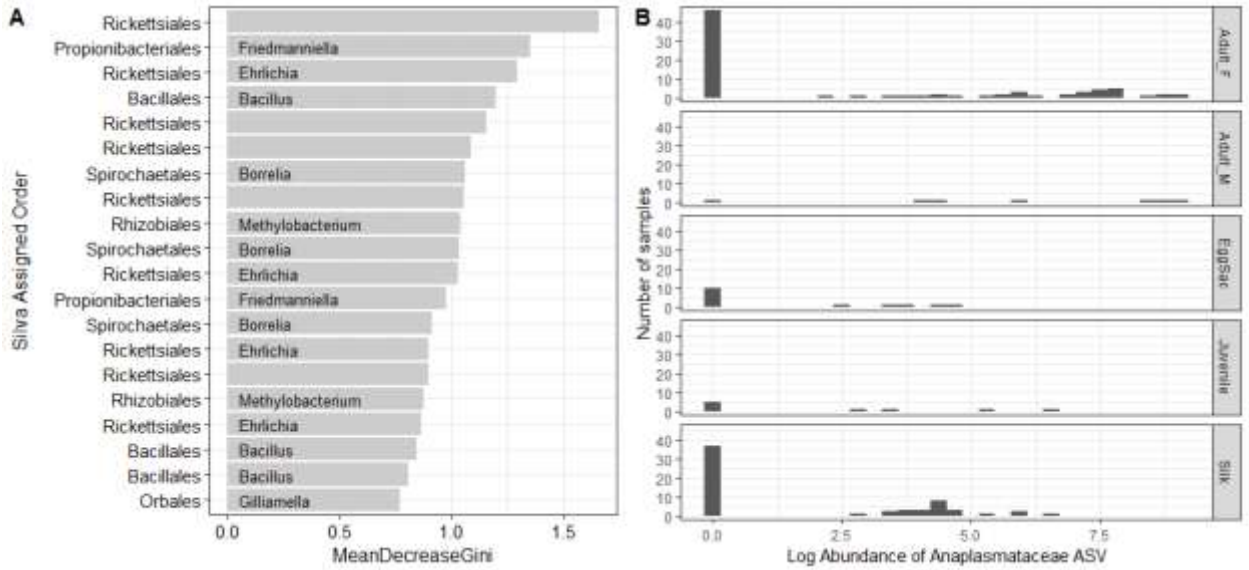

Figure A4.11 A) Most important predictors by location as determined by random forest analysis. B) Abundance of the Anaplasmataceae ASV across specimen types, that was found to be the most important predictor for specimen type in random forest analyses. Adult_F = adult female *A. studiosus*; Adult_M = adult male *A. studiosus*; EggSac = whole *A. studiosus* eggsacs; Juvenile = single *A. studiosus* juveniles of varying developmental stages; Silk = silk samples spooled from the webs of *A. studiosus* females.

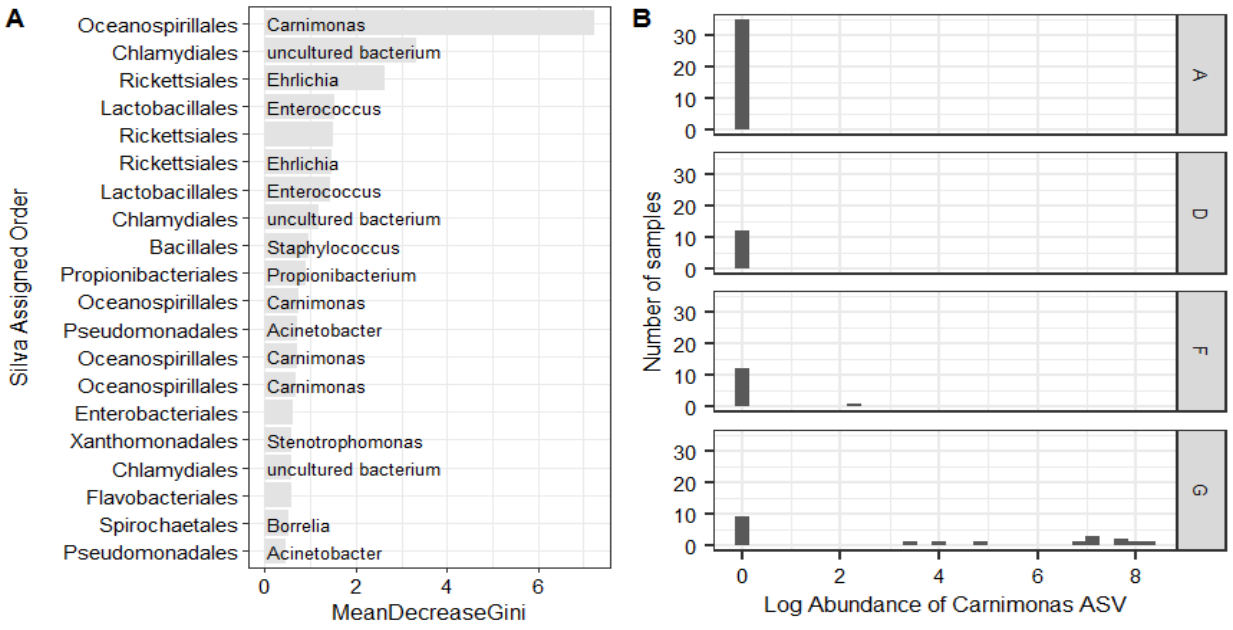

Figure A4.12 A) Most important predictors by location for adult females only as determined by random forest analysis. B) Abundance of the *Carnimonas* ASV across specimen types, that was found to be the most important predictor for specimen type in random forest analyses.

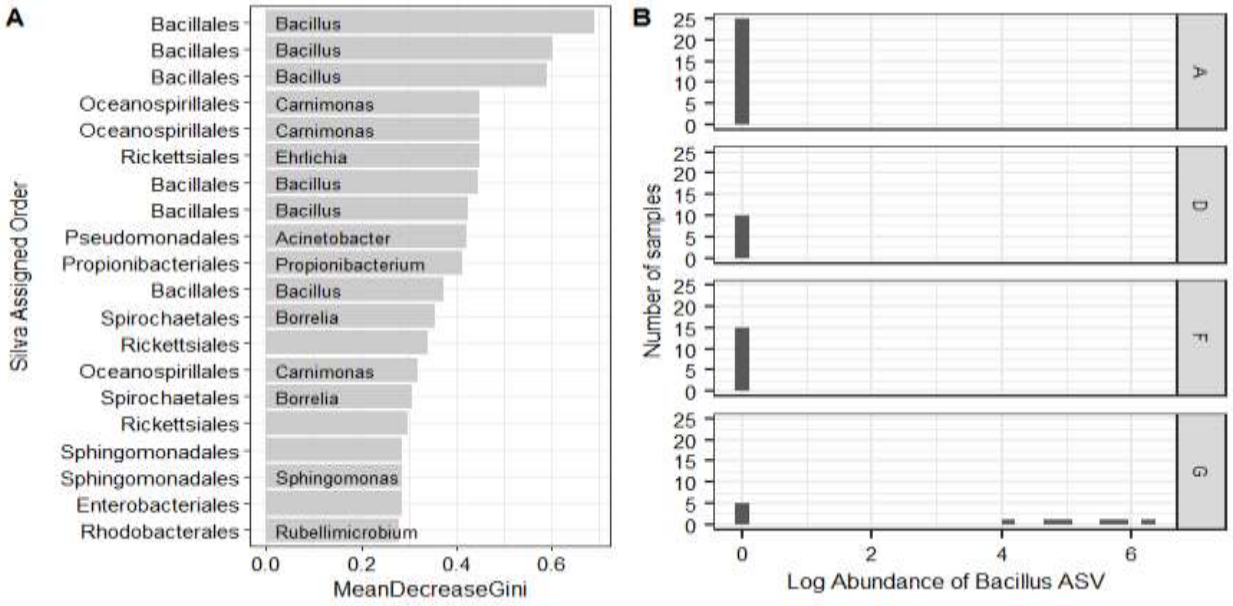

Figure A4.13 A) Most important predictors across location for silk samples only as determined by random forest analysis. B) Abundance of the *Carnimonas* ASV across specimen types, that was found to be the most important predictor for specimen type in random forest analyses.

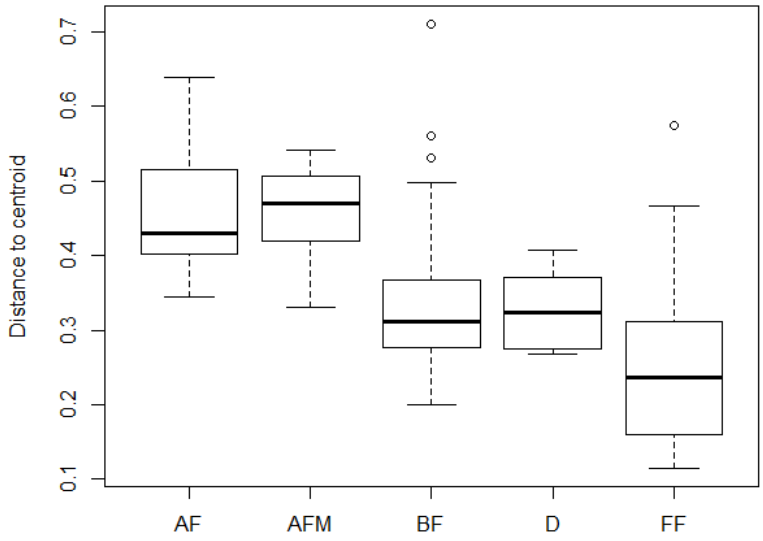

Figure A4.14 Beta dispersion estimates for samples from the diet and molting assay across treatments. Estimates were significantly different across locations (ANOVA, $p < 0.05$). BF = before feeding; AF = after feeding; AFM = after feeding and molting; FF = flies fed upon by spiders; D = *D. melanogaster* from stock.

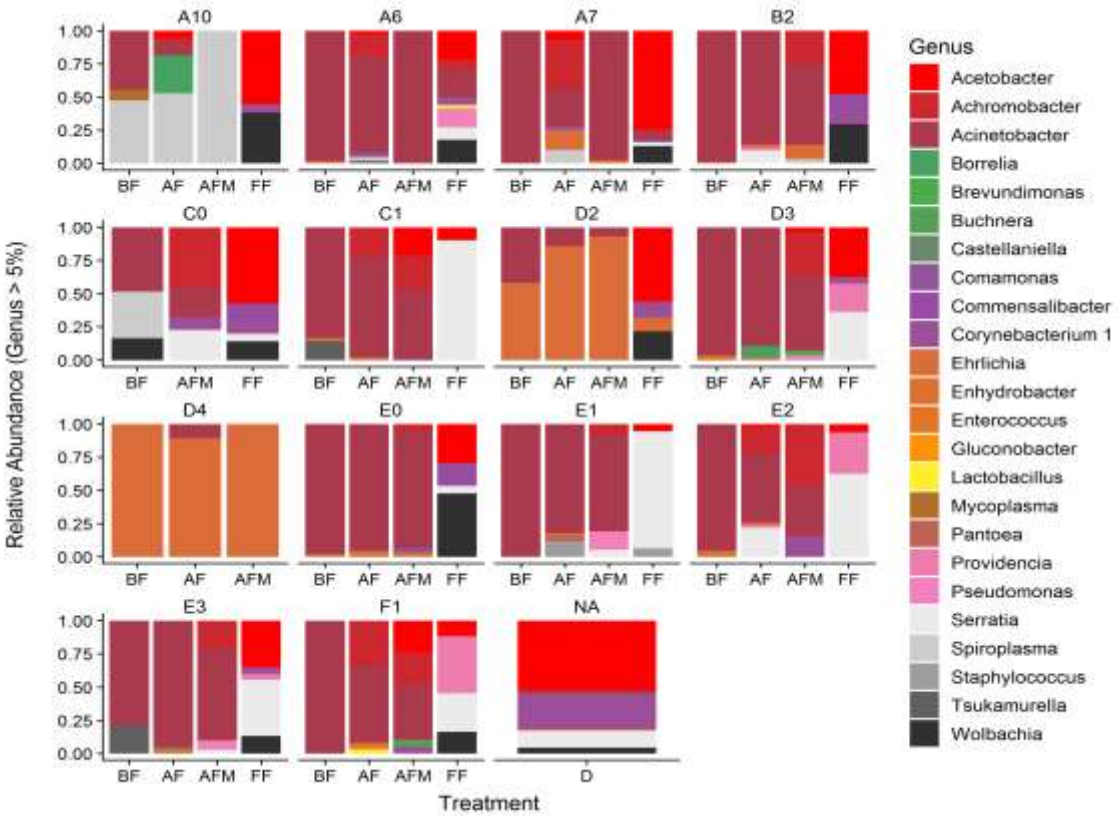

Figure A4.15 Relative abundance of genera present in more than 5% of all samples in the diet and molting assay, across treatment and family. BF = before feeding; AF = after feeding; AFM = after feeding and molting; FF = flies fed upon by spiders; D = *D. melanogaster*. Letters (A-F) represent maternal collection location, and numbers indicate web number from that site.

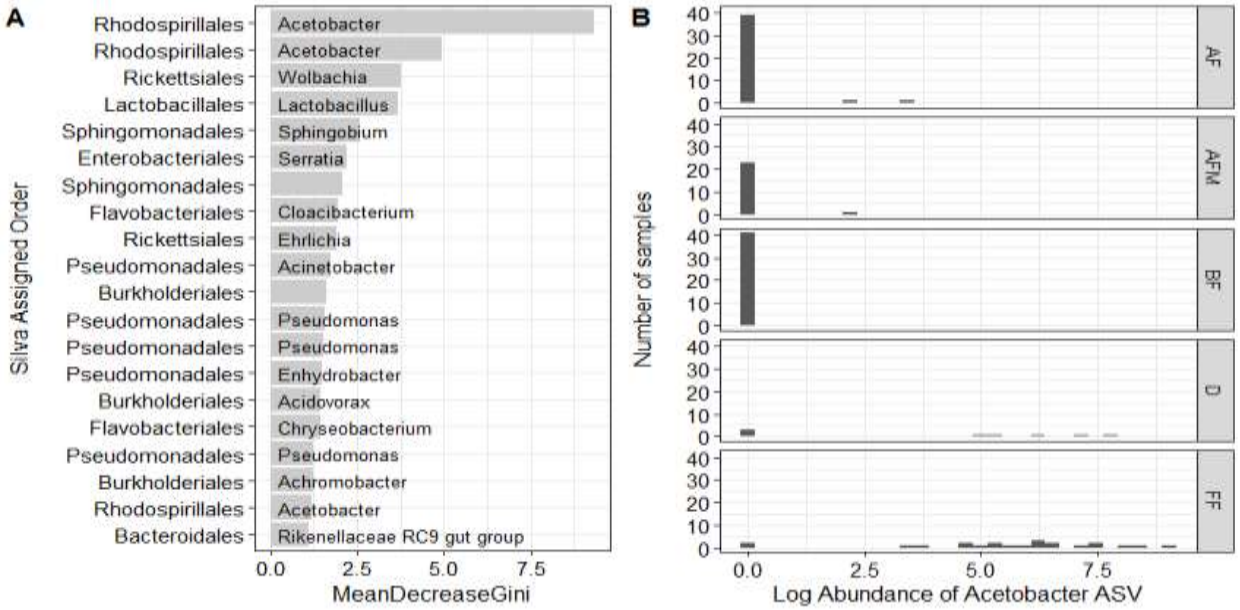

Figure A4.16 Results of random forest model for classifying treatments (*D. melanogaster* treatments included). Important predictors of treatment in the diet and molting assay, and the ln(abundance) of the top predictor across treatments.

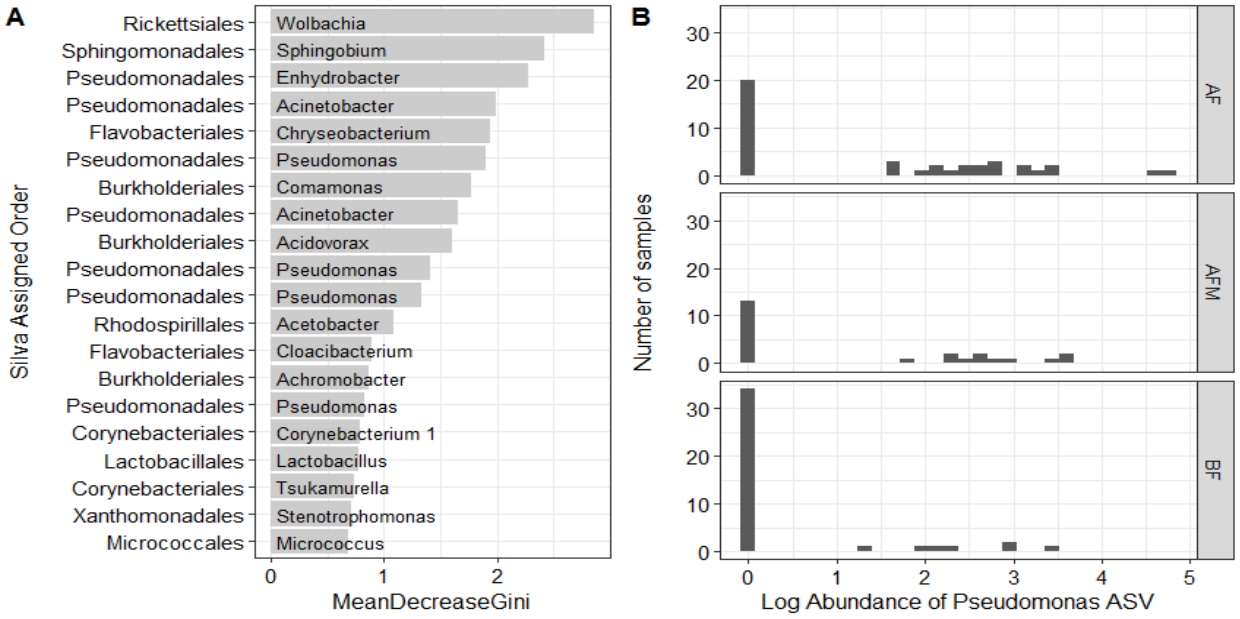

Figure A4.17 Results of random forest model for classifying treatments (*D. melanogaster* treatments not included). Important predictors of treatment in the diet and molting assay (left), and the ln(abundance) of the top predictor across treatments (right).

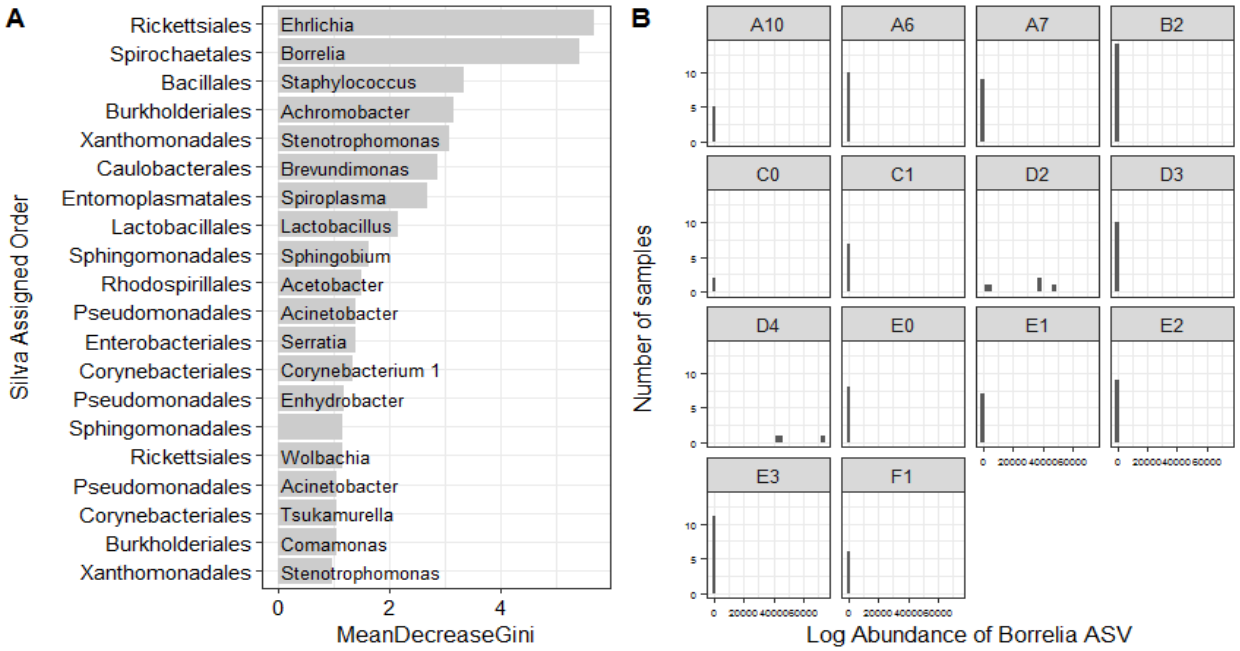

Figure A4.18 Results of random forest model for classifying families (*D. melanogaster* treatments not included). Important predictors of family in the diet and molting assay (left), and the ln(abundance) of the top predictor across treatments (right).

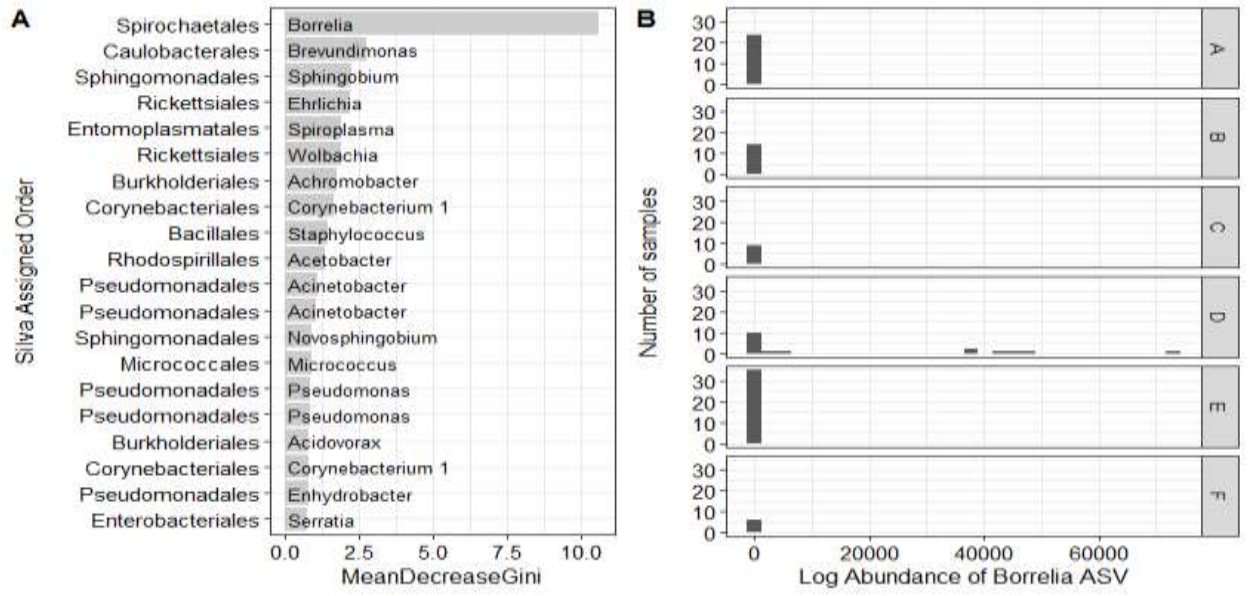

Figure A4.19 Results of random forest model for classifying maternal collection location (*D. melanogaster* treatments not included). Important predictors of location in the diet and molting assay (left), and the ln(abundance) of the top predictor across treatments (right).

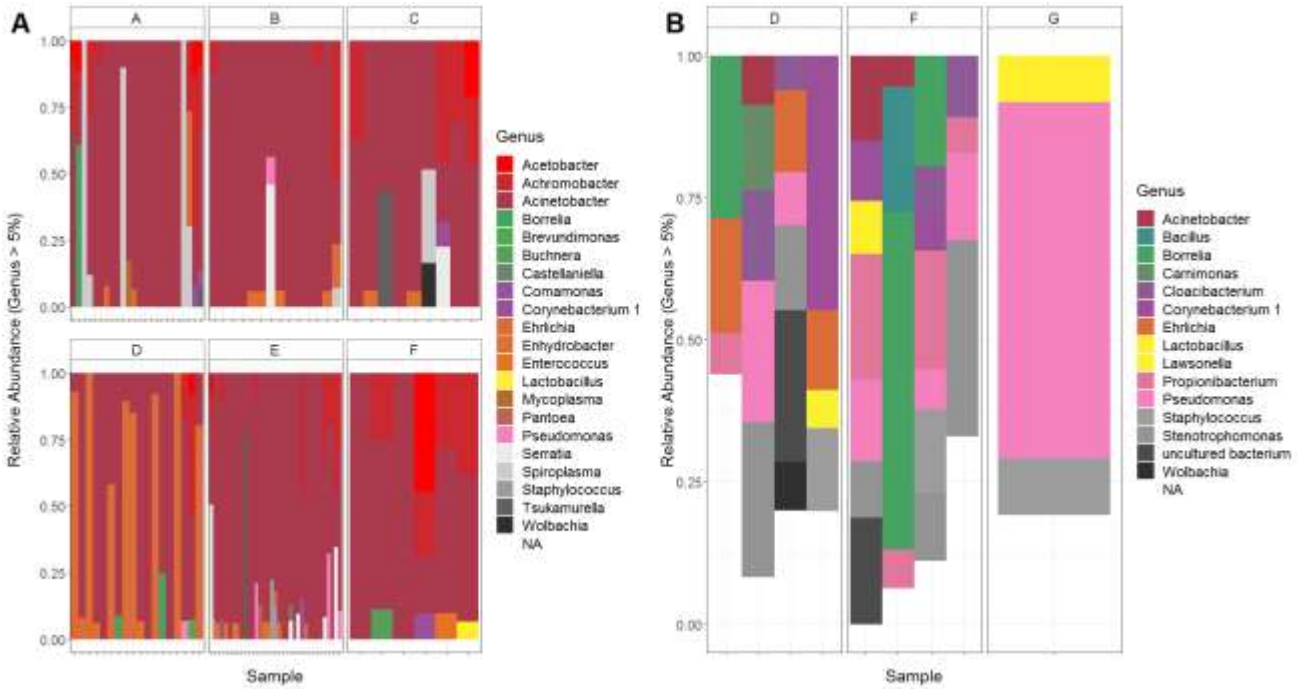

Figure A4.20 Relative abundance of bacterial genera present in >5% of juvenile samples only from each assay. A) Genera from the diet and molting assay across maternal collection locations. B) Genera from the wild-caught assay across collection sites.

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