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Valeria De Las Casas April 13, 2021

The Impact of Initial Symbiont Inoculation Density and Diversity on Fitness of the *Anasa tristis* Squash Bug

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Abstract
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Microbiome composition, which encompasses both the quantity and the diversity of microbes present, can have profound impacts on the function and development of surrounding biotic environments, including the living hosts within which microbiomes are often found. While the importance of host-associated microbes for hosts has been studies across systems, less is known about how the initial conditions at the onset of these interactions impact subsequent outcomes. I explored this question using a tractable insect-microbe system, *Anasa tristis* squash bugs and *Caballeronia* bacterial symbionts. I analyzed the impact of symbiont inoculation density on host and microbial fitness by comparing differences in host survival, host development time, and within host symbiont population size across three different bacterial inoculation densities. There were no statistically significant differences in measures of host and symbiont fitness across the treatments, suggesting that initial inoculation density may have little influence on final outcome. I also analyzed the impact of symbiont diversity on host fitness by comparing differences in host survival and development time across treatments varying from inoculation with single strains of bacteria to inoculation with a more complex microbiome. There was no statistically significant difference in host survival between the four treatment groups, though there were statistically significant differences in development time, suggesting that the composition of the initial symbiont inoculation may impact final outcome. Future studies need to continue to assess how microbiome composition affects host fitness and health.

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Introduction

Microbes are all around us and they have profound impacts on our world. Found in habitats ranging from the soil to the human body, crucial processes are driven by the intricate balance of microbial communities (Shade et al., 2012). The complex and dynamic microbial composition of an environment is referred to as the microbiome. Even though there are many transient members, some bacterial strains of the microbiome are constantly associated with hosts, including humans, and may evolve in response to host association (Bäckhed et al., 2005). In some cases, the microbes (termed "symbionts") form symbioses, life-long associations, with their hosts. Microbiome composition, which encompasses both the quantity and the diversity of microbes present, can have profound impacts on the function and development of surrounding biotic environments, including the hosts within which microbiomes are often found.

Microbial community composition plays a crucial role for environmental function, stability and productivity. For example, Lau and Lennon, 2011 demonstrated that below-ground microbial communities impact the productivity, diversity and composition of plants. Specifically, they found that diverse microbial communities better protect plants from environmental stress and result in more productive plant communities than microbial communities with little diversity (Lau and Lennon, 2011). Furthermore, microbial communities also play essential roles for animal hosts. Bäckhed et al., 2005, for example, demonstrated that the gut microbiota of mice provides essential metabolic functions by aiding the digestion of polysaccharides, otherwise indigestible by mice. Additionally, microbiome composition plays an important role for mouse health. Mice ingesting a higher proportion of bacteria from the Firmicutes relative to the Bacteroidetes, for example, are characterized by greater adiposity and increased weight, potentially resulting in negative health implications (Turnbaugh et al., 2008).

Additionally, microbial communities can also have important implications for host fitness by providing defense against pathogens. For example, the gut microbiome of the bumble bee *Bombus terrestris* protects it from the parasite *Crithidia bombi* (Mockler et al., 2018). Lower *Crithidia* infection loads are associated with greater microbiome diversity, larger gut bacterial populations, and the presence of specific bacterial taxa in the microbiome, including *Apibacter*, *Lactobacillus Firm-5*, and *Gilliamella spp.* (Mockler et al., 2018). This illustrates that both quantity and diversity have significant effects in microbiota-dependent protection against the *Crithidia* pathogen.

Similarly, microbial communities play vital roles in human hosts. For example, the human gastrointestinal (GI) tract is home to a complex, dynamic microbiome (Thursby and Juge, 2017). The diversity and stability of microorganisms in the GI tract results from a combination of stochastic uptake, host selection, and coevolution (Bäckhed et al., 2005). The GI tract contains more than 500 different bacterial species, some of which offer benefits to the host, strengthening the gut integrity, shaping the intestinal epithelium, providing nutritional support, protecting against pathogens, and regulating host immunity (Thursby and Juge, 2017). If the gut microbiome composition is altered and becomes imbalanced, host fitness may decline due to the loss of keystone members that provide nutritional resources, defenses against pathogenesis, and essential components of host development (Thursby and Juge, 2017). For example, antibiotic use can alter the microbial composition of the GI tract which can later result in inflammatory disease, such as *Clostridium difficile* infection (Valeria De Las et al., 2020).

Despite what is known about the importance of host-associated microbes for plant and animal health, there are many aspects of these systems that remain unknown. For example, in many systems, little is known about how initial conditions at the onset of a host-microbe

interaction influence subsequent outcomes. Additionally, in many systems, we know little about how specific taxa within the microbiome influence host fitness. To fill this gap, I asked the following questions. Does initial symbiont concentration impact host and microbial fitness? Does symbiont diversity impact host fitness? Here, in Chapters 1 and 2, I explore these questions using a tractable interaction between the squash bug, *Anasa tristis*, and its bacterial symbiont *Caballeronia*. In this mutualistic relationship, both partners presumably receive reciprocal benefits. While *Caballeronia* can survive outside their host, they benefit inside the host by growing to high titers and free from competition with other bacteria. *A. tristis* squash bugs rely on *Caballeronia* for survival and development. This system is ideal because squash bugs are easily reared in the lab, have cultivable bacteria, and have a symbiosis with a simple microbial community dominated by *Caballeronia*.

Chapter 1: The Impact of Symbiont Inoculation Density on Host and Microbial Fitness

Squash bugs horizontally acquired *Caballeronia* from the environment each generation. Hosts that environmentally acquire their bacterial symbionts may not have access to a reliable symbiont pool each generation. The lack of a reliable symbiont source may result in variation in the quantity of symbionts to which hosts are exposed. The implications of *Caballeronia* concentration for *A. tristis* fitness remains unknown. This chapter seeks to address whether the symbiont inoculation density the host ingests affects host and microbial fitness. Specifically, I test for differences in host survival and development time when they are given access to three different inoculation densities of symbionts (Low, Medium, and Standard). I also determine whether the final symbiont concentration in adult hosts varies in response to the initial inoculum concentration. I find no impact of inoculation density of these measures of host and symbiont fitness.

Methods

Collecting eggs

Eggs were collected from plants in a mesh cage into clean, plastic rearing boxes with vented lids. Prior to collection, the inside of the box and the lid were sprayed with ethanol (70%), and the box was wiped dry with a kimwipe. To remove eggs from the plant and mesh cage in which they were kept, fingers were gently run along the eggs to carefully knock the eggs off the surface of the plant and mesh and into the rearing box.

Sterilizing eggs

Ethanol (70%) was added until all eggs were just covered. The box was shaken a few times to ensure all surfaces of the eggs were exposed to the ethanol. The eggs were exposed to ethanol (70%) for about two minutes. Bleach (10%) was added until all the eggs were just covered. The box was shaken a few times to ensure all surfaces of the egg were exposed to the bleach. The eggs were exposed to bleach for about two minutes. Then, the eggs were exposed again to ethanol (70%), ensuring all surfaces were covered, for about 30 seconds for a final wash. With a kimwipe, the sides of the container were wiped to remove the excess liquid. The eggs were allowed to air dry.

Reviving *Caballeronia* from glycerol stocks

Glycerol stocks of *Caballeronia* sp. Sq4a, which was originally isolated from a squash bug, were removed from the -80°C freezer and placed in ice. Bacteria were streaked onto luria broth (LB) using a sterilized inoculating loop. Plates were incubated at 28°C for 48 hours or left at room temperature for five days.

Preparing bacterial feeding solutions and exposing bugs

Overnight cultures were prepared the day before the bugs were fed. A single colony from the revived bacteria was placed into LB broth (3 mL) and incubated at 28°C overnight with shaking. A two-hour culture was prepared using the overnight culture from the day before: 1) the overnight culture was mixed and 500 μL of culture was removed and placed into 2.5 mL of LB broth; 2) then, the tube was incubated at 28°C for two hours with shaking. The volume of *Caballeronia* for the final feeding solution was adjusted so that the "Standard" had a final concentration of 2×10^7 cell/mL, the "Medium" treatment had 1×10^7 cell/mL, and the "Low"

had 5 x 10^6 cell/mL solutions. Blue dye (1%) was added to the solution for visual confirmation of feeding solutions in the squash bugs' abdomens. Feeding solutions were carefully poured into the small sterile Petri dishes (10 mm). A sterile dental cotton swab was placed in each dish, ensuring that it hung over the side of the dish. The dishes were carefully wrapped with parafilm. The feeding solution was placed in a rearing cage with the second instar nymphs (30 nymphs/cage for each treatment), which had been starved eight hours prior. The rearing cages with nymphs were placed in a 28°C incubator. After 24 hours, the feeding solutions were removed.

Feeding *A. tristis* after inoculation

Squash bugs were separated into new rearing cages with a small piece of parafilmed-covered yellow squash or zucchini. To prepare this fruit, a knife and cutting board were sprayed with ethanol (70%) and wiped dry with a kimwipe. The fruit was cut to pieces about 5 mm thick. Each piece of fruit was wrapped with parafilm. A wrapped piece of fruit was placed in each rearing box container, each of which contained five squash bugs. Fruit was replaced with every two days.

Development time and survival assays

For each treatment group, there were a total of six rearing boxes, each of which contained five second instar nymphs (6 experimental rearing cages x 5 nymphs/cage = 30 squash bugs per treatment). There was another rearing box for the negative control (water), also containing five second instar nymphs. Host survival and development time was recorded every other day. A Cox Proportional-Hazard Model was performed to compare the effect of dose on survival. A

quasipoisson distributed generalized linear model was performed to compare the effect of each treatment on host development rate.

Dissecting squash bugs and assessing symbiont fitness

Once squash bugs reached adulthood, they were sacrificed and dissected. Squash bugs were anesthetized using CO_2 then surface sterilized in ethanol (70%) for five minutes. Excess ethanol was removed from squash bugs, which were then dissected to isolate the gut crypts where *Caballeronia* are stored. Then, 500 μL of 1x PBS was aliquoted into a microcentrifuge tube. The crypts were macerated in the tubes using a pestle. Crypt homogenates were serially diluted, plated onto LB, and grown at room temperature for 3-5 days. The number of colonies and colony morphology was recorded for CFU/crypt analysis. A quasipoisson distributed generalized linear model was used to compare the effect of treatment on the number of CFUs per crypt between treatment groups.

Results

Host Fitness

Squash bugs in three treatment groups were given access to varying symbiont densities (Low, Medium, and Standard) of *Caballeronia* sp. Sq4a. There was no statistically significant difference in survival between the three treatment groups (Figure 1: $\chi^2 = 2.78$, d.f. = 3, $p = 0.43$). There was also no statistically significant difference in development rate across the three treatment groups, including to third instar ($F = 0.1525$, d.f. = 2 , $p = 0.8592$), to fourth instar ($F = 2.3354$, d.f. = 2 , $p = 0.116$), to fifth instar ($F = 2.0589$, d.f. = 2 , $p = 0.1487$), and to adult ($F = 1.4951$, d.f. = 2 , $p = 0.2461$) (Figure 2).

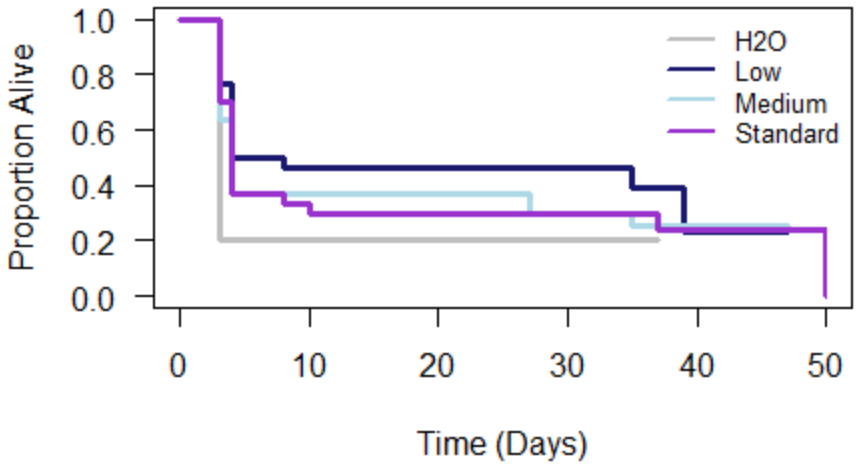

Figure 1. Survival. Host survival was recorded every other day following symbiont inoculation with either the Low, Medium, or Standard symbiont treatment. The water (H_2O) treatment group serves as a negative control. The graph above shows proportion alive vs. time (days) across the four treatment groups. There is no statistically significant difference in survival between the varying doses.

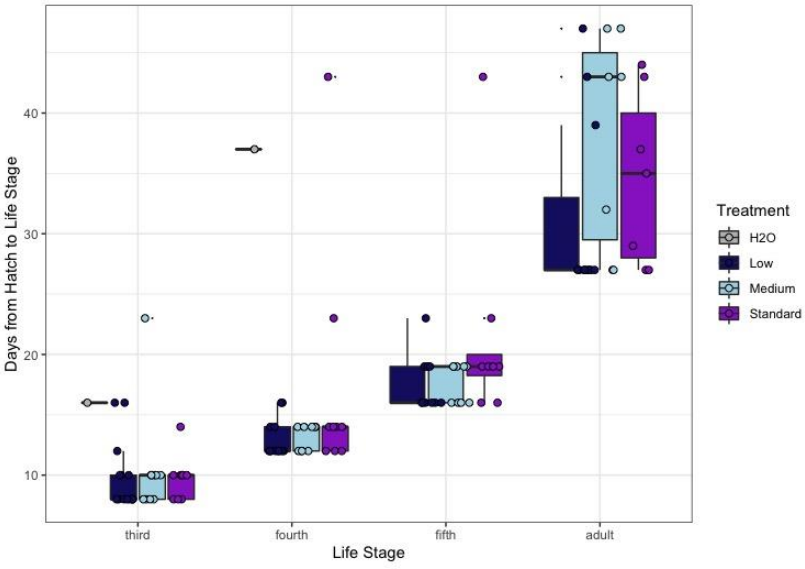

Figure 2. Development time. Development time (days to life stage) was recorded every other day following symbiont inoculation. The graph above shows days from hatch to life stage vs. life stage. Development time did not vary between symbiont doses. Th negative control, water (H_2O) treatment, was not included in the statistical analysis.

Symbiont Fitness

Adults that survived to adulthood were dissected. The number of CFUs per crypt did not vary between symbiont treatments (Figure 3). Regardless of treatment, host crypts were colonized, on average with 6.45 x 10^8 *Caballeronia* (estimated total CFU/crypt).

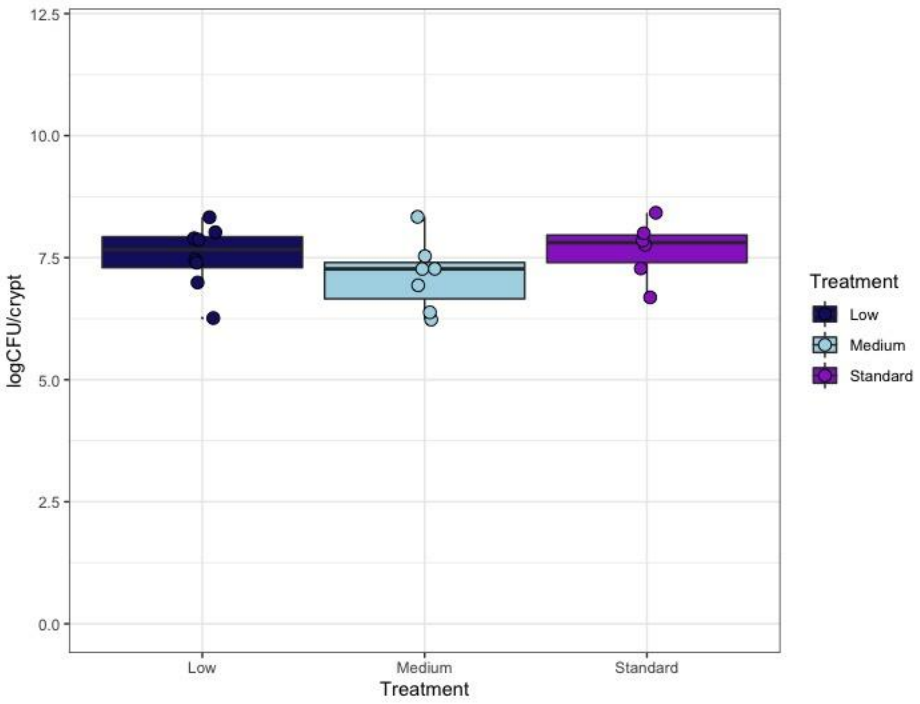

Figure 3. Symbiont Fitness. Adults that survived to adulthood were dissected. The graph above shows LogCFU/crypt vs. Treatment. There was no statistically significant difference in LogCFU/crypt between the varying inoculation doses.

Discussion

These results indicate both host and symbiont fitness are not impacted by inoculation dose. This may suggest that either the hosts or the symbionts or both have a mechanism to adjust symbiont density upon establishment of the symbioses, regardless of how many bacteria were initially consumed. Alternatively, it is possible that the squash bugs are capable of recognizing when they have taken up enough bacteria and stop feeding as soon as that is the case. Further work should explore these potential mechanisms.

One limitation of this study is that dilutions varied across a small scale. Since my experiment was a preliminary study to see if dose could play a potential role in host fitness, my dilutions were performed on a relatively small scale by performing two 1:1 serial dilutions to create the Medium and Low treatments. Future studies should assess the effects of dose with greater variance between inoculum concentrations by considering dilutions performed at a logarithmic scale.

Chapter 2: The Impact of Symbiont Diversity on Host Fitness

Previous experiments, like that of Chapter 1, have been conducted by inoculating *A. tristis* squash bugs with a single beneficial strain of *Caballeronia*. These strains were collected by dissecting bugs and isolating the most common *Caballeronia* symbionts. Such experiments indicate that *Caballeronia* provides the squash bugs with greater fitness benefits (faster development rate and increased survival) compared to squash bugs without the symbiont (see Chapter 1, Figure 2 as an example). The squash bug crypt, however, often contains multiple *Caballeronia* strains, as well as several additional bacterial taxa (Garcia et al., unpublished; Stoy et al., unpublished). Some of the *Caballeronia* strains are likely uncultivable. Therefore, the effects of the entire crypt microbiome for squash bug fitness remains unknown. Research in other systems has demonstrated an effect of bacterial diversity on microbiome function and host fitness (Lau and Lennon, 2011; Mockler et al., 2018; Turnbaugh et al., 2008). Therefore, the whole squash bug crypt microbiome may provide greater fitness than the most common single isolate strain.

This chapter seeks to address whether the diversity of beneficial microbes the host imbibes affects host fitness. Specifically, I sought to determine whether the whole crypt microbiome confers greater fitness benefits for the host than inoculation with a single beneficial strain. I tested for differences in fitness outcomes by comparing host survival and development time up to fourth instar between four treatment groups: Sq4a (a *Caballeronia* sp. bacteria that serves as our positive control), Smt4a (a *Paraburkholderia* sp. bacteria that is known to provide worse fitness benefits compared to Sq4a), a solution of undiluted crypt microbiome, and a solution of diluted crypt microbiome. Due to time constraints, the data included in this report was collected from February 20, 2021 to March 17, 2021. Previous studies have provided strong

evidence that the greatest differences in development rate between symbiotic and apo-symbiotic squash bugs occurs in their rate to fourth instar, making this a reliable fitness measure.

Methods

Collecting eggs

Eggs were collected from plants in a mesh cage into clean, plastic rearing boxes with vented lids. Prior to collection, the inside of the box and the lid were sprayed with ethanol (70%), and the box was wiped dry with a kimwipe. To remove eggs from the plant and mesh cage in which they were kept, fingers were gently run along the eggs to carefully knock the eggs off the surface of the plant and mesh and into the rearing box.

Sterilizing eggs

Ethanol (70%) was added until all eggs were just covered. The box was shaken a few times to ensure all surfaces of the eggs were exposed to the ethanol. The eggs were exposed to ethanol (70%) for about two minutes. Bleach (10%) was added until all the eggs were just covered. The box was shaken a few times to ensure all surfaces of the egg were exposed to the bleach. The eggs were exposed to bleach for about two minutes. Then, the eggs were exposed again to ethanol (70%), ensuring all surfaces were covered, for about 30 seconds for a final wash. With a kimwipe, the sides of the container were wiped to remove the excess liquid. The eggs were allowed to air dry.

Reviving *Caballeronia* from glycerol stocks

Samples of Sq4a and Smt4a in glycerol were removed from the -80°C freezer and placed in ice. Bacteria were streaked onto LB using a sterilized inoculating loop. Plates were incubated at 28°C for 48 hours or left at room temperature for five days.

Preparing bacterial feeding solutions and exposing bugs

Overnight cultures of Smt4a and Sq4a were prepared the day before the bugs were fed. A single colony from the revived bacteria was placed into LB broth (3 mL) and incubated at 28°C overnight with shaking. A two-hour culture was prepared using the overnight culture from the day before: 1) the overnight culture was mixed and 500 μL of culture was removed and placed into 2.5 mL of LB broth; 2) then, the tube was incubated at 28°C for two hours with shaking. The volume of *Paraburkholderia* (for the Smt4a treatment) or *Caballeronia* (for the Sq4a treatment) for the final feeding solution was adjusted to 2×10^7 cells/mL. Blue dye (1%) was added to the solution for visual confirmation of feeding solutions in the squash bugs' abdomens. The feeding solution was mixed by inverting the tube and poured into the small sterile Petri dish (10 mm). A sterile dental cotton swab was placed in the dish, ensuring that it hung over the side of the dish. The dishes were carefully wrapped with parafilm. The feeding solution was placed in the rearing cage with the second instar nymphs (25 nymphs/cage for each treatment). The rearing cages with nymphs were placed in a 28°C incubator. After 24 hours, the feeding solutions were removed.

Preparing the entire microbiota feeding solutions

Squash bugs were anesthetized using CO_2 then surface sterilized by being dropped in 70% ethanol for five minutes. Excess ethanol was removed from squash bugs, which were then

dissected to isolate the crypts. The crypts of ten bugs were carefully dissected and placed into 1x PBS. Crypts were macerated using a pestle. Undiluted crypt homogenate (100 μL) was added to zucchini juice (10 mL), which was extracted, using a juicer, from whole zucchini with the ends removed. Zucchini juice was used in order to stabilize the crypt, which often contains multiple *Caballeronia* strains, as well as several additional bacterial taxa. Blue dye (1%) was added to the solution for visual confirmation of feeding solutions in the squash bugs' abdomens. Diluted solutions were made by diluting crypt homogenates (1000x). The solutions were mixed thoroughly and carefully poured into the small sterile Petri dish (10 mm). A sterile dental cotton swab was placed in the dish, ensuring that it hung over the side of the dish. The dishes were carefully wrapped with parafilm. The feeding solution was placed in the rearing cage with the second instar nymphs (25 nymphs/cage for each treatment). The rearing cages with nymphs were placed in a 28°C incubator. After 24 hours, the feeding solutions were removed.

Feeding *A. tristis* after inoculation

Squash bugs were separated into new rearing cages with a small piece of parafilmed-covered yellow squash or zucchini. To prepare this fruit, a knife and cutting board were sprayed with ethanol (70%) and wiped dry with a kimwipe. The fruit was cut to pieces about 5 mm thick. Each piece of fruit was wrapped with parafilm. A wrapped piece of fruit was placed in each rearing box container, each of which contained five squash bugs. Fruit was replaced with every two days.

Analyses of Survival and Development Time

For each treatment group, there were a total of five rearing boxes, each of which contained five second instar nymphs (5 experimental rearing cages x 5 nymphs/cage = 25 squash bugs per treatment). Host survival and development time was recorded every other day following symbiont inoculation. A Cox Proportional-Hazard Model was performed to compare the effect of treatment on host survival. A quasipoisson distributed generalized linear model was performed to compare the effect of each treatment on host development rate. Tukey's posthoc analysis was used to compare fitness differences between treatment groups. Due to time constraints, the data included is from day 0 to day 25 of the Microbial Community Experiment. Because of this, the quasipoisson distributed generalized linear model was only used to compare the rate to third and fourth instar. Previous work has demonstrated that the most significant differences in development time occur in the rates to third and fourth instar.

Results

Host Survival

Up until day 25 post inoculation, there was no statistically significant difference in survival between the four treatment groups (Figure 4). It is important to note that 19 bugs are still alive in the Smt4a treatment, 18 in the Sq4a treatment, 16 in the Undiluted treatment, and 15 in the Diluted treatment.

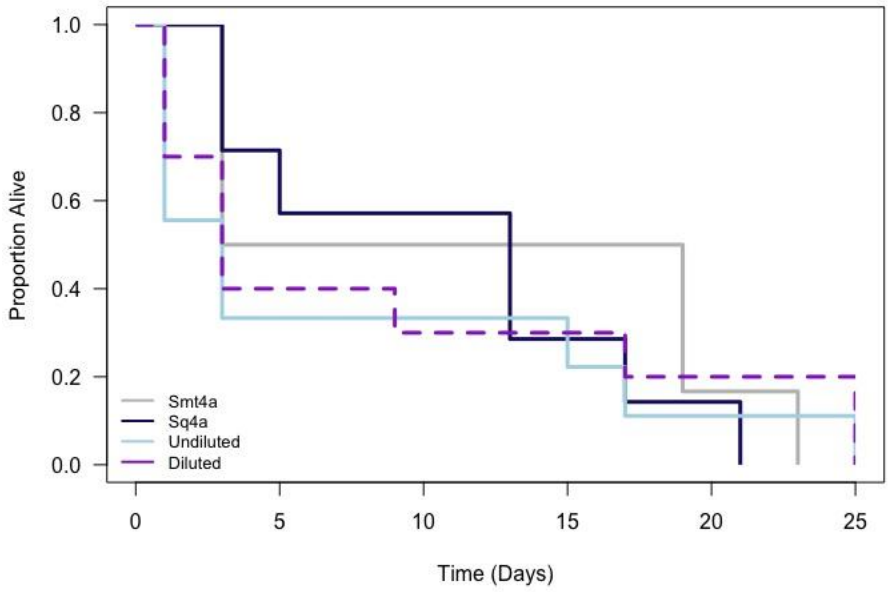

Figure 4. Host survival in relation to alternative symbiont inoculation compositions. The graph above shows proportion alive vs. time (days) across the four treatment groups. Due to time constraints, the data included in this graph is from day 0 to day 25 post inoculation. There is no statistically significant difference between treatments.

Host Development Time

Development Time to Third Instar. There was a statistically significant difference in the effect of treatment on the rate of development to third instar (Figure 5; $F = 9.56$, d.f.=3, $p < 0.001$). Squash bugs provided Sq4a developed more quickly than squash bugs provided Smt4a ($p < 0.001$) and those provided the Diluted microbiome treatment ($p < 0.001$). There was a marginally significant difference between crypt homogenate treatments (Undiluted vs. Diluted). Squash bugs provided the Undiluted microbiome treatment developed slightly faster than those provided the Diluted microbiome treatment ($p = 0.054$). There was no significant difference between the Undiluted microbiome and Sq4a treatments.

Development Time to Fourth Instar. There was a statistically significant difference in the effect of treatment on the rate of development to fourth instar (Figure 5; $F = 24.50$, d.f.=3, $p < 0.001$). Squash bugs provided the Diluted microbiome treatment developed slower than squash bugs provided Smt4a ($p = 0.016$), Sq4a ($p < 0.001$), and the Undiluted microbiome treatment ($p < 0.001$). Squash bugs provided Smt4a developed slower than squash bugs provided Sq4a ($p < 0.001$) and the Undiluted microbiome treatment ($p = 0.005$). There was no significant difference in development rate to fourth instar between squash bugs provided the Undiluted microbiome and Sq4a treatments.

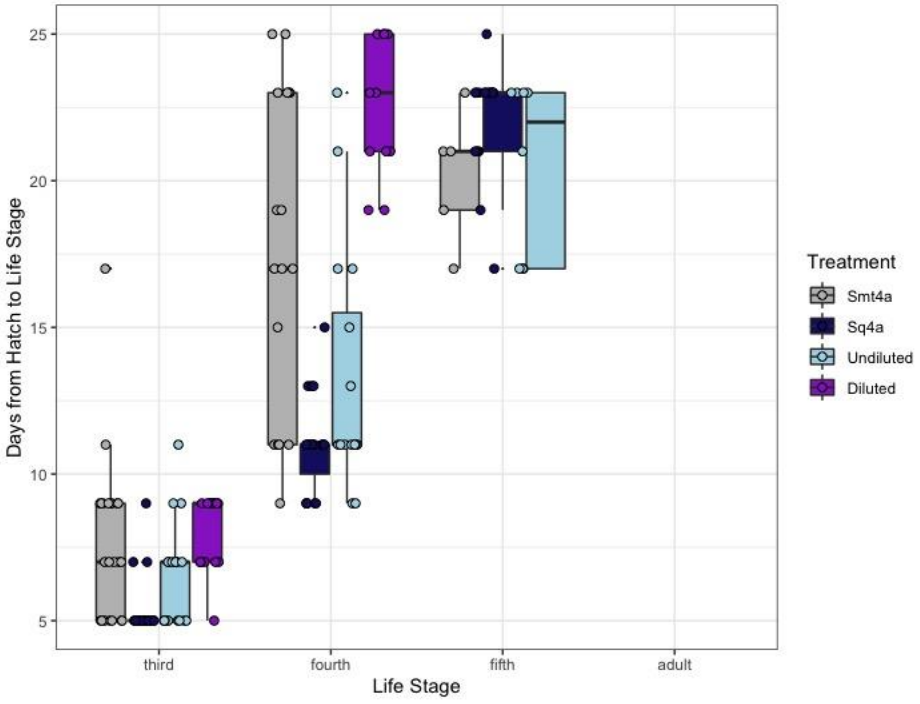

Figure 5. Development time in relation to alternative symbiont inoculation compositions. Development rate to fifth instar, also shown in the graph above, is gathered from preliminary data points collected up until day 25. Statistical analyses on rate to fifth instar and rate to adulthood have not been calculated due to time constraints.

Discussion

Up until day 25, there was no statistically significant difference in survival between the four treatment groups but there were statistically significant differences in development time to

third and fourth instar between the four treatment groups. Future work will compare the development rate to fifth instar and adulthood.

Interestingly, there was no statistically significant difference in rate to third and fourth instar between the Undiluted and Sq4a treatments. Sq4a has been previously shown to be a beneficial symbiont strain for *A. tristis* (Acevedo et al., in prep). There are several possible interpretations. First, a core bacteria colonizing the crypts in lab settings may be genetically very similar to Sq4a and thus may provide a similar level of benefit. Alternatively, the bacteria provided through the undiluted treatment may work together to provide the fitness benefits. Finally, it may be the *A. tristis* benefit from a widely diverse group of *Caballeronia*, such that most strains, whether consumed individually or as part of a consortium, provide similar levels of benefit. This is consistent with recent findings in our lab (Stoy et al., in prep).

There was a statistically significant difference in rate to third and fourth instar between the Undiluted and Diluted treatments. The differences between the Undiluted and Diluted crypt homogenate treatments could potentially be because fewer *Caballeronia* symbionts are colonizing the crypt, though results of Chapter 1 suggest that initial symbiont density should not impact host fitness. Another possibility is that less beneficial *Caballeronia* strains colonize the host when fewer symbionts are ingested initially.

To date, we have no information about the diversity of symbionts within the crypt homogenates. Since we do not know what exactly colonized the host in the Undiluted and Diluted crypt homogenate treatments, in future work it is imperative to perform 16S rRNA gene sequencing in order to determine crypt bacterial diversity for squash bugs inoculated with varying treatments. Furthermore, since this experiment is currently ongoing, in future work we

will perform symbiont fitness assays to determine the effect of treatment on the number of CFUs per crypt between the treatment groups.

Conclusion

Here, in Chapter 1, I show that initial symbiont inoculation density has little impact on subsequent measures of host and symbiont fitness in the squash bug system. In Chapter 2, I find no evidence that a more diverse inoculation source provides greater host benefit, though I do see that some inoculation sources provide greater host benefits than others.

There are still many unknowns about the role of the microbiome in *A. tristis* and in animal hosts more generally. We do not know, for example, how microbiomes maintain functional stability or if the whole microbiome is required for proper functioning inside of these and other hosts. Future studies need to continue to assess how microbiome composition affects host fitness and health.

Better understanding of microbiome establishment and functioning is critical for human health. In the future, I believe that the microbiome will become a critical component of personalized medicine. For example, in an *in vitro* experiment that I conducted on the effect of probiotics on *C. difficile* infection, we found both probiotic composition and *C. difficile* strain mattered when analyzing the inhibitory effects of probiotics on the neutralization of toxin and inhibition of sporulation, which are markers for its pathogenesis (Valeria De Las et al., 2020). This indicates that probiotics could be personalized to best serve as an additional course of therapeutic intervention for patients with recurring CDI along with the current standard of care. In addition, other studies have demonstrated the effect of diet on obesity (Turnbaugh et al., 2008), suggesting the microbes may influence the disease. Because of studies like these, the use of the microbiome as a biomarker of diseases and for assessing therapeutic interventions needs to continue to be explored.

References

Bäckhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., and Gordon, J.I. (2005). Host-bacterial mutualism in the human intestine. Science (80-.). *307*, 1915–1920.

Lau, J.A., and Lennon, J.T. (2011). Evolutionary ecology of plant-microbe interactions: Soil microbial structure alters selection on plant traits. New Phytol. *192*, 215–224.

Mockler, B.K., Kwong, W.K., Moran, N.A., and Koch, H. (2018). Microbiome structure influences infection by the parasite Crithidia bombi in bumble bees. Appl. Environ. Microbiol. *84*, 1–11.

Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Bürgmann, H., Huber, D.H., Langenheder, S., Lennon, J.T., Martiny, J.B.H., et al. (2012). Fundamentals of microbial community resistance and resilience. Front. Microbiol. *3*, 1–19.

Thursby, E., and Juge, N. (2017). Introduction to the human gut microbiota. Biochem. J. *474*, 1823–1836.

Turnbaugh, P.J., Bäckhed, F., Fulton, L., and Gordon, J.I. (2008). Diet-Induced Obesity Is Linked to Marked but Reversible Alterations in the Mouse Distal Gut Microbiome. Cell Host Microbe *3*, 213–223.

Valeria De Las, C., Sam, M., Herbert L., D., and Zhi-Dong, J. (2020). In vitro Effects of Probiotics on Clostridium difficile Toxin Production and Sporulation. Int. Arch. Public Heal. Community Med. *4*, 1–6.