Fitness Effects of *Burkholderia* Symbiosis of *Serratia* Infection Within the Broad-headed Bug System

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**ABSTRACT**

Symbiotic interactions are common in many insects, including the infraorder heteroptera, the focus of this study. Such interactions result in a variety of effects for the host. Certain symbionts benefit their host by providing essential amino acids and others harm their host by utilizing host resources. Of particular interest are endosymbiotic relationships in which symbionts convey pathogenic resistance to their hosts. *Burkholderia* species, bacterial symbionts of broad-headed bugs, members of Heteroptera, are known to increase fitness of their hosts. *Serratia*, a pathogen to broad-headed bugs, disintegrates the tissues of their hosts. *Serratia* are found throughout the gut, allowing for direct interaction between the two. We believe that *Burkholderia* convey pathogenic resistance to their host. The fitness benefits of *Burkholderia* to hosts post *Serratia* infection will be tested. Eggs of four species of broad-headed bugs will be sterilized and grown in artificial environments. Some will be reared in the presence of *Burkholderia* while others will not. During the second instar of development, the broad-headed bugs will be exposed to RHOD, a strain of *Serratia*, from a RHOD food juice mixture of Carlson’s solution, RHOD, and food juice as the primary form of nourishment. After infection, micro dissections will be performed and the infection status of the individual will be assessed. We will test whether *Burkholderia* provides increased fitness to broad-headed bugs by comparing the survival rates, infection rates, concentration of pathogen, and relative location of pathogen to the symbiont in broad-headed bugs post infection with *Serratia*.

Symbiosis is an interaction between one organism and another that can have a variety of evolutionary and fitness effects on both the symbiont, the smaller organism, and the host, the larger organism (Wilkinson, Sherratt 2001). Symbionts can exhibit a wide variety of relationships, and provide both positive and negative effects on the host, such as producing an essential amino acid or stressing host nutrient supply, respectively. Endosymbionts are symbionts that have been taken up and reside inside other organisms. There are many examples of microorganisms illustrating endosymbiosis. This project seeks to study a relationship in which the symbiont conveys pathogenic resistance to a host. Another example of such a relationship is an endosymbiont of honeybees, lactic acid bacteria, which benefit bee health by defending against pathogens (Vasquez et al. 2012). Such symbioses can be facultative or obligate. The method by which endosymbionts enter their hosts also vary. Many endosymbionts are transferred maternally from one generation to the next in a vertical fashion (Mira, NA Morgan 2002). Vertical transfer is an integral method by which intimate symbiotic relationships can coevolve as it ensures their ability to interact in future generations (Ewald 1987). One process of vertical transmission can happen, for example, through transovarial transfer, and many symbionts are so specialized that they cannot survive outside of the host (Wilkinson, Sherratt TN 2001). Symbionts can also be transferred horizontally from the external environment, though in most of these cases the symbiotic life is facultative, and the symbiont lives outside of the host (Bright, Bulgheresi 2010). Symbioses are common throughout many organismal systems. The “true bugs”, Heteroptera, encompass more than 40,000 species and are generally characterized by a distinct X-shaped design on their backs (Froeschner 2012). Symbionts have been found in all Heteroptera investigated to date, and often live in the gut (Garcia, unpublished data). Broad-headed bugs are classified within the order Hemiptera, and the sub-order Heteroptera. Broad-headed bugs provide an excellent model to study the ability of horizontally acquired symbionts to convey pathogenic resistance to their host. Broad-headed bugs contain endosymbionts throughout their gut. In particular, they contain multiple strains of the genus *Burkholderia* in their midgut crypts (Kikuchi et al. 2005). The fitness of broad-headed bugs increases with the presence of bacteria in the genus *Burkholderia*. Individuals with *Burkholderia* symbiosis have larger abdomen widths, thorax widths, body lengths, and body weights than uninfected individuals (Kikuchi et al. 2007). The absence of gut symbionts in a species of stink bugs, of the same infraorder as and closely phylogenetically related to broad-headed bugs, has been shown to have a negative effect on the nymphal development, survival, and reproduction (Prado, Almeida 2009). Such symbioses illustrate the possible benefit of symbionts found in the gut and provide a basis for our interest in this model as a means to study symbionts’ ability to convey pathogen resistance.

Broad-headed bug species acquire *Burkholderia*, from the external environment (Kikuchi et al. 2007). *Burkholderia* is transferred into a broad-headed bug from the soil through mechanisms that are not understood during specific periods of development. One such broad-headed bug species in particular, *R. pedestris* contains...
a window in which the transfer of symbionts occurs in the second instar (Kikuchi et al. 2011). Burkholderia can live within the soil and have formed diverse symbiotic relationships with many host plants (Coenye, Vandamme 2003). It is possible that through interactions with the soil and plants, the broad-headed bugs ingest Burkholderia either through the consumption of soil, surrounding plants, or through the rubbing of their rostrum with their front legs.

The pathogen Serratia is a genus of the Enterobacteriaceae family. Serratia can act as a pathogen to many species, growing within the gut of many of its hosts. Pea Aphids inoculated with Serratia marcescens undergo bacterial liquefaaction as Serratia marcescens disintegrated aphid tissues internally following infection (Mackauer, Albright 1973). Serratia are known to infect broad-headed bugs and can result in death of the host. Serratia are characterized by the production of a red protein, which can be used as a preliminary method of testing for the presence of Serratia to be followed by PCR analysis. Serratia is present in the same type of soil in which broad-headed bugs and is reported to be 4% of the total bacteria in the soil (Ashelford et al. 2003). Species of Burkholderia can be found in soil and are present in environments in which broad-headed bugs live (White 2003; Kikuchi et al. 2007). It has been observed that Serratia infect the entire midgut, a portion of which Burkholderia live in (Kikuchi et al. 2005). This provides the possibility of a direct interaction in which Burkholderia could indeed inhibit Serratia growth.

Symbiont Control. Symbiont Control. Sterilization of Broad-headed Bug Eggs. Eggs from four species of broad-headed bugs will be collected from dirt in bug cages. Onto the eggs, 500 μL of 70% EtOH will be added. The eggs sat for two minutes while being agitated in EtOH solution. The 500 μL solution of 70% EtOH will be removed. Onto the eggs, 500 μL of 10% bleach will be added. The eggs will sit for two minutes while being agitated in 10% bleach. The 500 μL of 10% bleach will be removed. Onto the eggs, 500 μL of pure H2O will be added. The eggs will sit for 2 minutes in the H2O while being agitated. Eggs will be removed, dried and isolated, to be distributed into broad-headed bug cages.

**Serratia Infection.** A glycerol stock, RHOD.G.1, was derived from a RHOD stock passaged through aphids and was collected by GPF. Five microliters of RHOD.G.1 will be plated onto an LB plate. LB broth will become inoculated with a single colony from the plated glycerol stock. This broth will have been growing shaksh at 200 rpm for 1 hour at 30 °C. Five microliters of this broth will be plated onto LB and grown at 30 °C overnight. From a scrape of this plate, 15 mL of LB broth will be inoculated and grown for 2 hours at 30 °C and 200 rpm. This bacterial stock will be diluted with LB to OD600=0.100. To a 15 mL falcon tube, 5 μL of the OD600=0.100 RHOD, 3,330 μL of Carlson’s solution, and 6,665 μL of food juice will be added, to create an RHOD food juice mixture. This mixture of liquids will be lightly agitated so as to ensure proper mixing. This mixture will then be poured onto a cut dried sponge to be placed in a broad-headed bug cage. The broad-headed bugs will have their source of liquid nourishment removed 12 hours before these bugs will be exposed to the RHOD food juice mixture. As needed, more RHOD food juice mixture will be added to the sponge whenever the sponge appeared dried out. In general, these bacterial concentrations will be determined by calculating CFU per mL which where determined by plating 5 μL of bacteria containing solution onto a LB plate and counting the colonies and determining how many cells per mL were present in solution. The desired bacterial concentrations were 10^7 CFU per mL and while higher than normal environmental levels, previous studies of symbiont infection involved levels 100 fold of natural levels with success (Kikuchi et al. 2011).

**MATERIALS AND METHODS**

**Symbiont Control.** Sterilized eggs were placed into dishes within a broad-headed bug boxes. A sterilized sponge will be combined with 10 mL of food juice and placed into each broad-headed bug box. Food containing peanuts, black-eyed peas, and small grain are to be added to a dish within each broad-headed bug box. To one half of the boxes, autoclaved...
soil, lacking natural symbionts, collected from broad-headed bug environments was added to one dish within broad-headed boxes. To the other half of the boxes, soil, with natural symbionts, collected from broad-headed bug environments will be added to one dish within broad-headed boxes.

**Broad-headed Bug Infection.** During the 2\textsuperscript{nd} instar of broad-headed bug development, broad-headed bugs will be exposed to Serratia in the following manner: after 12 hours of a lack of food juice, 10 mL of RHOD food juice will be added to the sterilized sponge in the broad-headed bug cages.

**EXPECTED RESULTS**

Noting the previous experiments done in this field and the outcomes of similar experiments on similar insect models, we expect that Burkholderia symbionts of broad-headed bugs will provide resistance to pathogenic Serratia. We expect this resistance to manifest itself in the form of increased survival rates, decreased infection rates, and a lower rate or speed of Serratia infection.

**NEXT STEPS FOR EXPERIMENTATION**

Four species of broad-headed bugs will be collected from a field in Stone Mountain State Park. According the methods outlined in Sterilization of Broad-headed Bug Eggs, eggs from the collected broad-headed bugs will be collected and sterilized. Broad-headed bugs will be reared with Burkholderia and infected with Serratia and the survival and infection rates will be compared to broad-headed bugs lacking Burkholderia that were also infected with Serratia. Such comparisons will be made by performing micro dissections on individual broad-headed bugs. Further experiments will depend on the preliminary results and infection rates.

**References**


Gregory Fricker is a sophomore in Emory College of Arts and Sciences from Hahira, Ga. He is currently pursuing a B.S. in Biology and plans on attending medical school after college. He is interested in how horizontally acquired symbioses can affect symbiont-host interactions and influence fitness. When he is not in the lab or the library, he is out taking pictures, enjoying the outdoors, hunting, or in a Bible study.