EFFECT OF QUORUM SENSING BY *STAPHYLOCOCCUS EPIDERMIDIS* ON THE ATTRACTION RESPONSE BY FEMALE ADULT YELLOW FEVER MOSQUITO, *AEDES AEGYPTI AEGYPTI* (LINNAEUS) (DIPTERA: CULICIDAE) TO A BLOOD-FEEDING SOURCE

A Thesis

by

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ABSTRACT

Aedes aegypti, the principle vector of yellow fever, is responsible for more than 30,000 deaths annually. Compounds like carbon dioxide, amino acids, fatty acids and other volatile organic compounds (VOCs) have been widely studied for their role in attracting *Aedes aegypti*. Many VOCs from humans are produced by skin microbiota, which consists of over 1000 species. *Staphyloccocus epidermidis*, although not the most abundant bacteria according to 16S ribosomal RNA research, commonly occurs on human skin. Quorum sensing (QS) by bacteria serves as a source for VOCs. This study determined if QS by bacteria serves as a mechanism regulating *A. aegypti* attraction to hosts.

Four pairwise tests examine the response of female *A. aegypti* to tryptic soy TSB (media for the bacteria) and *S. epidermidis* wildtype. And three replicates tests differential response between wildtype vs. *agr*- strains and *agr*- vs. TSB (the *agr* gene can express an accessory gene regulator for quorum sensing). Differential attractiveness by the wildtype and *agr*- was determined. The blood-feeder treated with wildtype *S. epidermidis* attracted about two times more *A. aegypti* than the *agr*- *S. epidermidis* ($P \leq 0.001$). Also, wildtype strain was more attractive by 20% to *A. aegypti* than the TSB. Replicate effects were detected demonstrating that some replicates were significantly different from others in the same experiment, resulting in heterogeneity among replicates. Future work should focus on reducing this variability in the assay and determining which

genes are responsible for the VOCs that interfere with the ability of *A. aegypti* to locate hosts, possibly by manipulating bacterial quorum sensing systems.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Yellow fever is an acute viral disease, principally affecting primates and is prevalent throughout the tropical and subtropical areas of the world. The devastating epidemics of hemorrhagic disease caused by the yellow fever virus, which can be potentially fatal, are indiscriminate of age, gender, race, or wealth (Gardner and Ryman 2010). It is now believed that yellow fever was originally imported into the Americas and Europe from Africa (Gardner and Ryman 2010). During the time when doctors were unable to clarify the cause or to treat the yellow fever, hundreds of thousands people were ill or fatally infected in America (Patterson 1992). The disease was widespread not only among civilian populations, but also among the troops during the Spanish-American War, and ultimately caused more soldiers' death than the war itself. It had been widely accepted that yellow fever was a result of miasmas before Dr. Walter Reed first confirmed Carlos Finlay's theory that the disease was transmitted to human by the mosquito. *A. aegypti* (Linnaeus) (Diptera: Culicidae) is the principle and very efficient vector responsible for the spread of yellow fever virus and has a close association with humans (Gubler 2004). In the modern world, this disease is estimated to affect over 200,000 people per year in tropical and subtropical regions with at least 30,000 fatalities (Monath 2001). Forty-four countries in Africa and South and Central

America are within the modern yellow fever endemic zone, with almost 900 million people at risk of infection. This total includes an estimated 508 million people in 32 African countries, and the remainder split between South and Central American countries (Monath 2001). Control of yellow fever mainly depends on vaccination and immunization. Despite massive vaccination programs to help controlled outbreaks, the risk of yellow fever epidemics has greatly increased in poor and dense human population recently (Gardner and Ryman 2010). The reinvasion of the urban environment by *A. aegypti*, makes control of this dreaded disease, along with control of *A. aegypti* in the United States, more urgent today than in past decade.

The mosquito originated in Africa and was imported to tropical and subtropical areas all around the world. They generally live two to four weeks and require aquatic conditions, but the eggs are resistant to desiccation when it is dry, allowing them to emerge later when conditions are favorable. The infected mosquitos transmit the yellow fever virus during piercing of the human skin to obtain a blood meal (Gardner and Ryman 2010). Female adults are typically nocturnal, though they can bite and spread infection at any time, locating their human hosts by tracking heat, carbon dioxide (CO_2), as well as volatile organic compounds (VOCs) emitted by their hosts (Gibson and Torr 1999).

A number of VOCs produced by humans have been examined to determine their role in mediating host-seeking behavior by mosquitoes. Compounds emanated by human skin and known to serve as mosquito attractants include members of diverse chemical groups: estrogens, amino acids, fatty acids, aldehydes, carboxylic acids, alcohols,

aliphatics/aromatics, amides, amines, esters, halides, heterocyclics, ketones, sulfides, and thioesters (Takken 1991, Geier, Bosch et al. 1999, Bernier, Kline et al. 2000). Other cues including L-lactic acid, 1-octen-3-ol, acetone, and ammonia play an important role in host-seeking behavior over longer distances (Takken 1991). The skin microbiota plays an important role in producing VOCs associated with the human body (Verhulst, Takken et al. 2010). Sweat-associated human volatiles are probably the primary determinant factor in the host preference of anthropophilic mosquitoes (Smallegange, Verhulst et al. 2011).

It was demonstrated that microorganisms on human skin are responsible for the conversion of fresh sweat, collected right after exercise, into sweat attractive to *Anopheles gambiae* (Giles) (Diptera: Culicidae) (Braks and Takken 1999). Previous work has shown that fresh sweat is odorless and results in limited mosquito attraction unless it is incubated with bacteria (Braks and Takken 1999). Sterile blood agar plates incubated with skin bacteria are also more attractive to the mosquito than these plates with no bacteria. (Verhulst, Beijleveld et al. 2009).

Besides products of bacteria metabolism, VOCs produced by bacteria serve a function within the bacterial community. The cell-cell communication system between bacteria is called quorum sensing (QS) (Waters and Bassler 2005). QS has been recognized as important regulator of virulence and biofilm formation in response to fluctuations in cell-population density in many bacteria, thus producing variety kinds of VOCs. As a predominant bacterium associated with human skin (Schleifer and Kloos 1975), *S. epidermidis* was demonstrated contributing to the formation of volatile fatty

acids (James, Hyliands et al. 2004), containing a VOC has distinct sweaty odor and attractive to mosquitoes (Bernier, Kline et al. 2000). Currently, two QS systems of Gram-positive *Staphylococci* have been studied: accessory gene regulator (*agr*) and *lux*S systems (Kong, Vuong et al. 2006), which can regulate a diverse array of physiological activities, including symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation (Waters and Bassler 2005). In QS, varieties of compounds are generated and released by the cells, many of which are known to volatilize. Interspecies interactions regulated by QS compounds produced by microbes have been determined for a number of systems. Such systems exist in both interspecies communication and interkingdom communication. They have been proposed in a wide variety of bacteria and also extend to relationships between bacteria and eukaryotes and host–pathogen interactions in both clinical and agricultural settings are of particular interest (Lowery, Dickerson et al. 2008). Similar research have been performed with blow flies (*Lucilia sericata*) and mutated *Proteus mirabilis*, which is unable to swarm-a flagella-driven movement of differentiated hyperflagellated, elongated and multinucleated swarmer cells by which bacteria spread as a biofilm over a surface. The flies showed a significant preference to wildtype *P. mirabilis*, which are able to swarm (Ma, Fonseca et al. 2012). The *agr* operon, which is important for encoding *S. epidermidis* QS systems, was deleted to construct a mutant *S. epidermidis* strain (Tü3298) that perform quorum sensing defectively due to missing the *agr* pathway (Vuong, Götz et al. 2000). Despite the recent research on how bacterial quorum sensing relates to other insects, such as flies, how QS volatile compounds produced by *S.*

epidermidis influences the attraction response of the mosquito vectoring yellow fever remains unknown. In this project, the *agr* deletion mutant will be used to test if the deletion of one of the QS operons of *S. epidermidis* contributes to attracting *A. aegypti*.

Objectives and Hypotheses

Objective:

Detect the impact of *agr* system of quorum sensing of *Staphylococcus epidermidis* on adult female *Aedes aegypti* attraction behavior.

Hypothesis:

H_0: Wildtype and *agr*- strains of *Staphylococcus epidermidis* in association with a blood meal do not differentially attract female adult *Aedes. aegypti*.

H_a: Wildtype and *agr*- stains of *Staphylococcus epidermidis* in association with a blood meal can differentially attract female adult *Aedes. aegypti*.

CHAPTER II

RESEARCH, CONCLUSION, AND DISCUSSION

Introduction

Mosquitoes are serious vectors responsible for the transmission of viruses, bacteria or other organisms, which cause fatal disease such as malaria, dengue or yellow fever (Eldridge and Edman 2000). Among all vectors that cause vector-borne disease, mosquitoes are widely regarded as the most dangerous one to human in terms of spread, mortality, incapacitation, and economic losses. More than three billion people are still under the threat caused by mosquitoes (Becker, Petrić et al. 2010). Pesticides and management of the environment are still primarily used to eliminate mosquitoes regardless of environment-unfriendly effects (Eldridge and Edman 2003). Not only genetic manipulation using molecular biology techniques, but also development of new efficient repellents have been increasingly important for controlling mosquito-borne diseases.

Besides heat and carbon dioxide, mosquitoes also locate their hosts through olfaction systems sensing chemical cues emanated by human (Takken and Knols 1999). As Shelley pointed decades ago, human sweat is odorless unless incubated with bacteria (Shelley, Hurley Jr et al. 1953), especially bacteria on human skin playing an significant

role in the interactions between mosquitoes and their hosts by producing odors (Verhulst, Takken et al. 2010). It is believed that many of volatiles emitted by the human body to which mosquitoes respond are originally produced by bacteria (De Jong and Knols 1995). Many chemical compounds have been demonstrated to be able to attract mosquitoes, such as estrogens, amino acids, fatty acids, aldehydes, carboxylic acids, alcohols, aliphatics/aromatics, amides, amines, esters, halides, heterocyclics, ketones, sulfides, and thioesters (Takken 1991, Geier, Bosch et al. 1999, Bernier, Kline et al. 2000). Other cues include L-lactic acid, 1-octen-3-ol, acetone, and ammonia play an important role in host-seeking behavior over longer distances (Takken 1991).

S. epidermidis from human skin can attract mosquitoes (Verhulst, Beijleveld et al. 2009). *Anopheles gambiae*, a species that causes malaria and dengue fever, were attracted more by blood agar plates incubated with *S. epidermidis* than by sterile blood agar plates (Verhulst, Beijleveld et al. 2009). Volatile organic compounds produced by bacteria can be produced either by metabolism or by QS, a cell-cell communication system in bacteria (Waters and Bassler 2005). As a predominant bacterium associated with human skin (Schleifer and Kloos 1975), *S. epidermidis* contributed to the formation of volatile fatty acids (James, Hyliands et al. 2004), a VOC has distinct sweaty odor and attractive to mosquitoes (Bernier, Kline et al. 2000).

Currently, two QS systems of Gram-positive Staphylococci have been studied: accessory gene regulator (*agr*) and *lux*S systems (Kong, Vuong et al. 2006), which can regulate a diverse array of physiological activities, including symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm

formation (Waters and Bassler 2005). In quorum sensing, varieties of compounds are generated and released by the cells, many of which are known to volatilize. Interspecies interactions regulated by QS compounds produced by microbes have been determined for a number of systems (Lowery, Dickerson et al. 2008). Such systems exist in both interspecies communication and interkingdom communication. They have been proposed in a wide variety of bacteria and also extend to relationships between bacteria and eukaryotes and host–pathogen interactions in both clinical and agricultural settings are of particular interest (Lowery, Dickerson et al. 2008). Similar test has been previously performed on flies, and it has been demonstrated that a mutant *rfal* strain of *Proteus mirabilis*, which is unable to swarm normally, is less attractive to blow flies, *Lucilia sericata* (Diptera: Clliphoridae). (Ma, Fonseca et al. 2012). Also, compared to the wildtype *Proteus mirabilis*, the *rfal* strain can reduce the number of eggs laid by the flies. The *agr* operon, which encodes a *S. epidermidis* QS system, was deleted to construct the mutant *S. epidermidis* strain (Tü3298) that can not regularly perform quorum sensing (Vuong, Götz et al. 2000). The goal of my research was to determine if the *agr* deletion mutant will be used to test if the deletion of one of the QS operons of *S. epidermidis* contributes to attracting *A. aegypti*.

Materials and Methods

Mosquito Colony

A. aegypti eggs were hatched in a container with 1 L distilled water at 25°C, 70%

RH and 12:12 L:D photoperiod. Larvae at approximately 2-d-old were separated into containers at a density of 100-200 larvae/L, which is a proper condition for larval growth (Clements 1995). Larvae were fed on a diet of 3 g fish food (TetraMin diet by Tetra) every other day. After pupation, the mosquitoes were transferred into small plastic cups (60 ml) with distilled water at a density of 50 mosquitoes/cup. Plastic cups containing pupae were placed inside a metal cage with cotton sleeves on one side. Adults were collected every day after emergence by using an aspirator, separated according to gender. Fifty Females were placed in one paper cup with mesh top and were provided a 5% sucrose solution with damp cotton wool ad libitum before use. Adult mosquito colonies were maintained under conditions of 25°C, 80% RH and 12:12 L:D photoperiod. Females, starved for 24 h, at 5-8-d-old (Dekker, Takken et al. 2001) were used in the attraction assay.

Bacteria Culture

The *agr* deletion mutant *S. epidermidis* strain (TüF38) was used in this experiment due to its mutation of the QS agr gene. Both the mutant (agr^-) and wildtype *S. epidermidis* strains were grown in mannitol salt agar (MSA), because MSA contains a high concentration of NaCl (approximately 7.5%-10%), it is inhibitory to most other bacteria, but selective for gram-positive bacterium *Staphylococci* (Mannitol salt agar 7143, Neogen Corp. 2008). The bacteria were diluted to a concentration of 10^7 cfu in TSB, for use in the experiments.

Blood-Feeder Design

Blood feeders used in the experiments were made by 25 cm^2 cell culture flask (Corning Incorporated NY) (Fig. 1). Parafilm were used to cover the surface of the blood feeder (Fig. 2), so that there was a gap space between the parafilm and the surface of blood feeder. A piece of 100% cotton cloth was cut into 4.5 cm x 9.5 cm pieces and autoclaved. Then the sterile cotton cloth was soaked with 500 µl containing 10^7 cfu of *S. epidermidis* wildtype or mutant TSB solutions that are in exponential growth phase and covered outside the parafilm with rubber band.

Experiment Design

A clear Plexiglas cage (Fig. 1) containing 50 5-8-d old female mosquitoes that had not received a previous blood meal was put in the incubator room under conditions previously described. Mosquitoes were placed in the cage 15 min prior to each replicate to allow acclimation to the environment. In each replicate, two blood-feeders were placed parallel to one another on opposite ends of the top of the cage (Fig. 2). The two blood-feeders were connected by tubes to a water bath at 37°C. Each blood-feeder was either covered with cloth (Kaufman) soaked with 0.5 ml of 10^7 colony forming units (cfu) *S. epidermidis* wildtype solution, mutant (*S. epidermidis* TüF38) solution or 0.5 ml TSB solution respectively. An aliquot of 1 ml of rabbit blood (HemoStat Laboratories) was added in the gap space between the blood feeder and the parafilm. Initial experiments indicated there was no bias towards either side of the cage (see below); however,

treatments were still rotated after each replicate. Furthermore, the cage and feeders were cleaned using vacuum and ethanol between replicates.

Figure 1. The olfaction device used to conduct behavioral assays with *A. aegypti*.

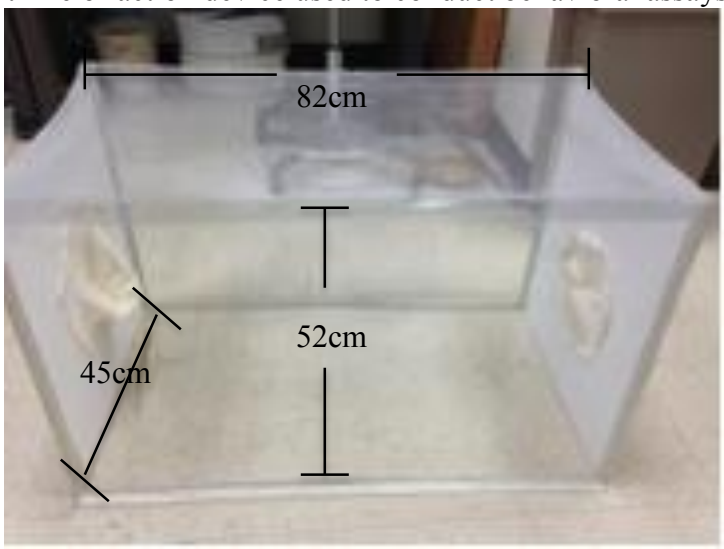
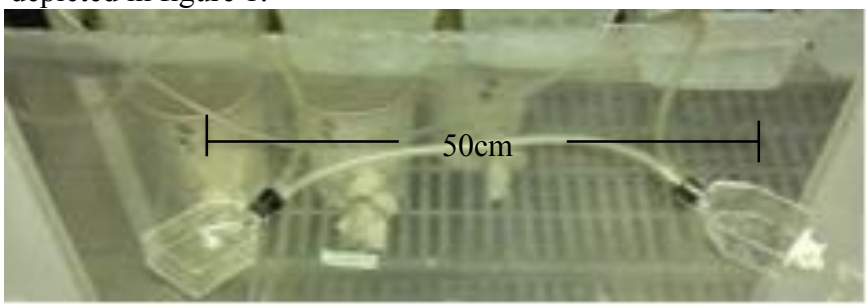


Figure. 2 The blood-feeders used to conduct the behavioral assays with *A. aegypti* in the device depicted in figure 1.



The experiments were performed 3-4 h before dark till dark of the chamber (12:12 L:D), based on the fact that *A. aegypti* females are actively seeking hosts several

hours before sunset (Yasuno and Tonn 1970). A camcorder (SONY HDR) was placed outside the cage and aimed to shoot up towards the underside of the feeder. The mosquitoes were recorded for 15 min after introduction of the feeder for every replicate. The number of mosquitoes feeding on either treatment was counted at each minute over the 15 min recording. Response data indicate the mosquito response by minute was consistent over the course of the experiment. Therefore, total mosquitoes present on each feeder were determined by summing the total number of mosquitoes present at each one-minute observation period. Four replicates of two experiments (TSB vs TSB; mutant vs TSB) and three replicates of other two experiments (wildtype vs TSB; wildtype vs mutant) were conducted with each replicate representing a different mosquito generation. Experiments were conducted as outlined in Table 1.

Table 1. Treatments in experiments examining female adult *A. aegypti* responses to blood-feeders with different microbial treatments in 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min replicate in a laboratory at approximately 25°C and 80% RH.

Name	Treatment 1	Treatment 2	Purpose
TSB vs TSB	TSB	TSB	Optimize the conditions
TSB vs Wildtype	TSB	wildtype *S. epidermidis*	If wildtype attracts *A. aegypti*
TSB vs Mutant	TSB	*agr-* *S. epidermidis*	If mutant repels *A. aegypti*
Wildtype vs Mutant	wildtype *S. epidermidis*	*agr-* *S. epidermidis*	Differential attractiveness of *A. aegypti* to wildtype and mutant

Data Analyses

In order to determine if mosquito response was significantly different between treatments and replicates, the data were analyzed using the Cochran-Mantel-Haenszel test ($P \leq 0.05$) using Excel (McDonald, J.H. 2009).

Results and Conclusion

Mosquito Response to Blood-Feeders Treated with TSB

Four replicates of this experiment were conducted. Average number of adult female mosquitoes to respond to the blood-feeders treated with TSB per minute for 15 min is shown in Figure 3. The blood-feeder located on the top left and right sides of the cage attracted similar (around 50%) mean percent of mosquitoes per minute (Figure 4). Average percent response of adult female mosquitoes to the blood-feeders treated with TSB for each replicate is presented in Figure 5. Statistical analysis indicates a significant difference between replicates ($G_H = 9.479$, df = 3, $P = 0.023$) (Table. 2). No replicate resulted in a significant difference in attraction of *A. aegypti* between blood-feeders on the left or right side of the cage indicating no bias ($G_p = 0.011$, $P = 0.9165$). However, treatments in the following experiments were still rotated across replicates to avoid potential bias.

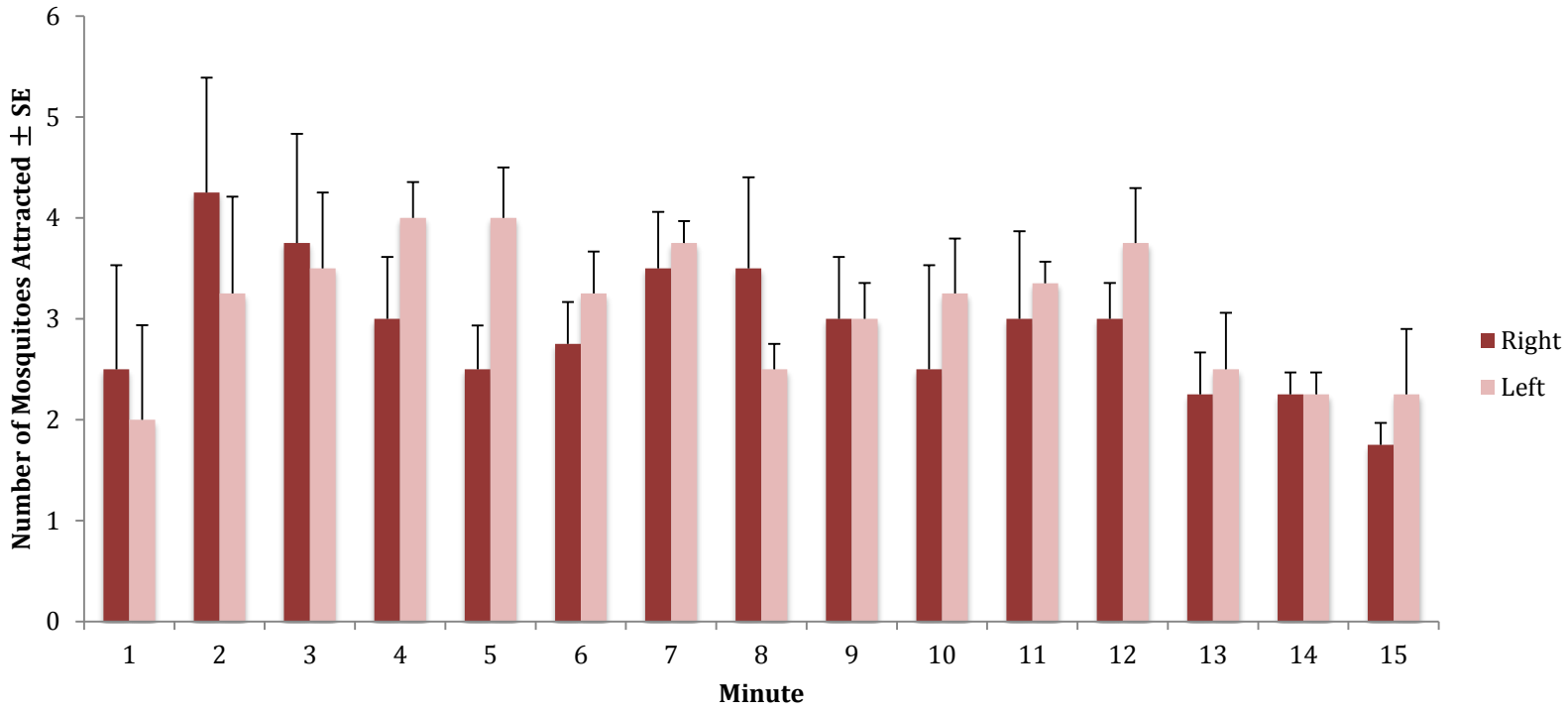


Figure 3. Mean number of adult female *A. aegypti* mosquito attraction per minute ± SE to blood-feeders treated with TSB and located on the right and left sides of the top of a 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min replicate (n = 4) at approximately 25°C and 80% RH.

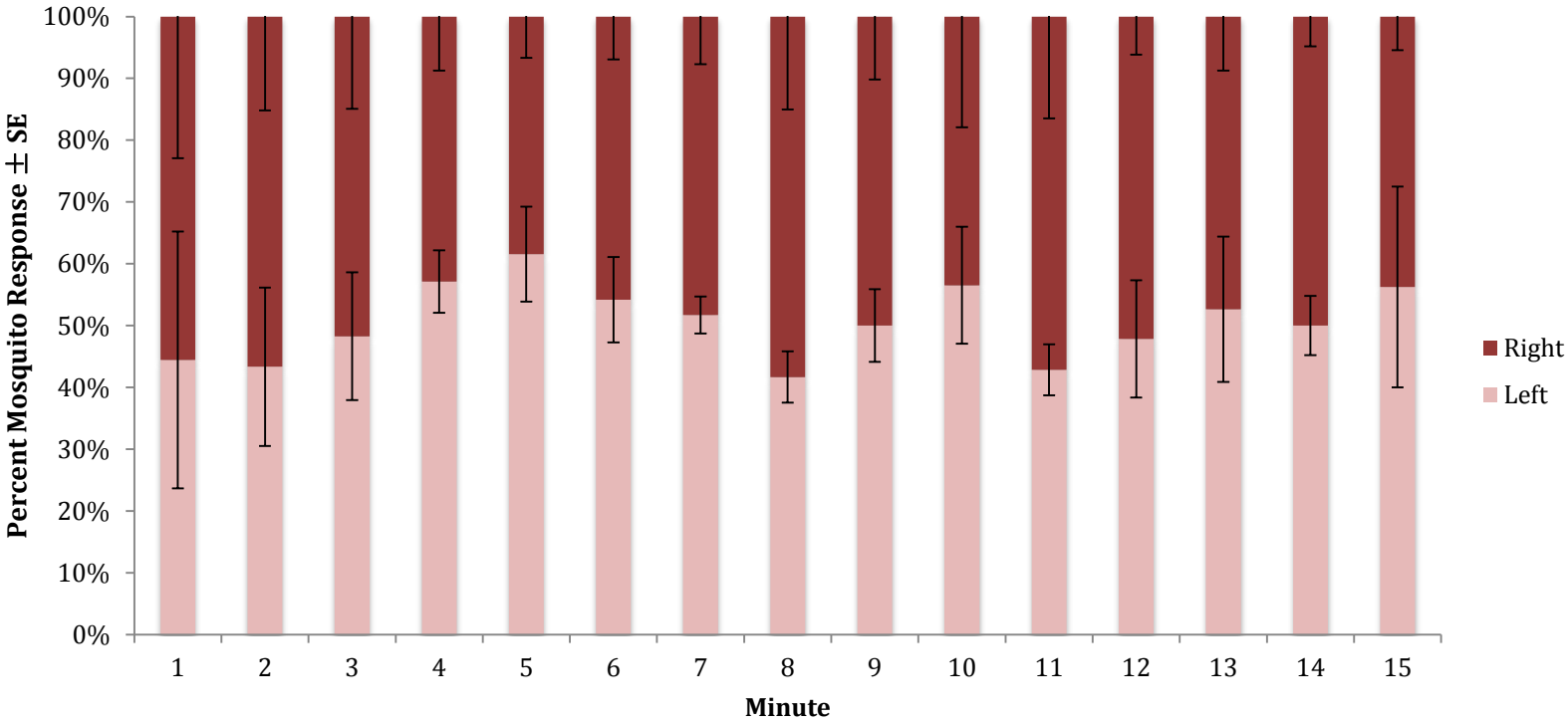


Figure 4. Mean percent of adult female *A. aegypti* mosquito attraction ± SE to blood-feeders treated with TSB and located on the right and left sides of the top of a 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min replicates (n = 4) at approximately 25°C and 80% RH.

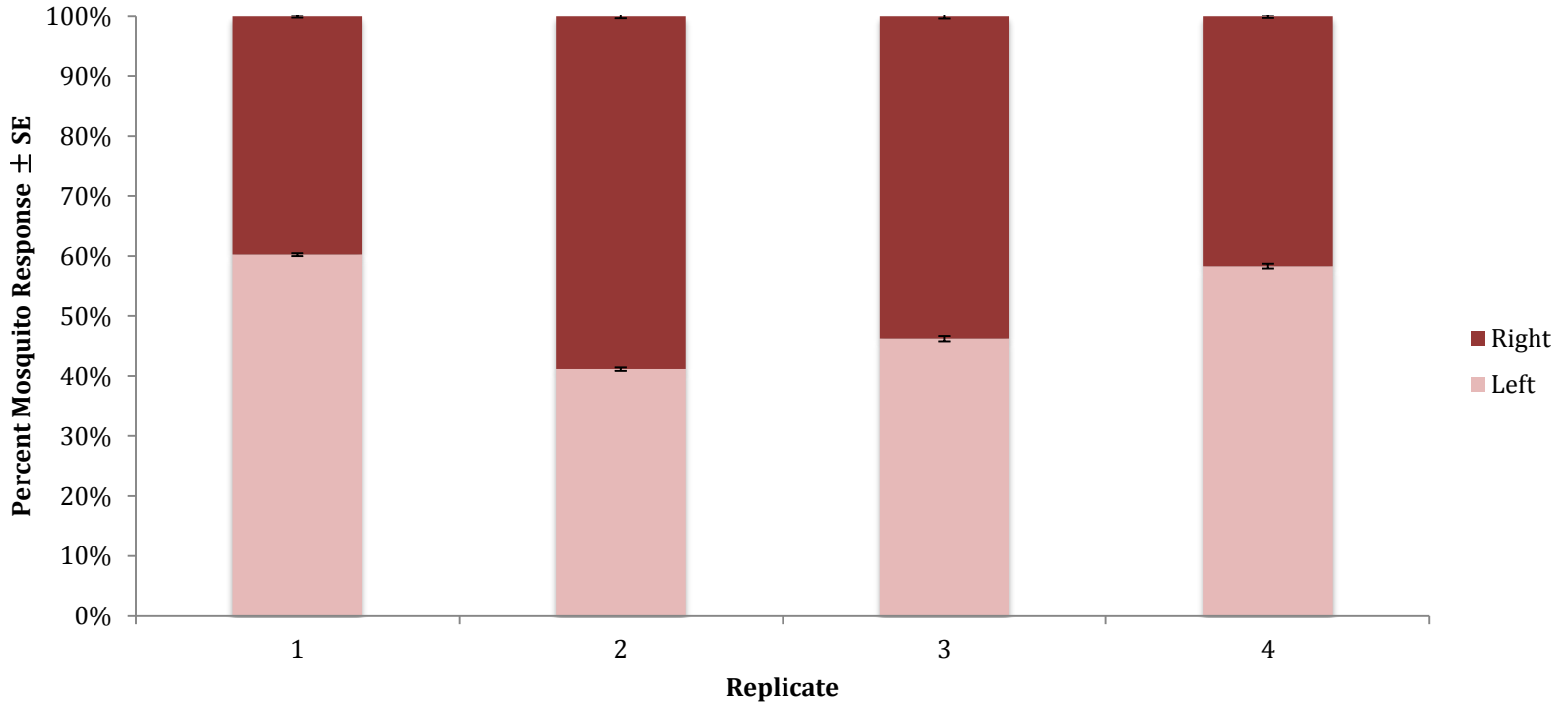


Figure 5. Mean percent attraction ± SE of adult female *A. aegypti* mosquitoes to blood-feeders treated with TSB and located on the right and left sides of a 82 cm x 45 cm x 52 cm Plexiglas cage of each replicate during 15-min replicates at approximately 25°C and 80% RH.

Table 2. Cochran-Mantel-Haenszel test of the feeding response of 5-8-d old *A. aegypti* (replicates, N=4; n =50) adult female attraction to blood-feeders located on opposite sides of the top of a 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min experiments under 25°C and 80% RH and treated with TSB.

	G value	P value
Replicate1	3.708	0.054
Replicate2	3.603	0.057
Replicate3	0.515	0.472
Replicate4	1.664	0.197
Total G	9.49	0.049
Pooled G	0.011	0.916
Heterogeneity G	9.479	0.023

Mosquito Response to Blood-Feeders Treated with Wildtype S. epidermidis and TSB

Three replicates of this experiment were conducted. Average number of adult female mosquitoes to respond to the blood-feeders treated with TSB and wildtype strain *S. epidermidis* per minute for 15 min is shown in Figure 6. The blood-feeder treated with wildtype *S. epidermidis* attracted more than 20% of the mosquitoes than the TSB treated mosquito-feeder (P = 0.025) (Figure 7). Statistical analysis indicates significant difference between replicates (G_H = 9.033, df = 2, P = 0.011) (Table. 3).

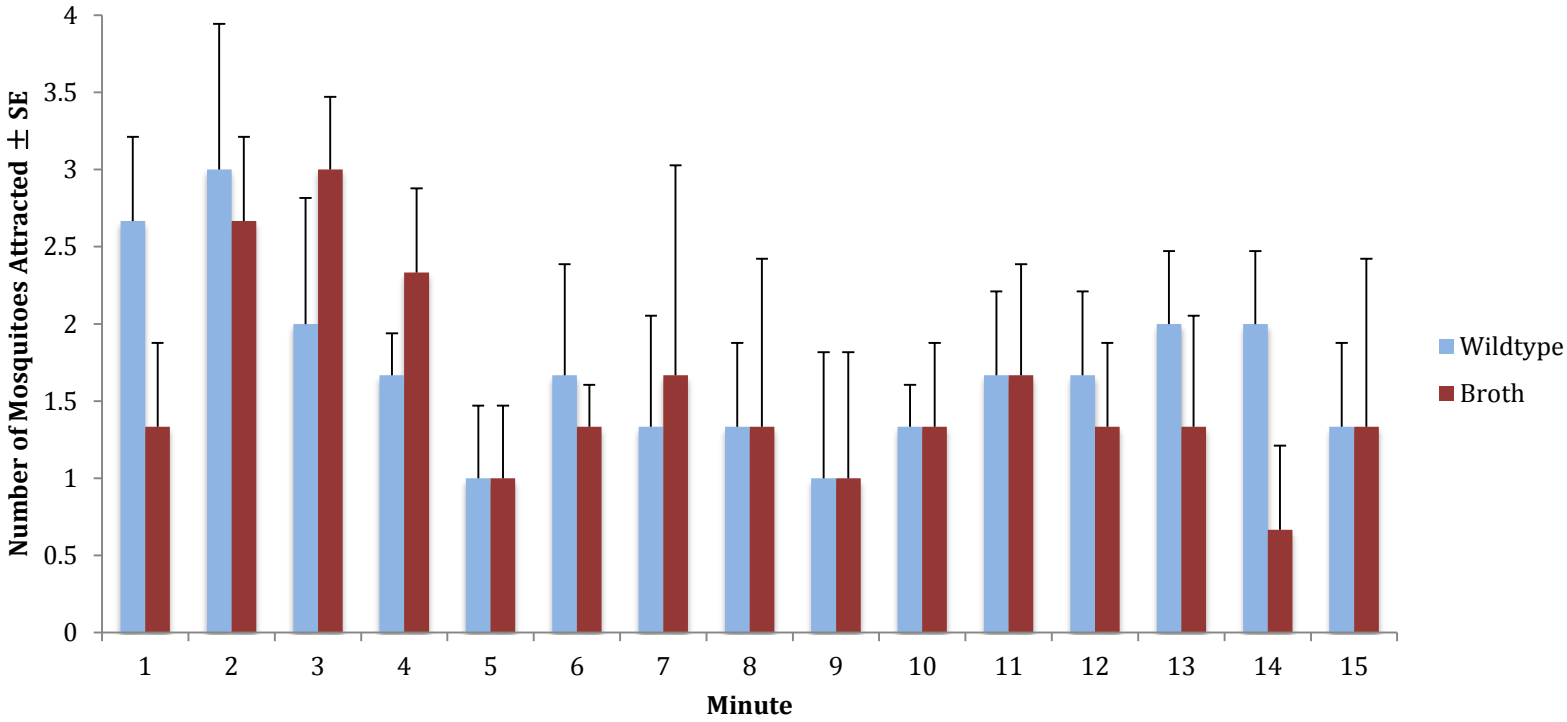


Figure 6. Mean number of adult female *A. aegypti* mosquito attraction per minute ± SE to blood-feeders treated with TSB and wildtype strain of *S. epidermidis* and located on the right and left sides of the top of a 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min replicate (n = 3) at approximately 25°C and 80% RH.

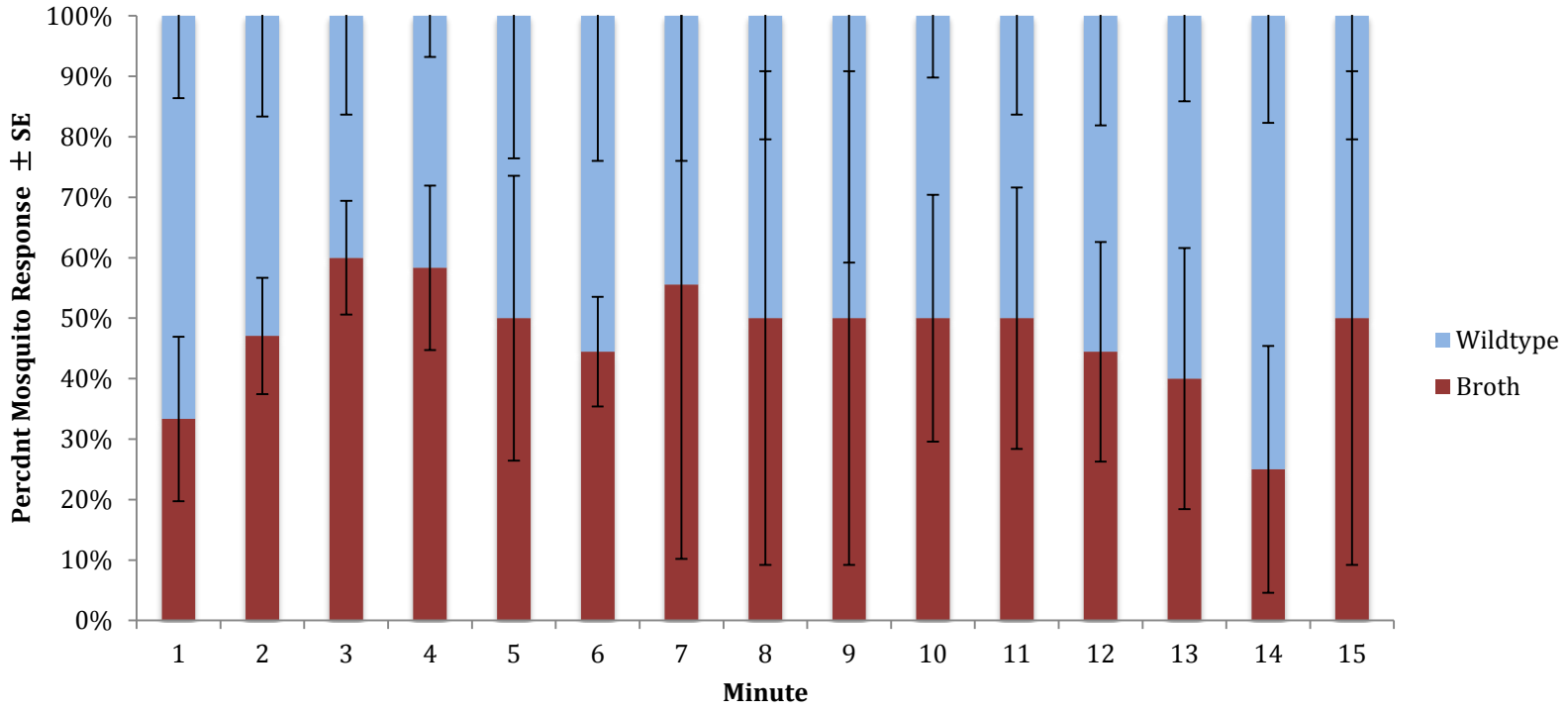


Figure 7. Mean percent of adult female *A. aegypti* mosquito attraction ± SE to blood-feeders treated with TSB and wildtype strain of *S. epidermidis* and located on the right and left sides of the top of a 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min replicates (n = 3) at approximately 25°C and 80% RH.

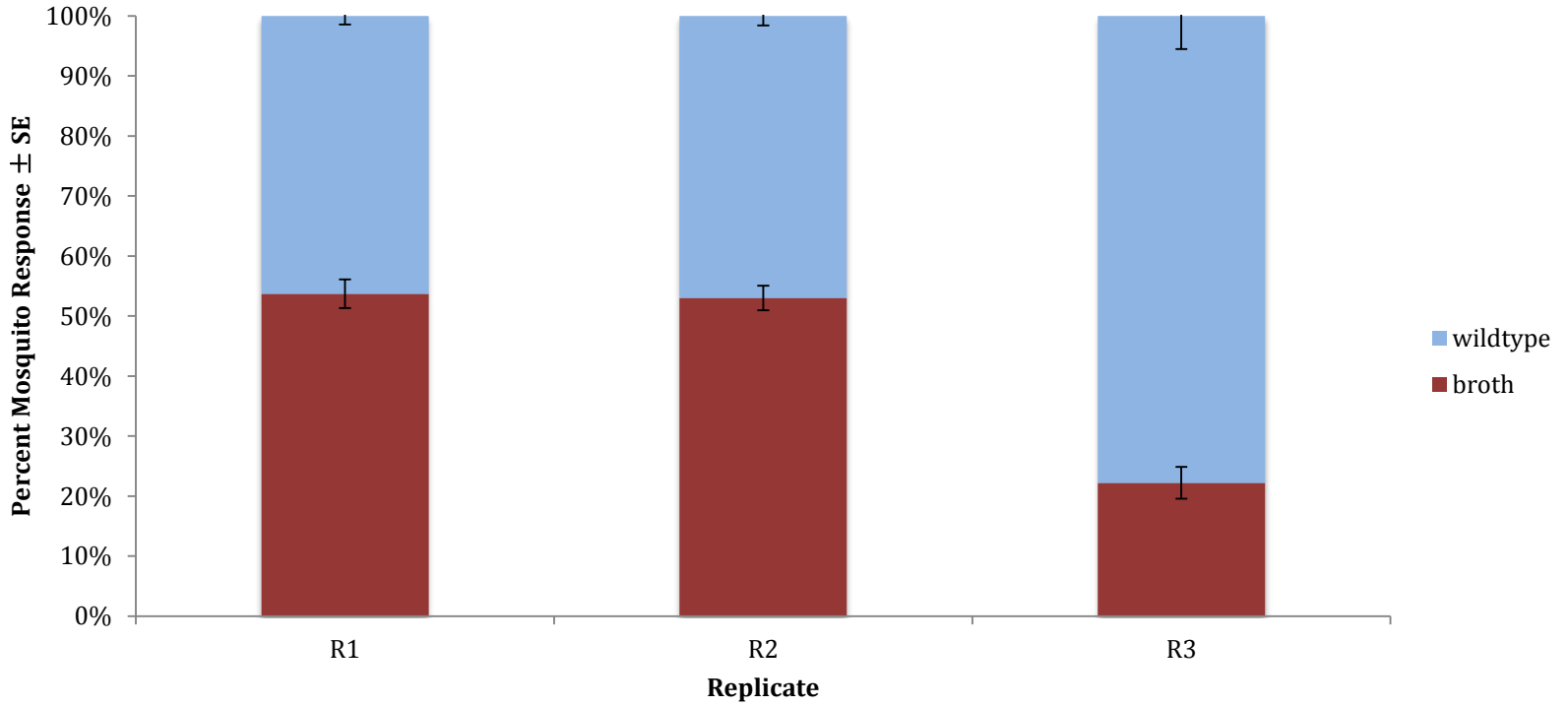


Figure 8. Mean percent attraction ± SE of adult female *A. aegypti* mosquitoes to blood-feeders treated with TSB and wildtype *S. epidermidis* located on the right and left sides of a 82 cm x 45 cm x 52 cm Plexiglas cage of each replicate during 15-min replicates at approximately 25°C and 80% RH.

Table. 3 Cochran-Mantel-Haenszel test of the response of 5-8-d old *A. aegypti* (replicates, N=3; n =50) adult female attraction to blood-feeders located on opposite sides of the top of a 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min experiments under 25°C and 80% RH and treated with wildtype *S. epidermidis* and TSB.

	G value	P value
Replicate1	0.297	0.586
Replicate2	0.243	0.622
Replicate3	8.826	0.003
Total G	9.366	0.025
Pooled G	0.333	0.564
Heterogeneity G	9.033	0.011

Mosquito Response to Blood-Feeders Treated with agr- S. epidermidis and TSB

Four replicates of this experiment were conducted. Average number of adult female mosquitoes to respond to the blood-feeders treated with TSB and *agr*- strain *S. epidermidis* per minute for 15 min is shown in Figure 8. The blood-feeder treated with *agr*- mutant *S. epidermidis* did not attracted significantly more mosquitoes than the TSB treated mosquito-feeder (P = 0.076) (Figure 9). Statistical analysis not indicates significant difference between replicates (G_H = 6.122, df = 3, P = 0.106) (Table. 4).

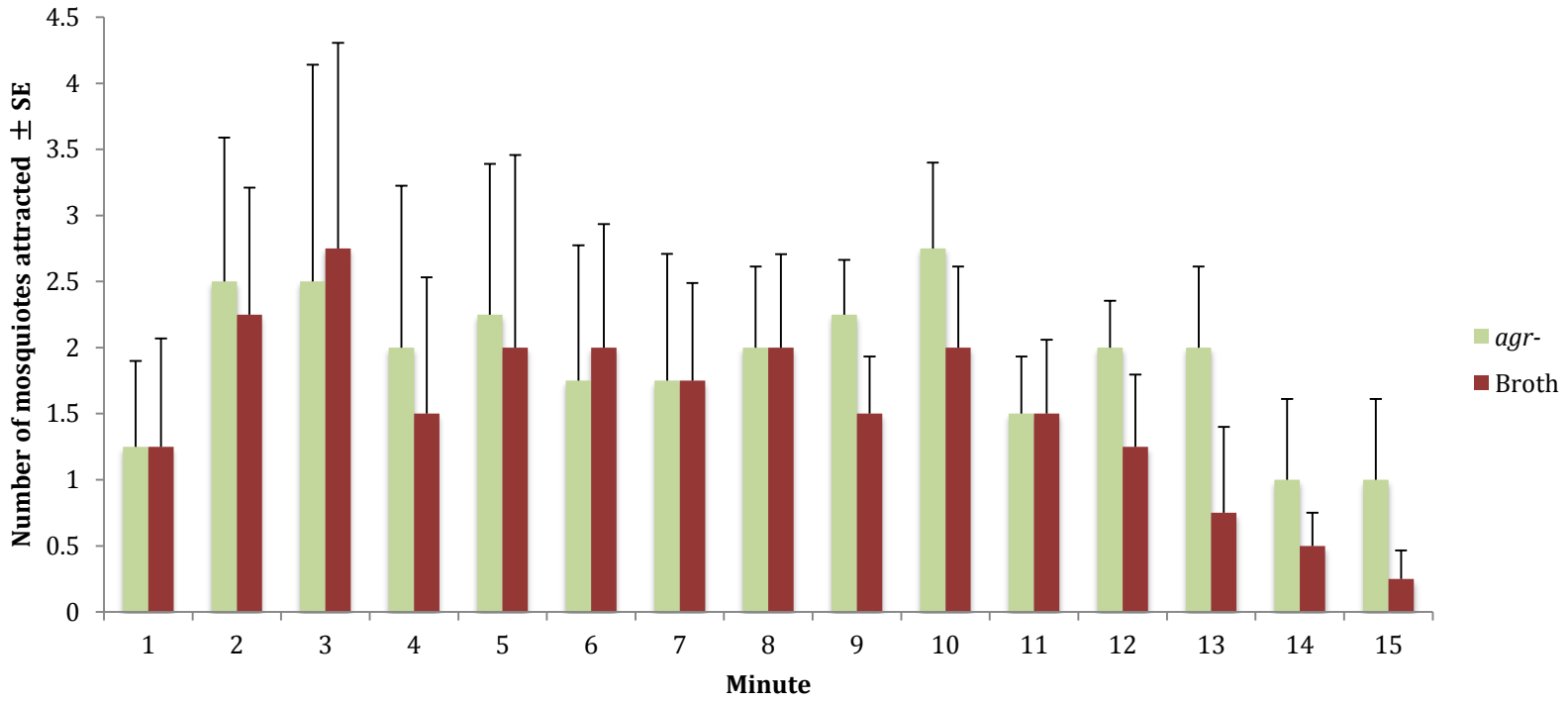


Figure 9. Mean number of adult female *A. aegypti* mosquito attraction per minute ± SE to blood-feeders treated with TSB and *agr*- strain of *S. epidermidis* and located on the right and left sides of the top of a 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min replicate (n = 4) at approximately 25°C and 80% RH.

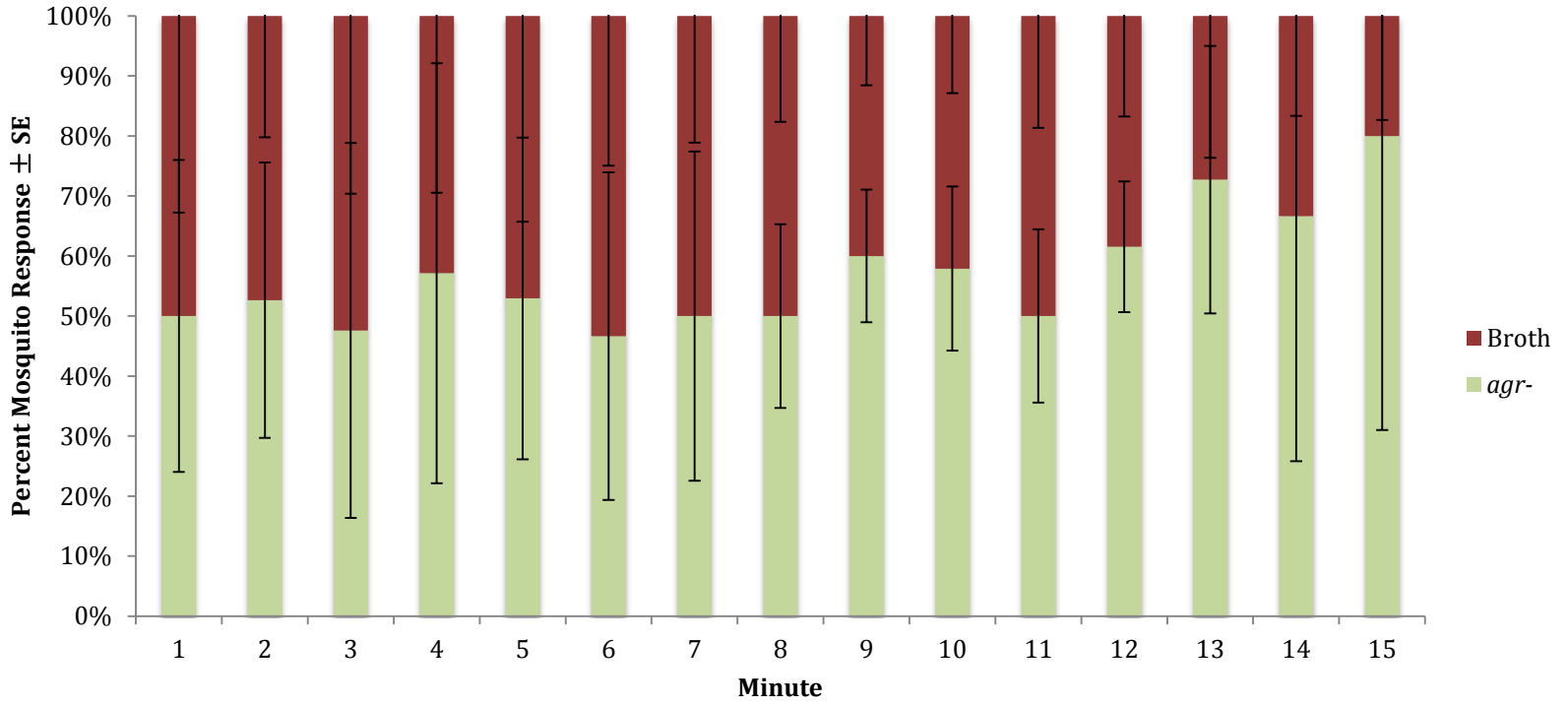


Figure 10. Mean percent of adult female *A. aegypti* mosquito attraction ± SE to blood-feeders treated with TSB and *agr*- stain of *S. epidermidis* located on the right and left sides of the top of a 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min replicates (n = 4) at approximately 25°C and 80% RH.

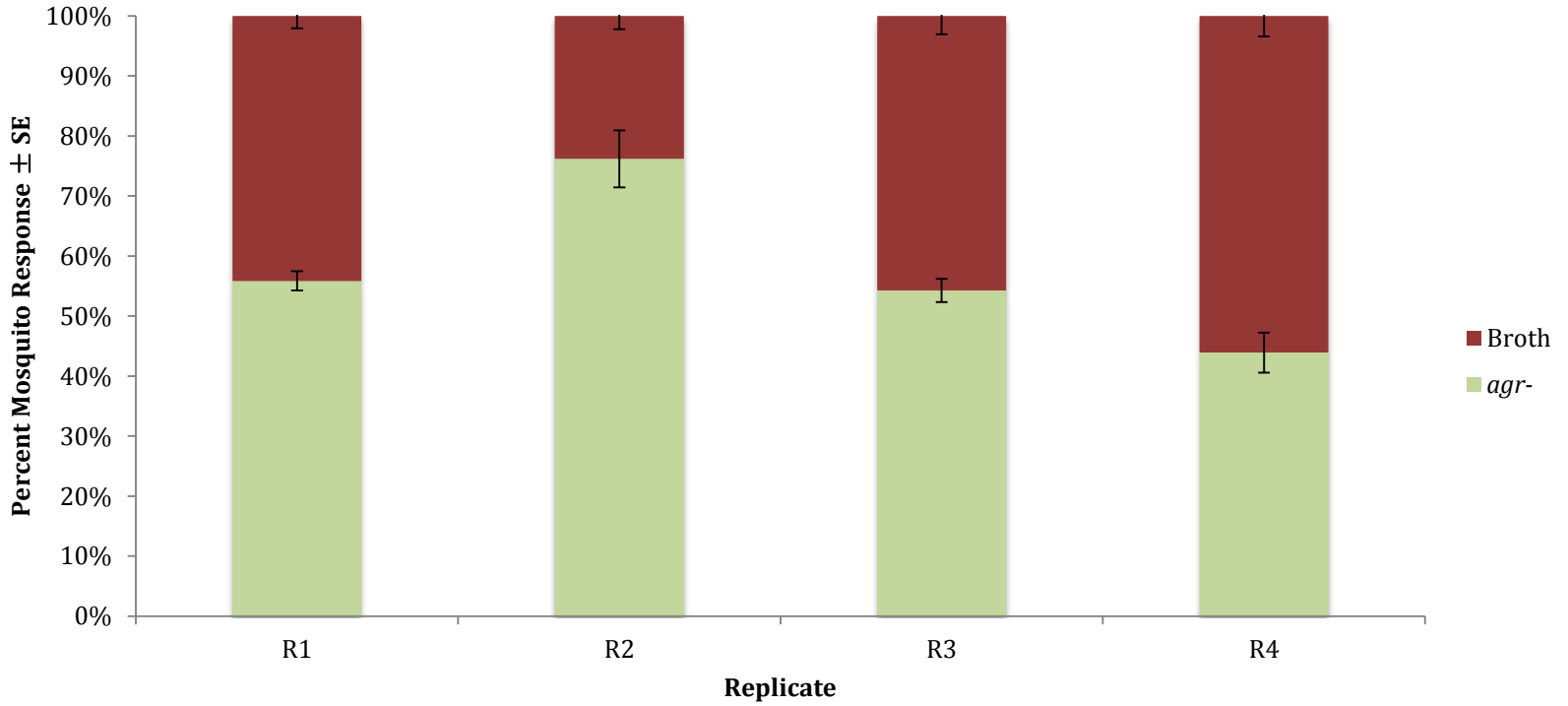


Figure 11. Mean percent attraction ± SE of adult female *A. aegypti* mosquitoes to blood-feeders treated with TSB and *agr- S. epidermidis* located on the right and left sides of a 82 cm x 45 cm x 52 cm Plexiglas cage of each replicate during 15-min replicates at approximately 25°C and 80% RH.

Table. 4 Cochran-Mantel-Haenszel test of the response of 5-8-d old *A. aegypti* (replicates, N=4; n =50) adult female attraction to blood-feeders located on opposite sides of the top of a 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min experiments under 25°C and 80% RH and treated with *agr*- mutant *S. epidermidis* and TSB.

	G value	P value
Replicate1	1.526	0.217
Replicate2	6.059	0.014
Replicate3	0.257	0.612
Replicate4	0.611	0.434
Total G	8.453	0.076
Pooled G	2.331	0.127
HeterogeneityG	6.122	0.106

Mosquito Response to Blood-Feeders Treated with Wildtype and agr- S. epidermidis

Four replicates of this experiment were conducted. Average number of adult female mosquitoes to respond to the blood-feeders treated with TSB and *agr*- strain *S. epidermidis* per minute for 15 min is shown in Figure 10. The blood-feeder treated with wildtype *S. epidermidis* attracted about 2 times more *A. aegypti* than the *agr*- mutant treated mosquito-feeder (Figure 11) ($P \leq 0.001$). Average percent response of adult female mosquitoes to the blood-feeders treated with wildtype and *agr*- strains of *S. epidermidis* for each replicate is presented in Figure 12. Statistical analysis not indicates a significant difference between replicates ($G_H = 2.258$, df = 2, $P = 0.323$) (Table. 5).

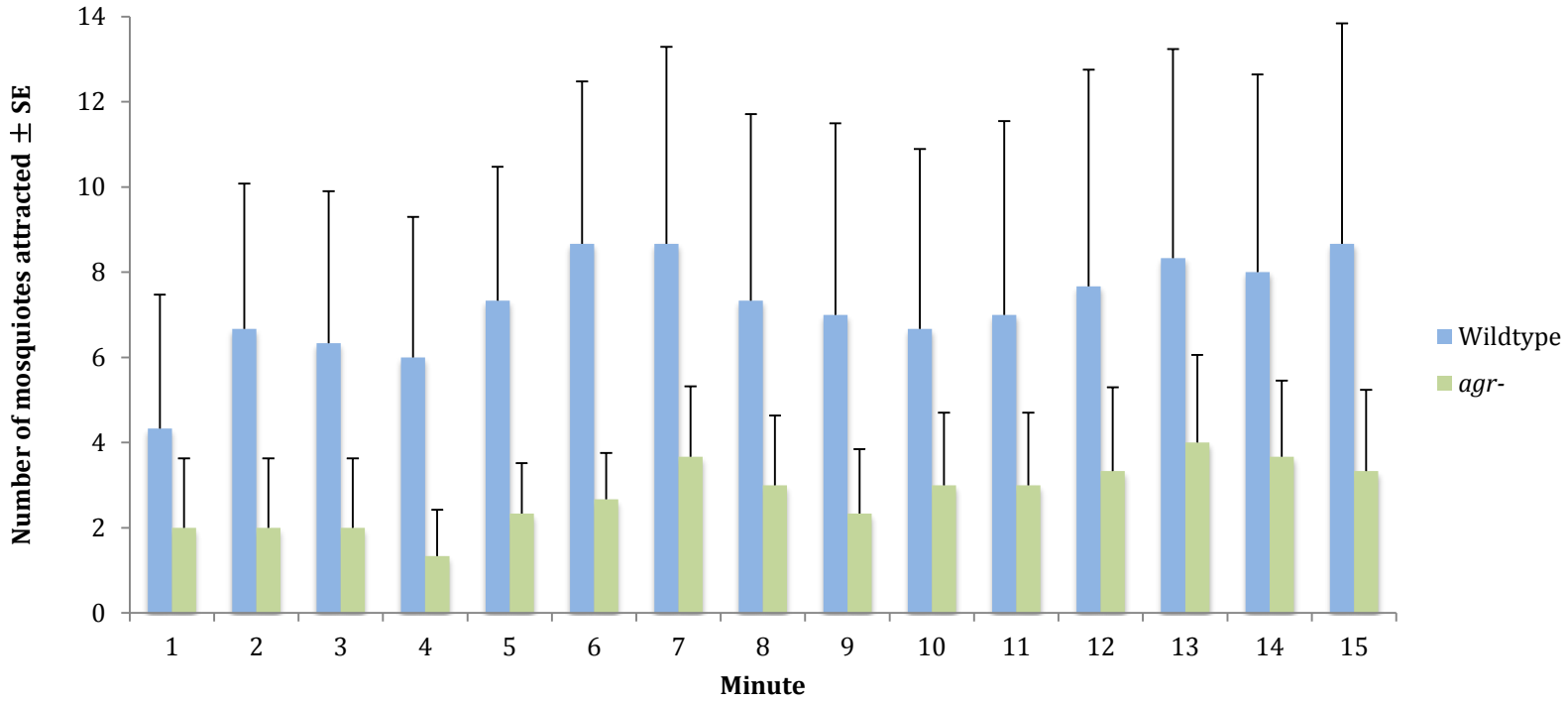


Figure 12. Mean number of adult female *A. aegypti* mosquito attraction per minute ± SE to blood-feeders treated with wildtype and *agr-* strain of *S. epidermidis* and located on the right and left sides of the top of a 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min replicate (n = 3) at approximately 25°C and 80% RH.

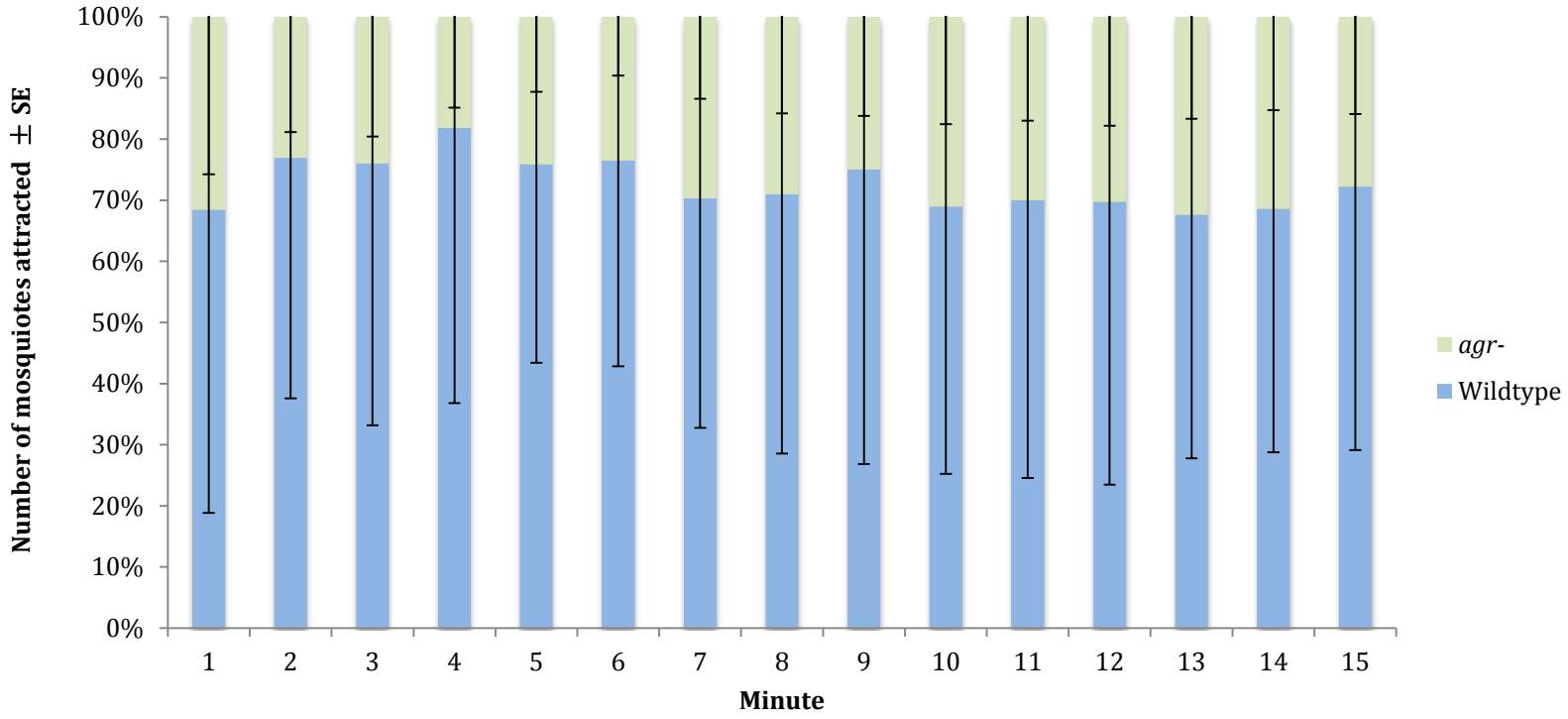


Figure 13. Mean percent of adult female *A. aegypti* mosquito attraction ± SE to blood-feeders treated with wildtype and *agr*- stains of *S. epidermidis* and located on the right and left sides of the top of a 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min replicates (n = 3) at approximately 25°C and 80% RH.

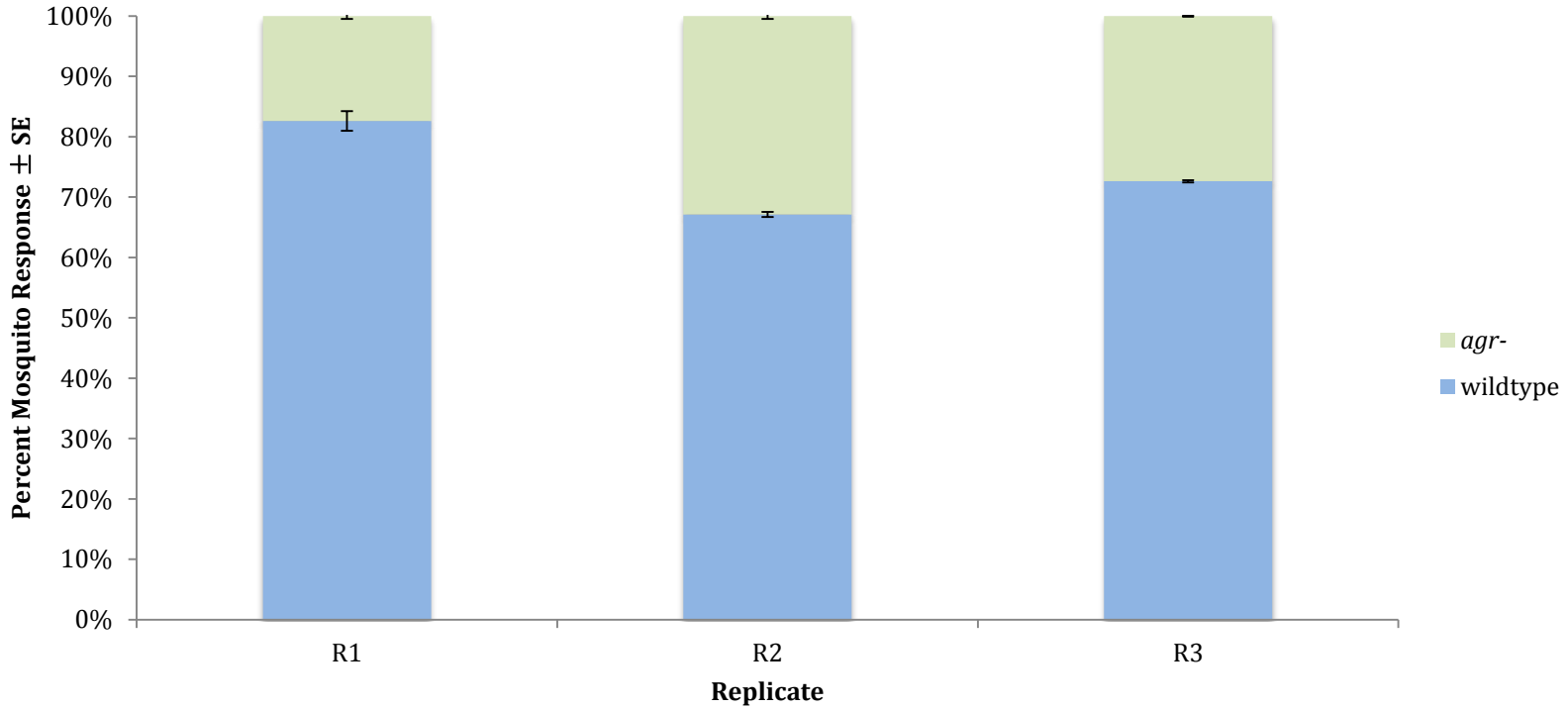


Figure 14. Mean percent attraction ± SE of adult female *A. aegypti* mosquitoes to blood-feeders treated with wildtype and *agr*- strains of *S. epidermidis* and located on the right and left sides of a 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min replicates at approximately 25°C and 80% RH.

Table 5. Cochran-Mantel-Haenszel test of the response of 50 5-8-d old *Aedes agypti* (replicates, N = 3;) adult female attraction to blood-feeders located on opposite sides of the top of a 82 cm x 45 cm x 52 cm Plexiglas cage during 15-min experiments under 25°C and 80% RH and treated with wildtype and *agr*- mutant *S. epidermidis*.

	G value	P value
Replicate1	10.631	0.001
Replicate2	8.398	0.004
Replicate3	76.040	< 0.001
Total G	95.069	< 0.001
Pooled G	92.811	< 0.001
Heterogeneity G	2.258	0.323

Discussion

VOCs produced by humans play a role in regulating host-seeking behavior by mosquitoes. For example, Carbon dioxide (CO_2) is a strong volatile cue for mosquito attraction over long distances (Gillies 1980). Ammonia, lactic acid and carboxylic acids, all of which can be produced by microorganisms, are also demonstrated to be able to attract mosquitoes (Verhulst, Beijleveld et al. 2009). In addition to different types of volatile organic compounds, various concentrations can also result in differential attractiveness to mosquitoes. For example, a higher concentration of butyl 2-methylbutanoate, butyl butyrate or butyl acetate at the 1:100 dilution caught significantly more mosquitoes than trapping devices baited with the basic blend alone

(Verhulst, Beijleveld et al. 2009). However, for many of these VOCs, their sources are not known.

Communication between human-associated bacteria plays an important role in attracting insects. *Corynebacterium minutissimum* and *Bacillus subtilis* were proved having significant attractiveness to *A. gambiae* (Verhulst et al. 2011). However, VOCs produced by *P. aeruginosa* were not attractive, and may even attenuate the effect of the volatiles from the other bacterial species (Verhulst et al. 2011). The role of *Staphylococci*, involved in converting branched-chain amino acids to highly sweaty-odorous short-chain volatile fatty acids and regulating host-seeking behavior of *A. gambiae* also cannot be ignored (Smallegange, Verhulst et al. 2011).

Bacteria have social behaviors, such as QS, to communicate intra-, and inter-species, and even interkingdom (Ng and Bassler 2009, Stevens, Schuster et al. 2012, González and Venturi 2013). Evidences supporting the concept of interkingdom communication by bacteria and eukaryotic organisms continues to increase (Hughes and Sperandio 2008). Hosts are able to respond to bacterial QS and conversely produce molecules that are sensed directly by bacteria (Wang, Dufour et al. 2010). For example, *Salmonella typhimurium* can detect host hormone norepinephrine by QS regulator QseC (Moreira and Sperandio 2012). An earlier research showed the marine macroalga *Delisea pulchra* can block bacterial QS by producing halogenated furanones that act as signal mimics (Hentzer, Wu et al. 2003). Furthermore, by producing acetate, one of the short-chain fatty acids which have major consequences for health and behavior, gut bacterium *Acetobacter* is able to promote host growth rates and reduce sugar and lipid

levels (Shin, Kim et al. 2011). Recent studies show connections between the gut microbiota and animal behavior (Collins, Surette et al. 2012). For example, depression-like behaviors reduce in germ-free mice with defects in the brain regions that control anxiety (Heijtz, Wang et al. 2011) that are fed probiotic bacteria (Lee, Menezes et al. 2011, Mayer 2011).

The role of bacterial QS in attracting insects has been detected in blow fly (Diptera: Calliphoridae) by Ma et al. (2012). The blow fly, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), showed 38% less attraction and 63% less oviposition to a mutated *Proteus mirabilis*, which was unable to swarm (QS response), than to the wildtype *P. mirabilis* (Ma, Fonseca et al. 2012). I recorded similar results for *A. aegypti* attraction to blood-feeders treated with wildtype and *agr- S. epidermidis* strains with mosquitoes demonstrating a 40% percent greater attraction to the wildtype with normal QS function (Fig.12).

In spite of the demonstration of a significantly better attractiveness to *A. aegypti* with the wildtype strain, replicate effects existed that influenced the results in some experiments, so the Cochran-Mantel-Haenszel test was used for statistical analysis. For the tests comparing wildtype and TSB treated blood-feeders, there was significant heterogeneity across all three replicates ($P < 0.05$). Replicate three was the major contribution to this heterogeneity ($P = 0.003$). Heterogeneity was also found in the experiment comparing both TSB. However, there were no heterogeneity in experiments comparing mutant vs TSB and wildtype vs mutant ($P = 0.106$, 0.323 respectively). The duration of starvation used before every replicate was inconsistent and could have been a

factor contributing to the heterogeneity. Abiotic factors such as, uneven lighting may also have contributed along with a possible variation in age stages of mosquitoes used. Other factors (temperature, humidity and nutrition) should be taken into account for the next set of experiments in order to reduce the heterogeneity problem.

Future work can be developed focusing on detection of types and amount of VOCs produced by human skin bacteria with and without modified QS system. Novel repellents might be developed based on such research. Furthermore, the quorum sensing of bacteria associated with a mosquito locating an oviposition site should also be explored for the development of new and efficient methods for mosquito control.

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