Investigation of Host Hormone Impact on Mosquito (Diptera: Culicidae) Scent Preferences

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Abstract: Mosquitoes pose an immense threat to human life because of their capability to both locate and feed upon hosts. Though plenty is known about the methods used by these arthropods to seek out hosts, less is known regarding the impact each method has upon successful host location. The degree of host-specificity between species is known to vary, and to what extent host-specific qualities affect this is not fully apparent. Specimens of medically important mosquito species known to inhabit Brazos County, Texas, including *Aedes aegypti, Aedes triseriatus, Culex quinquefasciatus* and *Psorophora columbiae* were collected in the spring season of 2020. Samples were evaluated based upon apparent preferences for various simulated host signals, by baiting traps with deer pheromone or sweaty clothes. The data obtained demonstrated preferences in-line with natural populations of *A. aegypti* and *P. columbiae,* with other species lacking statistically significant results or sample sizes for proper analysis. Results provide incentive for further ecological research into the impact of isolated host-seeking signals in initiating host-seeking behavior, as well as characteristics of said isolated signals that induce stages of behavior.

Keywords: mosquito, Culicidae, host-seeking, specificity, secretion

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Mosquitoes (Diptera:Culicidae) are established as the most important arthropod vectors in the field of medical entomology. Mosquitoes are vectors for many fatal diseases. Female mosquitoes utilize various methods of detection to find suitable hosts for acquiring protein through blood meals. This host-finding behavior is what makes mosquitoes so suited to the transmission of disease-causing pathogens. Mosquitoes must find hosts to give a host a pathogen. (Dekker et al. 2005). Studies and experiments have demonstrated that for both human and non-human hosts, variations in certain physiological factors can affect the preferences of these mosquitoes in seeking out certain hosts. These factors can include carbon dioxide levels. Carbon dioxide is known to attract mosquitoes and is used to bait traps when collecting mosquitoes (Becker 1995). Mosquitoes are also known to have species preferences in hosts, which can alter mosquito behaviors. *Aedes aegypti* (Linnaeus) (Diptera:Culicidae) is known to show a strong preference for human hosts, while *Psorophora columbiae* (Dyar & Knab 1906) (Diptera:Culicidae), despite feeding on numerous vertebrate hosts, has been shown in studies to favor livestock, such as cattle, and even smaller mammals like rabbits (Bibbs et al. 2019; ECDC 2016). Knowing what other factors play a potential

role in mosquito host-finding may enable experts to craft more effective control methods. Experts may be able to manipulate mosquito attractants in a way that prevents vector-host interactions altogether rather than having to provide expensive healthcare as a result of mosquito-borne illness. By analyzing the feeding preferences of mosquitoes based on human behavior, researchers can gain a greater understanding of what factors have a greater effect on mosquito host-finding and feeding. The purpose of this study is to determine the existence of a preference of mosquitoes between the scent of potential hosts or a control. This experiment is also designed to evaluate the correlation of mosquito host-seeking and host preference between human and non-human animals, to determine if the presence of these scents have a significant impact on mosquito behavior. Mosquito activity and preferences were analyzed via the use of secretions from humans and deer, as well as simple carbon dioxide as a control. The presence of standard carbon dioxide will also serve as a negative control in determining if host secretions from both the human and deer have a negligible impact on mosquito host-seeking. The variation of mosquito species collected may further prove a correlation. Specimens collected were from the species *Aedes aegypti*, *Psorophora columbiae*, *Aedes triseriatus* (Say 1823) (Diptera:Culicidae), and *Culex quinquefasciatus* (Say 1823) (Diptera:Culicidae). This variation in host preference may prove insightful, as these collected species have different preferences for hosts. Mosquito species that have preferences for humans, like *A. aegypti*, should be more attracted to traps baited with human sweat.

Materials and Methods

Collecting Procedures

Mosquitoes were collected from two different parks in College Station, Texas using standard CDC mosquito traps (Bioquip). The two parks were Wolf Pen Creek (30°37’5.451” N, 96°18’14.5866” W) and Bee Creek Park (30°36’6.7206” N, 96°18’30.8622” W), located approximately two miles from each other. Mosquito traps were set up under similar weather conditions, using weather data obtained through several different local weather stations. Wind speed was at an average of five miles per hour or less with a temperature of 75° to 95° F. Traps were placed a minimum of 60 feet away from each other to avoid overlap (Kline, D.L. 1999). Traps were checked every hour over a total time span of five hours where trapped specimens were collected. These collections continued for a minimum of seven days.

Specimen Preservation, Identification, and Imaging

Collected specimens were then identified using a dichotomous key (Department of

Entomology, 2020), where identifications were verified by two other researchers as well. Mosquito specimens were used to analyze their feeding behavior in response to the presence or absence of different host products. Three mosquito traps were placed in a 10x10 room in a manner that allowed a simple epicenter to be marked. Trap 1 had clothing partially saturated with a sample of human sweat to simulate aspects of natural signals used for human host-seeking. Trap 2 contained a sample of deer pheromone to simulate aspects of natural signals utilized by mosquitoes for finding larger mammals as hosts. Trap 3 lacked any sort of animal product to serve as a negative control. All three traps were partially filled with carbon dioxide (Becker et al. 1995). Each of the traps was placed on a platform to ensure all traps were stationary, as movement may alter results. (Lorenz et al. 2013). The release point for mosquito specimens was marked at a point equidistant from the three traps. Specimens were exposed to the traps in nine trials, each trial containing a separate sample of 10 specimens. During a trial, one researcher was stationed at each trap over a period of 20 minutes, recording of mosquitoes entering the traps. Samples for Trap 1 and Trap 2 were checked and replaced as needed between trials to ensure consistency of both samples and results. Traps and release areas were isolated from researchers and held at room temperature to prevent bias from temperature gradients aiding in host-detection (Corfas and Vosshall 2015). When samples had completed testing, mosquitoes were anesthetized and placed into a freezer and later stored for future reference and study. Results were collected and statistically analyzed using JMP.

Results

A total of ninety mosquitoes were collected and analyzed (Table One). A total of 50 mosquitoes were found in the traps containing sweaty clothes. Out of that 50, 40 were *A. aegypti*, one was identified as *A. triseriatus*, seven were collected as *C. quinquefasciatus*, and two were *P. columbiae* (Table One). A total of 25 mosquitoes were found in the traps containing deer pheromone. Out of those 25 mosquitoes, 18 were shown to be *P. columbiae*, five were identified as *A. aegypti*, two were C. *quinquefasciatus*, and zero were found as *A. triseriatus* (Table One). A total of fifteen mosquitoes were found in the control traps containing only carbon dioxide. Ten were identified as *A. aegypti*, one was known as *A. triseriatus*, three were classified as *C. quinquefasciatus*, and one was known to be *P. columbiae* (Table One). In total, Fifty-five *A. aegypti* specimens were found, and 72.72% were attracted to sweaty clothes, 9.09% attracted to the deer pheromone and 18.18% to the control (Figure One). Of the two *A. triseriatus* found in total, 50% were located with the sweaty clothes and 50% were located with the control (Figure One). Of the 12 total *C. quinquefasciatus* specimens found, 58% were attracted to the sweaty clothes, 16.67% to the deer pheromone and 25% to the control (Figure One). Lastly, of

the twenty-one *P. columbiae* specimens captured, 9.52% were found with sweaty clothes while 85.71% were attracted to the deer pheromone and 8.33% were found by the control (Figure One). Overall, 55.56% of the mosquitoes collected preferred sweaty clothes, 27.28% chose the deer pheromone and only 16% went for the control (Figure One).

Table 1. Collection Results of Different Mosquito Host Selection Behaviors

Treatments = CO2 +	**Sweaty Clothes**	**Deer Pheromone**	**Control (CO2 only)**
Total # of Mosquitoes	50	25	15
Aedes aegypti	40	5	10
Aedes triseriatus	1	0	1
Culex quinquefasciatus	7	2	3
Psorophora columbiae	2	18	1

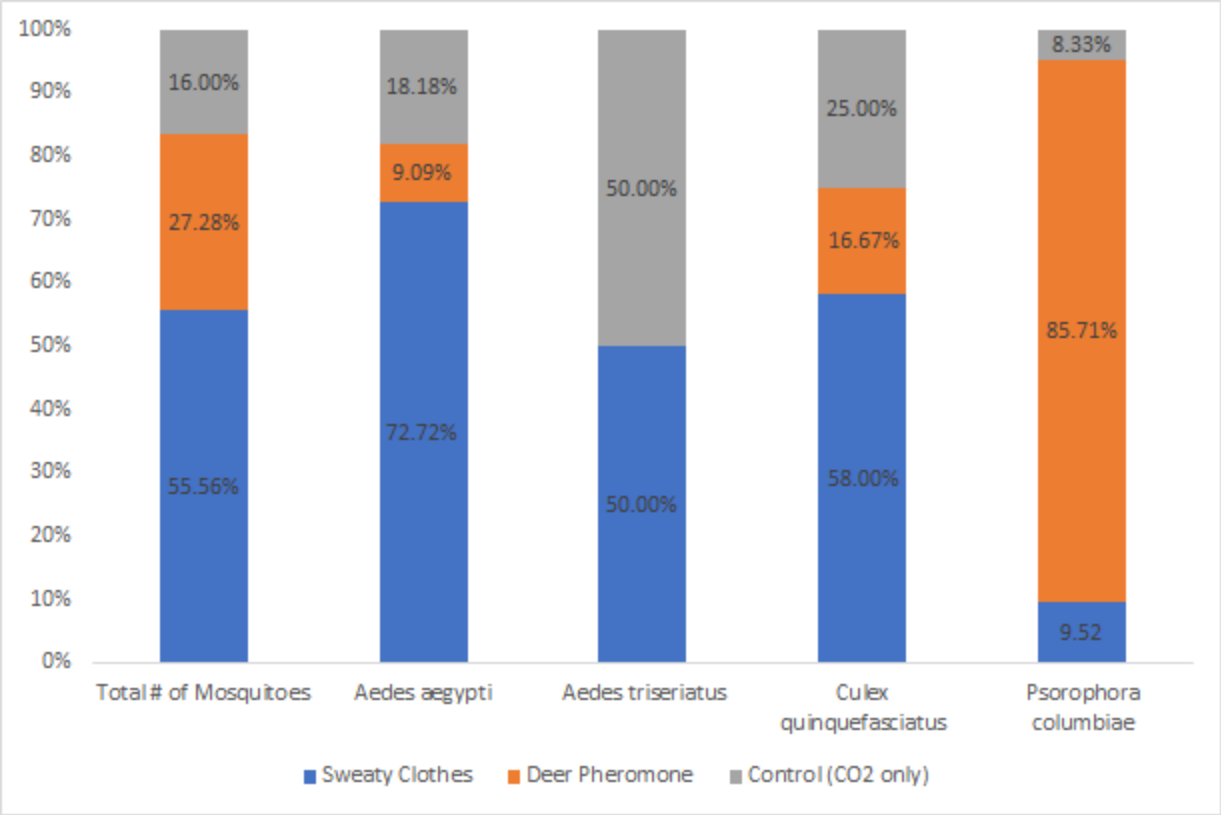

Figure 1. Percentages of Selectivity for Various Species of Mosquito

Discussion

Mosquitoes have the capability to both locate and feed upon hosts based upon numerous characteristics. Though the ways that arthropods seek out hosts are known, the impact that each independent method utilizes for the success of the host location is vague. Females have various methods to detect suitable hosts for acquiring blood meals, which also make them perfect vectors for several disease-causing pathogens (Dekker et al. 2005). Numerous experiments have demonstrated that variations in certain physiological factors can affect the favorability of mosquitoes. In the experiment, three samples were offered to mosquitoes which allowed the testing of specific host secretions and determined whether they have an impact on host-seeking behavior. Viewing the data, it would seem apparent that secretions from potential hosts, even when isolated from a true host, provide a significant signaling factor for mosquito host-seeking. When viewing the table presented above through a lens of species-dependent specificity, results may imply the following: (1) High preference for the human sweat sample by *Aedes aegypti* supports the prior knowledge of human host preference by the species, and the idea that these host secretions play a significant role in host detection (McBride, C., Baier, F., Omondi, A. *et al.* 2014). It is also noteworthy that secretions or pheromones from hosts not associated with

Aedes aegypti in this case being deer pheromone, may serve as negative signals for this species, and possibly others with this being indicated by Aedes *aegypti* being attracted more to the negative control rather than being tempted by the trap with deer pheromone (Figure One). (2) A similar trend can be seen with *P. columbiae* with deer and other large mammals known to be a preference for this mosquito, with humans not known to be a preferred host (Michael J. Turell, Seth C. Britch, Robert L. Aldridge, Rui-De Xue, Mike L. Smith, Lee W. Cohnstaedt, Kenneth J. Linthicum. 2015). However, data was not obtained regarding whether *P. columbiae* would prefer the negative control to the human variable. (3) The evident tendency of some specimens to prefer the carbon dioxide negative control supports prior knowledge concerning the use of carbon dioxide exhaled from potential hosts serving as another signal for host-seeking behavior (Sukumaran, D., S. Ponmariappan, A.K. Sharma, H.K. Jha, Y.H. Wasu, and A.K. Sharma. 2016). These trends support the claim that certain secretory products of host physiology have significant effects on host-seeking behavior without the presence of other typical host characteristics present (Liu, X., Liu, Q., Guo, Y. *et al.*2011). It is clear from Table One most specimens caught were the species *Aedes aegypti*, followed by *Psorophora columbiae*, with *Aedes triseriatus* and *Culex quinquefasciatus* having similarly low numbers obtained. Most *A. aegypti* specimens were found in traps baited with sweaty clothes. (Table One). To collect more precise results, a larger array of species needs to be obtained and analyzed. Due to these specimens being acquired during the pandemic of the coronavirus human activity outside may be low which could have resulted in a diminished food supply ultimately leading to a low specimen collection. Further research could show the implications of certain behaviors and how best to recognize and prepare for them. Further analysis regarding the significance of these factors combined with other host factors or isolating other variables of vector-host interactions have the potential to give greater insight into the significance of certain characteristics, as well as a deeper understanding of how these variables interact and modify one another.

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