J.N. Andrews Honors Program
Andrews University

Proposal for Senior Honors Thesis

HONS 497 Senior Honors Thesis **Credits ___2___** (2 minimum required)

Directions: Please return signed proposal to the Honors Office **at least one week prior to your scheduled meeting with the Honors Council**. This proposal must be accepted by Honors Council the semester before presentation.

Student's Name: Janelle An

Primary Advisor: Dr. Benjamin Navia
Secondary Advisor:
Thesis Title: Evaluation of Phonotactic Behavior in Male-exposed Female *Acheta Domesticus*
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Expected date of Graduation: May 2021

I. Provide goals and brief description of your project or research.

The goal of my research is to determine whether direct contact (mating) with male *Acheta domesticus* is required to elicit a decrease in the selectivity of young virgin female *A. domesticus* to male calling songs.

Phonotaxis can be defined as the movement of organisms in response to an auditory stimulus. As such, it plays an important role in helping organisms find a potential mate, and thus it is worthwhile to understand what factors may influence phonotaxis. Stout *et al.* (2010) first described phonotaxis in hundreds of virgin females as either selective or unselective depending on whether females were able to discriminate phonotactically between less than five or more than five synthetic calls which intended to mimic that of the males'.

Previous studies involving female cricket *A. domesticus* found that internal factors such as Juvenile Hormone III (JHIII) and external factors such as temperature influenced phonotactic behavior in these females (Atkins *et al.*, 2008; Navia *et al.*, 2015). More recently, Kent & Dosunmu (2018) working on the same species provided preliminary evidence that as a result of exposure to males, females became less selective. However, due to the experimental design of their study, they could not determine if the cause of this change in female selectivity was the result of mating. Thus, this proposal seeks to answer the question: Is mating necessary to elicit a decrease in female phonotactic selectivity? By preventing mating between male and female *A. domesticus*, I will test the hypothesis that mating is required to elicit a decrease in the phonotactic selectivity of females. This research project will expose virgin females to males while preventing mating, and then determine the effects of such exposure on female phonotaxis.

II. Outline your methodology. **Please be specific**. How does this achieve your goals and how reliable is it?

<u>Establish non-direct exposure to male crickets:</u>

To answer the question of whether mating is necessary to elicit a decrease in female's phonotactic selectivity to model calls, indirect exposure to males will first be established. To allow indirect exposure to

occur, males and females will be placed in a plastic container and segregated by gender using a perforated divider. This divider will prevent the crickets from mating but will allow indirect forms of communication such as acoustic stimuli and pheromone signaling to occur. Because this study seeks to further investigate possible causes that result in females becoming less selective after exposure to males as observed by Kent & Dosunmu (2018), it is advantageous to use young female crickets who are naturally more selective in order to identify a change in selectivity (Stout *et al.*, 2010). Thus, young female 4-10 days old will be tested in this study. To establish a control for this study, females of the same age group will be placed in an environment absent of males to avoid exposure. Females will be exposed to males for 3 days in order to ensure sufficient exposure, after which both experimental and control groups will be tested to determine whether significant differences in female's phonotactic selectivity have occurred.

Record female cricket selectivity:

Phonotaxis must be quantified in order to determine whether there are significant differences in selectivity between exposed and non-exposed females. The protocol to quantify phonotaxis described in Samuel *et al.* (2010) will be used to test phonotactic behavior. Female crickets are fixed onto a non-compensating spherical treadmill that gives the crickets free mobility to move and turn in all directions on the rotatable ball. After crickets are given time to adjust to this new environment for 5 minutes, their movements in response to computer-generated model calls (which mimic those of males) are tracked and quantified using a computer software called Optical Kugel. Seven different syllable periods ranging from 30-90 milliseconds in increments of 10 milliseconds are played in a nonsequential order (50, 90, 70, 30, 60, 80, 40). For each syllable period, phonotactic behavior is recorded for 5 minutes with a 3-minute break in between recording sessions. Crickets are said to exhibit phonotaxis when they display both an average angular orientation between -60 and +60 degrees and movement towards the speaker that is twice as far as movement away from the speaker. These criteria indicate that the cricket was moving intentionally towards the speaker and did not arrive closer to the source of the sound through random motion. After cricket phonotactic behavior has been recorded in response to all 7 syllable periods, crickets that respond to 5 or less of the calls presented are classified as selective, while crickets that respond to more than 5 are classified as unselective. Statistical analysis will be conducted to determine whether there are significantly different phonotactic responses between exposed and non-exposed female *A. domesticus*.

Use statistical analysis to identify significant differences in selectivity:

From the data collected during cricket recordings, ratios between selective and unselective females will be determined for exposed (experimental) and non-exposed (control) groups. A Chi-squared test will be conducted to test whether there is a significant difference in selectivity between the two groups. If there is a significant difference in selectivity between experimental and control groups, then it is evident that another factor besides mating had an influence on female selectivity and phonotaxis. Thus, we would reject our hypothesis that mating is required to elicit a decrease in the selectivity of females in response to male calling songs. But if there is no significant difference between experimental and control groups in phonotactic behavior, then it would be evident that mating is necessary to elicit a change in female selectivity. In this case, we would agree with our hypothesis that mating is required to elicit a decrease in the selectivity of female *A. domesticus* to male calling songs.

The method used to quantify phonotaxis outlined in Samuel *et al.* (2010) is a well established procedure used in several published articles by various researchers (Atkins *et al.*, 2008; Choi *et al.*, 2012; Samuel *et al.*, 2013; Stout *et al.*, 2010). This method will allow me to quantify the phonotactic behavior of exposed and non-exposed female crickets in order to achieve my goal to determine whether female phonotaxis is significantly different.

III. Explain in what sense your project is original, unique, or beyond normal senior expectations. How does it relate to current knowledge in the discipline?

Only recently have studies begun to explore how an external factor, mating with male crickets, might affect female cricket behavior. In the past, studies involving female crickets were conducted using virgin females that had not come into contact or mated with males. But because a majority of females will come into contact with males sometime during their lifespan, it is beneficial to study whether mating may alter the female's phonotactic behavior and therefore modify underlying neural mechanisms that regulate such behavior.

In an unpublished, preliminary study, Kent & Dosunmu (2018) reported that in comparison to young virgin females, young females that were placed in an environment where they could mate displayed a significantly lower level of selectivity and were less able to discriminate between attractive and unattractive calls. Although it appears that exposure to males modified female phonotaxis much like the factors of temperature and JHIII, due to the nature of their experimental conditions, it is not yet clear whether this effect was caused by mating between males and females or whether some other signal, such as a pheromone, may have been responsible for the change in behavior (Atkins *et al.*, 2008; Navia *et al.*, 2015). The methodology of Kent & Dosunmu (2018) allowed males and females to mate. However, because they could not verify whether the tested animal had actually mated, they were not able to rule out what factors affected phonotaxis.

My proposed research is unique in that discriminates between direct (mating) and indirect male-exposure, and seeks to clarify Kent & Dosunmu's research (2018) by determining whether mating is necessary to change the phonotactic behavior of females. In order to isolate the effects of mating on female phonotaxis, females will be kept in close proximity with males without allowing mating to occur. If these females are found to have statistically significantly different levels of selectivity than that of non-exposed females, then we can conclude that mating was not necessary to change the phonotactic behavior of these females. This finding would lead us to then explore the role of other male/female interactions such as acoustic communication and pheromones in altering female selectivity. Because changes in selectivity at the behavioral level have implications on the neuronal level, my research will open the door to further investigations involving underlying neural signaling and connectivity of networks that control such behavior.

IV. Include a substantive annotated bibliography of similar or related work.

Atkins, G., Kilmer, J., Scalfani, M., Navia, B., & Stout, J. (2008). Modulation of syllable period-selective phonotaxis by prothoracic neurones in crickets (*Acheta domesticus*): juvenile hormone, picrotoxin and photoinactivation of the ON1 neurones. *Physiological Entomology, 33*(4), 322-333.

This study showed the effects of three different treatments on female *A. domesticus* phonotaxis. JHIII injection increased female *A. domesticus* selectivity, and phonotaxis occurred most frequently within the 50-70 ms syllable period range. Picrotoxin (or PTX) is a plant-based substance that has been shown to block chloride channels and alter auditory neurons in insects. PTX also caused an increase in old female *A. domesticus* selectivity. Photoinactivation of ON1 neurons in previously unselective old female *A. domesticus* significantly increased their selectivity, to the point where they showed phonotactic behavior similar to that of young selective females. PTX injection and ON1 photoinactivation also significantly increased the activity of the L3 neuron (a neuron in the prothoracic ganglion that plays a role in phonotactic behavior of female crickets) further supporting the idea that JHIII, PTX, and ON1 inactivation have an influence on the auditory processing of *A. domesticus*. All three treatments had a similar influence on female *A. domesticus* phonotaxis. This study provided a robust background demonstrating the plasticity of female phonotaxis and how it is affected by different factors. This article served as a basis to explore other factors, including male exposure, that may influence female phonotaxis and selectivity.

Choi, R., Atkins, G., & Stout, J. (2012). The effects of injecting Juvenile Hormone III into the prothoracic ganglion on phonotaxis by female crickets *Gryllus bimaculatus*. *Physiological Entomology, 37*(2), 201-205.

This article outlines the work of Choi *et al.* (2012) in exploring the effects of Juvenile Hormone III (JHIII) on female *Gryllus bimaculatus* De Geer phonotaxis. Researchers observed that female crickets of *G. bimaculatus* were more selective (defined as responding with positive phonotaxis to 2 or less of 8 of syllable periods) when JHIII was injected into their prothoracic ganglion. When JHIII was injected, female *G. bimaculatus* exhibited phonotaxis only towards a limited range of syllable periods between 30-50 ms, correlating with those produced naturally by male *G. bimaculatus*. Choi *et al.* (2012) confirmed JHIII was the agent causing this change in female phonotaxis by testing the effects of the solvent acetone without JHIII, and they confirmed acetone did not elicit a similar change as was observed during JHIII injection. JHIII injection into the metathoracic ganglion did not significantly affect feamle cricket selectivity, showing that JHIII influences strutures found in the prothroacic ganglion. The methodology of recording cricket phonotaxis in this study was used in my research to test the species *A. domeseticus*. This study showed that female phonotaxis can be influenced by external factors, and thus provided a foundation for the idea to test the effects of other factors such as male exposure on female phonotaxis.

Navia, B., Burden, C., Steely, T., Hasegawa, H., Cha, E., Henson, S. M., . . . Atkins, G. (2015). Parallel effects of temperature on the male cricket calling song, phonotaxis of the female and the auditory responses of the L3 neurone. *Physiological Entomology, 40*(2), 113-122.

Navia *et al.* (2015) demonstrates how temperature affects three factors that play important roles in acoustic commincation and processing. These factors include length of male calling songs, female phonotaxis, and L3 auditory processing. As temperature increased from 17-33°C, the syllable period of the calling song decreased in length by 2.5 $ms°C^{-1}$. Females also displayed signicantly greater levels of phonotaxis towards calling songs that were most likely to be produced by males at similar temperatures. During L3 neuronal processing, peak levels of neuronal decrement are correlated with calls that females find most attractive. In this study, peak levels of L3 decrement were shown to vary across a range of temperatures (22-33°C) that were tested. For the purposes of my research, this study demonstrates the plasticity of female phonotaxis and how it can be influenced by factors such as tempature. This article served as a basis to then explore other factors, including male exposure, that might influence female phonotaxis and selectivity.

Navia, B., Kent, C., & Dosunmu, S. (2018). *P-31 Neural plasticity and behavioral changes as a result of male-exposure in females of an insect model.* Paper presented at the Honors scholars and undergraduate research poster symposium, Berrien Springs, MI.

Kent & Dosumnu (2018) demonstrated that male-exposed female *A. domesticus* exhibit significantly different phonotactic behavior compared to that of non-exposed females. Specifically, male-exposure reduced selectivity in young females between 5-14 days old. Preliminary data also indicates that L3 neurons of young, male-exposed

females responded with higher levels of decrement to a wider range of calling songs than those of virgin (non-exposed) females. Since higher levels of decrement are associated with calls females find more attractive, a high level of decrement over a wide range of syllable periods would imply that a female is attracted to a broad range of syllable periods and is thus less selective. The observed L3 neuronal activity (which exibits a high level of decrement over a wide range of syllable periods) is therefore consistent with the phonotaxis that would be expected in male-exposed females (reduced selectivity). My research seeks to build upon and clarify Kent & Dosumnu's findings. Specifically, I will determine whether the decrease in female selectivty observed in male-exposed females is a result of mating, or whether other factors such as acoustic communication or pheremone signalling play a more significant role.

Samuel, L., Stumpner, A., Atkins, G., & Stout, J. (2013). Processing of model calling songs by the prothoracic AN2 neurone and phonotaxis are significantly correlated in individual female *Gryllus bimaculatus*. *Physiological Entomology, 38*(4), 344-354.

Although previous studies had demonstrated the plasticity of female *G. bimaculatus* selectivity in either AN2 nueron processing (nueronal activity) or female phonotaxis, research conducted by Samuel *et al.* (2013) demonstrated that there is a significant correlation between the selectivity of the AN2 neuron and phonotaxis within individual female *G. bimaculatus*. AN2 activity and phonotactic selectivity were highly correlated across all syllable periods, excluding the shortest syllable period recorded.

The methodology for testing phonotaxis in my research project is primarily based on the detailed setup and procedure described in this paper. Along with the many studies that have used crickets to explore neurobiological processes, Samuel *et al.* (2013) describes crickets as a model organism for auditory processing studies, due to their simple nervous systems and the small number of neurons that are involved in auditory processing.

Stout, J., Navia, B., Jeffery, J., Samuel, L., Hartwig, L., Butlin, A., . . . Atkins, G. (2010). Plasticity of the phonotactic selectiveness of four species of chirping crickets (Gryllidae): Implications for call recognition. *Physiological Entomology, 35*(2), 99-116.

Stout *et al.* (2010) demonstrated that there is significant variability in female selectivity of crickets within a single species. This variability was seen in four different species of crickets, where previously it was thought that phonotactic behavior was fixed. Stout *et al.* (2010) attributed this earlier understanding to be partly due to small sample sizes and pre-selective screening methods that determined which crickets were fit to be included in the studies.

Stout *et al.* (2010) contributed to our decision to test young female *A. domesticus* crickets between 4-10 days old rather than testing over the entire range of ages. Their study found that while 90% (171/190) of young female *A. domesticus* between 5-7 days old were selective (responded to no more than 5 of 7 syllable periods presented), only 53% (170/318) of old female *A. domesticus* between 21-31 days old were selective. Should a decrease in female selectivity occur in our study, by using the naturally more selective young female *A. domesticus* we could be more certain that a change in their selectivity would be due to their exposure to males rather than an age related decline in selectivity.

V. Provide a statement of progress to date and list the research methods coursework completed.

To date, a total of 25 male-exposed virgin female *A. domesticus* have been tested. Of these crickets tested, four displayed no phonotaxis. Of the remaining 21 crickets, 16 were selective (responding to 5 or less syllable periods), while 5 were unselective. Throughout this semester and the following semester, I will continue testing male-exposed female crickets and analyzing my data.

The following research methods coursework was completed, along with other classes that have prepared me for this research project through reading scientific articles and writing formal lab reports:

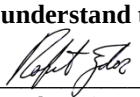
* Biostatistics & Research Design (BIOL 280) – Spring 2020
* Research Pro-seminar (HONS 398H) – Spring 2019

* Scientific Communication (BIOL 305) – Spring 2020
* Systems Physiology (BIOL 464) – Fall 2019
* Organic Chemistry Lab I & II (CHEM 241 & 242) – Fall 2018/Spring 2019
* General Chemistry I & II (CHEM 131 & 132) – Fall 2017/Spring 2018

* Foundations of Biology I & II (BIOL 165 & 166) – Fall 2017/Spring 2018

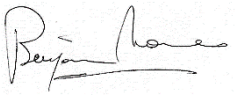
Department Chair Approval

- **This student's performance in his/her major field is acceptable.**
- **He/she has completed the requisite research methods coursework for the research to be pursued.**
- **I understand that he/she plans to graduate with Honors.**



Department Chair (signature)

Research Advisor Approval



I have read and support this proposal: _____

Primary Advisor (signature)

I have read and support this proposal: _____

Secondary Advisor (signature)

If human subjects or if live vertebrate animals are involved, evidence of approval from the Institutional Review Board or an Animal Use Committee is needed through the campus scholarly research offices (Ext. 6361).