Research reports from undergraduate students receiving support from the Shackouls Honors College via the Summer Research Fellowship

Summer 2022

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Differences in Recombination Rates of *Caenorhabditis elegans*

Introduction:

This summer, I worked under the guidance of Dr. Amy Dapper and graduate student Dharani Matharage in the Dapper Lab. I worked with four lab strains of the model organism *Caenorhabditis elegans* that are generated by the Dapper Lab and contain fluorescent markers (GFP or tdTomato) at specific genomic locations. Two of these strains are derived from N2 (a laboratory strain established in the 1950's) and two are derived from CB4856 (a more recently wild-derived strain collected from Hawaiian pineapples) (Frézal et al.,2015). Previously, the Dapper Lab found that the probability that a recombination event occurs between the fluorescent markers is significantly higher in CB486 than in N2. The goal of my project was to sequence the genome of four strains of *Caenorhabditis elegans* to test predictions of alternative hypotheses proposed to explain the difference in recombination rate between these strains.

Method and Progress:

Caenorhabditis elegans undergoes a relatively unique form of mating system called androdioecy, that allows the species to reproduce by either self-fertilizing (selfing) hermaphrodites (XX) or by hermaphrodites (XX) breeding with males (XO) (Frèzal et al., 2015). The strains that we use in the Dapper Lab have been introduced to a specific loss-of-function mutation, *fog2*, that alters the reproductive mechanism from androdioecy to gonochoristic reproduction, meaning that the strains can only reproduce through the mating of a hermaphrodite

(XX) and male (XO) (Katju et al., 2008). Several mating strain samples were created, in agar plates seeded with *Escherichia coli* as a food source, for each strain of *C. elegans* to be used for genomic DNA extraction. In every sample plate, 5-6 hermaphrodites and males each were introduced and allowed to breed for 3-4 days to produce worms needed to yield sufficient DNA for genome sequencing. The sample was prepared by washing the plates with chilled M9 solution, layered on a 5% sucrose solution and pelleted by centrifugation. The pellets of worms were washed several times with M9 medium and frozen as pellets at -80°C in 2.0 mL microfuge tubes. The same process of preparing the sample was used for all DNA extraction protocols tested.

As we did not have an established protocol designed to extract the DNA from *C. elegans* in the lab, we tested various methods to find a protocol that would fit our requirements. The first DNA extraction in the lab was done using the Monarch HMW DNA Extraction Kit. This test was performed only on one strain of the worm to test whether it would produce enough yield, it produced 32 ng/mL of DNA which was less than what was required to move on to the next stage of the project. Due to the less-than-ideal result of the first experiment, we decided to go on a more traditional route and tried a different protocol for DNA extraction using chemical reagents available in the Dapper Lab. This process was also not adequate to produce the yield we required from the worms. As we did not get the results we hoped to achieve, we decided to take a step back and do a DNA extraction on fruit flies as it was a quicker approach than using worms, to troubleshoot our protocol. To determine whether the problem was in the phenol we were using, this extraction was done two separate times simultaneously, a) using phenol from the Dapper Lab, and b) using phenol from the Ballinger Lab. After comparing the results from both extractions, there wasn't a significant difference in DNA yield. So, we concluded that phenol

was not the problem. After this, we proceeded to try to amplify the DNA through the Amp extraction process. The yield was higher than but not significantly different than our previous yields to integrate this approach into our protocol, especially because the yield obtained from Amp extraction was not pure DNA. Instead, it was a mixture of other nucleic acids combined with DNA. We suspected that after purifying the DNA from the product, the yield would be similar to our previous results. Moving forward, Dr. Dapper consulted with Dr. Levi Morra, an associate professor from Emory University, and he suggested a DNA extraction protocol by The Herman Lab, which functioned by using a phenol/chloroform extraction. Contingent on the reagents we had in the lab, we attempted several versions of this protocol and were able to derive sufficient DNA yield using this method.

All of the DNA concentration yields were obtained through the use of Qubit 2.0 Fluorometer. After we acquired sufficient DNA through the Qubit readings, we moved on to the next stage of preparing and organizing the raw data using the Native Barcoding Kit 24 V14. We are currently troubleshooting this genomic library construction in preparation for sequencing.

Future Direction:

The project is still ongoing as we experienced several obstacles at various stages. Currently, I am working on troubleshooting problems with the Library Preparation of extracted DNA. This correct application of this stage is crucial to move on to the next step of the project, which is to sequence the genome. I will be working on the project throughout the remainder of this semester and hope to have quality DNA to continue with the next stage of the project in Spring 2023 through a Directed Individual Study (DIS) with Dr. Dapper. The DIS will be focused on the computational analysis of the genome by comparing it to a reference genome to determine the reason for difference in recombination rates of different strains of *C. elegans.* This

will be done using MinION sequencing device and other reagents purchased by the Honors Research Fund which will sequence the genome and collect the raw data. Using this data, I will use bioinformatics to filter out low quality data that exists in the genome. I will compare the data acquired after this process and map it to a reference genome to mark how many base pairs exist between two fluorescent markers. I will compare the data acquired after this process and map it to a reference genome to mark how many base pairs exist between two fluorescent markers. By calculating the base pairs in the genomic sequence, I will calculate the physical distance between two markers and determine whether they are constant in all strains or not. I will use this data to determine whether:

1. The likelihood of recombination rate is higher in CB4856 strain, OR
2. The markers are farther apart in the genome of CB4856 strain.

I will also be participating in the Spring 2023 Research Symposium to present the work I have done since summer.

Project Significance:

C. elegans is regarded as a premier model organism in biological science due to the extensive body of knowledge that is currently available on molecular, cellular, developmental, and behavioral biology (Frèzal, et. al, 2015). It is also the first multicellular organism whose genome was sequenced. Naturally, there is a lot that can be learned and discovered by observing patterns in *C. elegans.* For example, researchers have used chemical and genetic screens in *C. elegans* to investigate molecular pathways involved in Parkinson's disease (Harrington et al., 2010). To make *C. elegans* a more effective model organism, it is essential for researchers to understand the functions and mechanisms of *C. elegans* as best as we can. This research project

holds significance as it is an attempt to build a better understanding of the recombination rates of *C. elegans* in the scientific community to strengthen its status as a model organism.

Protocols used:

C. elegans DNA extraction protocol - The Herman Lab

https://hermanlab.unl.edu/protocols/GenomicPrep.html

Monarch HMW DNA Extraction Kit: Tissue Protocol https://www.neb.com/-/media/nebus/files/protocols/t3060_quick_protocol_card_monarch_hmw_dna_extraction_tissue.pdf?rev=258f2c5785fe4d8abf0763f67a478682&hash=CF45DC1093D7D616EAB232E95D8C896B

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Name: Miller, Julie Anne

Faculty Advisor: Dr. Hilary L. DeShong

Project Title: *Personality and Childhood Experiences*

Introduction

Previous research has shown that childhood factors like emotional vulnerability and parental invalidation can predict future symptom presentations of borderline personality disorder (BPD; DeShong et al., 2019). Additionally, BPD has been linked to anxious and avoidant adult attachment styles. As such, investigating childhood risk factors as they relate to maladaptive attachment styles and BPD traits in adulthood may provide insight into potential prevention and intervention strategies. Specifically, childhood emotional sensitivity and parental invalidation might have differential relations in the development of adult maladaptive attachment styles and BPD. Furthermore, some research suggests the potential for parental validation to be a protective factor against BPD (Gill & Warburton, 2014). Notably, these childhood risk factors have not been directly assessed in relation to attachment styles. Thus, the current study aimed to investigate the relations between childhood factors (adverse childhood experiences, parental validation, parental invalidation, and emotional sensitivity), maladaptive adult attachment styles, and BPD.

Method

Participants (n = 245) were recruited via Amazon's MTurk and compensated $6.50 for their participation in the study. Participants completed the following measures: Adverse Childhood Experiences-International Questionnaire (ACES-IQ; WHO, 2018), Highly Sensitive Person Scale (HSPS; Aron & Aron, 1997), Experience in Close Relationships-Revised (ECR-R:

Fraley et al., 2000), Five-Factor Borderline Inventory-Short Form (FFBI-SF: DeShong et al., 2016), and the Socialization of Emotion Scale (SES: Krause et al., 2003).

We conducted model trimming in SPSS AMOS using a theory-driven model. Specifically, childhood sensitivity (HSPS; childhood biological factor) was entered as the predictor (X) variable, parental validation (SES) and parental invalidation (SES; childhood environmental risk/protective factors) as mediators followed by adulthood attachment style (ECR-R; adult adjustment) as a secondary mediator, and BPD as the outcome (Y) variable. We examined all direct and indirect pathways between each variable (e.g., sensitivity directly to BPD and indirectly through parental invalidation).

Results

Overall, the final model had excellent fit (TLI = 1.04; CFI = 1.00; RMSEA =.00) and accounted for 60.6% of the variance for BPD, 30.4% of the variance for adult attachment, 15% of the variance for parental invalidation, and 0% of the variance for parental validation. Adult attachment styles ($\beta = .481$, $p < .001$) and sensitivity ($\beta = .297$, $p < .001$) were significant direct predictors of BPD. Additionally, sensitivity was a significant direct predictor of adult attachment styles ($\beta = .437$, $p < .001$). Sensitivity was a significant indirect predictor of BPD through parental invalidation and adult attachment styles ($\beta = .318$, $p < .001$). Parental invalidation was also a significant indirect predictor of BPD through adult attachment styles only ($\beta = .099$, $p < .001$).

Discussion

Consistent with the literature, parental invalidation and childhood sensitivity were significant predictors of BPD. Contrary to previous research, parental validation did not emerge as a protective pathway for BPD. Therefore, an individual who is genetically predisposed to be

more sensitive to stimuli might be at a higher risk for developing BPD, and this may be exacerbated by growing up in a consistently invalidating environment.

Future Directions

The results from these analyses were submitted as a poster presentation to the 2023 Society for Personality Assessment convention. We have a variety of future directions for this project:

1) We plan to add Adverse Childhood Experiences (ACES) to our model as a mediator between childhood sensitivity and attachment styles. We will rerun this as Model 2 to see if ACES is a more salient predictor of attachment styles and BPD.
2) Next, we will rerun both Model 1 and Model 2 with depressive symptoms as the outcome variable in place of BPD. We will compare the BPD and depressive symptoms models to see if these social and environmental factors are unique to BPD or if they are transdiagnostic factors, meaning that they can predict a wide range of psychopathology.
3) To increase the generalizability of these findings, we also collected data from a student sample at Mississippi State University. This data will be cleaned and analyzed in Spring 2023 prior to thesis defense in April 2023. Results from the student sample will be compared to results from the community sample.

Overall, this funding allowed me to collect additional data that will greatly strengthen the validity and generalizability of my Senior Honors Thesis. This project is helping me prepare for the research demands of graduate school. Through working on this project, I have grown in my statistical and writing skills as well as more finely tuned my long-term research interests. The potential findings of this research project will help guide me in future research projects in graduate school (e.g., Master's Thesis). Furthermore, I am looking forward to presenting the

results of this project in my Senior Honors Thesis defense in April 2023 and submitting a first-authored manuscript to a personality journal in the Summer of 2023.

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Madison Brode

Faculty Mentor: Dr. Kristine Evans

Project Title: The Impact of Anthropogenic Noise on Avian Social Learning

Introduction:

It has been increasingly observed that anthropogenic noise from sources such as transportation and human activity has the ability to disrupt biological processes and interactions across a variety of taxa (Sordello 2020). One aspect of noise disturbance that has been particularly well studied is the impact of ambient noise on organismal communication. In the investigation of this question, a taxonomic group that is being particularly well studied is passerine birds (Gilbert 2017). While a large body of research is available documenting the influence of ambient noise on the production of and response to both conspecific and heterospecific vocal signals in passerines, there is limited research available regarding the influence of anthropogenic noise on the transfer of social information, which can be a critical aspect of learning, especially in novel environment.

I am working under the mentorship of Dr. Kristine Evans in the Department of Wildlife, Fisheries, and Aquaculture to investigate the impacts of anthropogenic noise on the social learning abilities of a captive population of European starlings (*Sturnus vulgaris*)---a gregarious species invasive to North America whose use of social information in responding to environmental cues such as the presence of predators or food resources has been documented (Brush 2016, Rafacz 2003). If ambient noise interferes with the social learning of European starlings either through distraction or cross-sensory interference, we predicted that individuals

exposed to noise will require a longer time when learning through the use of conspecific social information.

Materials and Methods:

This study is taking place at the Avian Science Center at the College of Forest Resources Blackjack Road facility on the Mississippi State University campus. Individuals used in this study are being locally-caught European starlings. We have been using baiting in combination with walk-in funnel traps and mist netting to capture starlings. All trials will take place in an isolated 8'x10' climate-controlled enclosure, and birds will be housed in partially-screened 8'x10' enclosers exposed to the outdoor environment. For this study, we are dividing approximately 80 starlings into four groups with 20 individuals in each group. A randomly selected group of demonstrator birds will be trained to complete a simple foraging puzzle. The foraging puzzle as well as video recordings of successful demonstrations will be presented to naïve conspecifics in either a control group or experimental noise group. The time required to successfully manipulate the foraging apparatus will then be recorded.

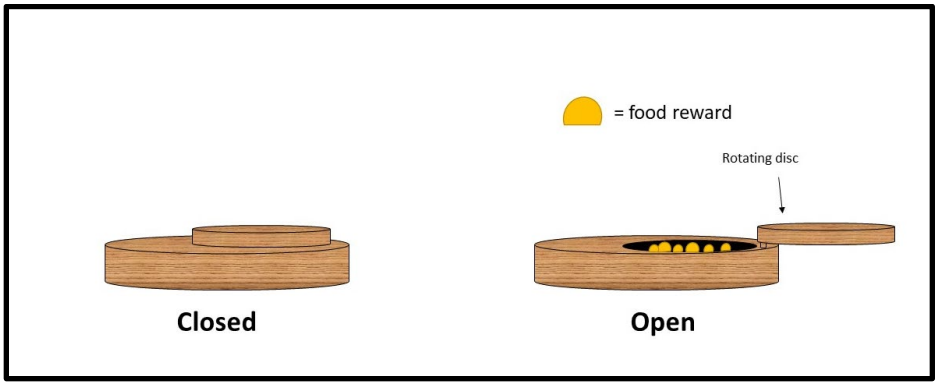

Figure 1. Model of the novel foraging apparatus that will be used in each trial

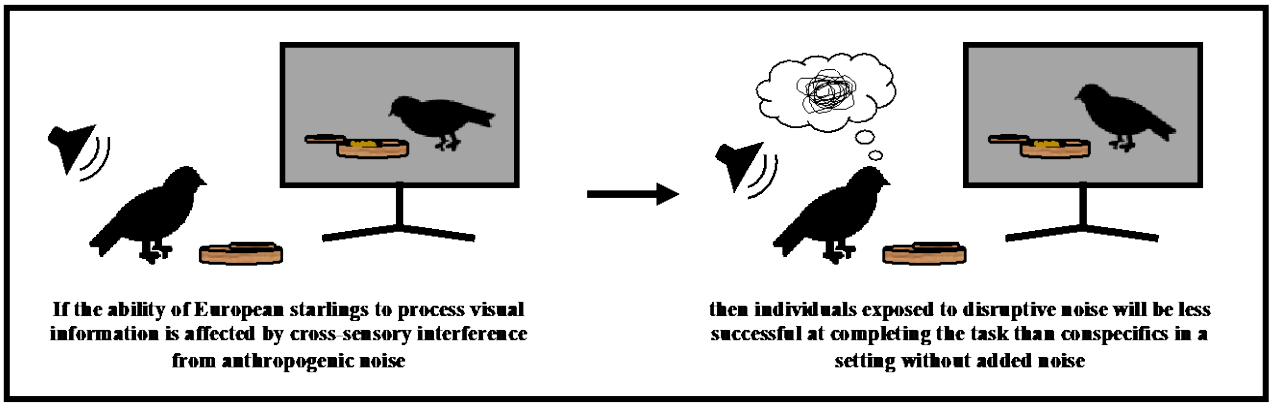

Figure 2. Illustration of our hypothesis and the set-up of an experimental trial

Accomplishments:

This summer I worked on preparing materials and enclosures in the Avian Science Center so that we would be ready to care for European starlings and begin experimental procedures in the future. Additionally, my mentor and I submitted an amendment to our IACUC protocol in order to begin capture of the European starlings. Since the approval of our updated protocol, we have been actively working on baiting and attempting to capture starlings located at MSU North and South Farms. Additionally, we are planning on beginning data collection in the Fall 2022 semester.

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